Comparative Analysis of the Capacity of Elite Suppressor CD4+ and CD8+ T Cells To Inhibit HIV-1 Replication in Monocyte-Derived Macrophages

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ABSTRACT
Elite controllers or suppressors (ESs) are HIV-1-infected individuals who are able to maintain viral loads below the limit of detection of clinical assays without antiretroviral therapy. The mechanisms of virologic control are not fully understood, but ESs have been shown to have a more effective CD8+ T cell response to infected CD4+ T cells than chronic progressors (CPs). While macrophages are another cell type productively infected by HIV-1, few studies have examined the ability of primary effector T cells to suppress HIV-1 replication in these target cells. Here, we compared the ability of unstimulated primary CD4+ and CD8+ effector T cells to suppress viral replication in monocyte-derived macrophages (MDMs) in ESs and CPs. While CD4+ effector T cells were capable of inhibiting viral replication in MDMs, the magnitude of this response was not significantly different between ESs and CPs. In contrast, the CD8+ T cells from ESs were significantly more effective than those from CPs at inhibiting viral replication in MDMs. The CD4+ T cell response was partially mediated by soluble factors, while the CD8+ T cell response required cell-to-cell interaction. Our results suggest that the individual contributions of various effector cells should be considered in rational vaccine design and in ongoing eradication efforts.

IMPORTANCE
Elite suppressors are individuals capable of maintaining low-level viremia in HIV-1 infection without antiretroviral drugs. Their T cell responses have been implicated in eliminating infected CD4+ T cells, and as such, elite suppressors may represent a model of a functional cure of HIV-1 infection. Here, we sought to determine whether the suppressive T cell responses against infected CD4+ T cells also apply to infected macrophages by comparing the responses of elite suppressors and HIV-1-positive individuals on highly active antiretroviral therapy (HAART). Our results show that the CD8+ cells but not CD4+ T cells from elite suppressors have a response against infected macrophages superior to the response of CD8+ cells from patients on HAART. Our results suggest that the induction of a CD8+ T cell response effective against infected macrophages is an outcome to consider in rational vaccine design.

Elite suppressors (ESs) are rare patients who control human immunodeficiency virus type 1 (HIV-1) replication without antiretroviral therapy (1). Many studies have shown that CD8+ T cells from ESs are more effective at inhibiting viral replication in CD4+ T cells than CD8+ T cells from chronic progressors (CPs) (2–11). Furthermore, HIV-1-specific CD4+ T cells from ESs have high-avidity T cell receptors and are more likely to maintain responses that are either proliferative, polyfunctional, or cytotoxic than effector CD4+ T cells from CPs (12–19).

While HIV-1 also infects macrophages, these target cells are rarely examined in the context of immunologic control. Macrophages are thought to be more difficult to infect with HIV-1 than activated CD4+ T cells, in part due to differences in the level of expression of retroviral restriction factors, such as tetherin, SAMHD1, and APOBEC3 (20–22). SAMHD1 specifically contributes to the lower concentration of deoxynucleoside triphosphates already found in macrophages, greatly inhibiting reverse transcription (23, 24). Even though CD4+ T cells are the major reservoir of HIV-1 infection, the infection of macrophages remains a concern, especially since these cells can directly infect CD4+ T cells with HIV-1 in an efficient manner (25, 26). Thus, examining the cellular immune response to HIV-1-infected macrophages will contribute to the rational design of an HIV-1 vaccine.

While some CD8+ and CD4+ T cell clones and cell lines have previously been shown to suppress HIV-1 or simian immunodeficiency virus (SIV) replication in infected macrophages (27–30), less is known about the inhibitory capacity of unstimulated primary T cells. Interestingly, in the macaque model of elite suppression, freshly isolated SIV-specific primary CD8+ T cells were able to inhibit viral replication in CD4+ target cells but not in macrophages (31).

In order to determine whether primary human ES T cells were capable of suppressing viral replication in macrophages, we compared the replication kinetics of a laboratory HIV-1 isolate in monocyte-derived macrophages (MDMs) in the presence and absence of freshly isolated primary CD4+ and CD8+ T cells.
results provide guidance for the development of an effective therapeutic vaccine against HIV-1 infection that can elicit immune responses similar to those observed in ESs.

MATERIALS AND METHODS

Patients. All blood was obtained from patients and healthy donors (HDs) after they provided written and informed consent and was handled as recommended by the Institutional Review Board of the Johns Hopkins University. The ESs (n = 12) had viral loads of less than 50 copies per ml, and the virus in highly active antiretroviral therapy (HAART)-treated CPs (n = 11) had been fully suppressed with antiretroviral therapy for at least 1 year. Seronegative controls comprised 20 healthy HIV-1-negative HDs.

Cell isolation and tissue culture. Peripheral blood mononuclear cells (PBMCs) isolated from whole blood via Ficoll-Paque Plus gradient centrifugation (GE Healthcare Life Sciences) underwent positive selection for CD14– monocytes using a magnetically activated cell sorting system (CD14 microbeads; Miltenyi Biotec). Monocytes were plated at 10^6 cells per well in a flat-bottomed 96-well plate with macrophage differentiation medium (RPMI 1600, 20% type human AB serum [U.S. origin; GemCell], 1% HEPES, 50 ng/ml human recombinant macrophage colony-stimulating factor [R&D Systems]) and incubated for 7 days at 37°C (32). PBMCs collected 7 days prior to infection were stimulated with phytohemagglutinin in activating medium (100 units interleukin-2 [IL-2]/ml) for 3 days before CD4+ T cell targets were isolated by negative selection.

CD4+ and macrophage suppression assay. PBMCs isolated from whole blood via Ficoll gradient centrifugation on the day of infection underwent positive selection for CD8+ T cells (median purity, 94.3%; CD8 microbeads; Miltenyi Biotec) and negative selection for CD4+ T cells (median purity, 95.8%; CD4 T cell isolation kit; Miltenyi Biotec) to isolate the effector cells used in the suppression assay. MDM and CD4+ T cell targets were spinoculated with HIV_HAD (500 ng p24 of virus stock per 10^6 cells was used for the standard assay; half that dose was used for MDMs in the experiment for the comparison with CD4 targets [32]) for 2 h at 1,200 x g and 37°C (33). Autologous CD4+ and/or CD8+ effectors were added to 10^5 target MDMs or target CD4+ T cells at ratios of 1:1, 1:2, and 1:4. CD4+ T cell and MDM targets were cultured in RPMI 1640–10% fetal bovine serum (FBS). Nonsuppressed targets cells incubated with effectors were used to control for background HIV production. The same number of infected target cells used in the experimental conditions was included to determine the maximum amount of virus production for calculating percent inhibition, which was calculated as 100% · [1 – (experimental value/maximum virus production)]. All effectors remained in culture with infected target cells for the entire time span of each experiment. CD4+ T cells and MDMs were cultured postinfection with 10 units/ml of IL-2, which was added every other day solely for the experiment comparing CD8+ T cell–mediated effector inhibition between target cell types. All other suppression assays were conducted with RPMI 1640–10% FBS without IL-2. Culture supernatant was collected for analysis immediately after the addition of effectors (day 0) and on days 3, 5, and 7 postinfection.

Macrophage infection without spinoculation. Monocyte–derived macrophages were incubated with HIV_HAD (500 ng p24 per 10^6 cells) for 4 h at 37°C. Three-quarters of the virus inoculum was removed and replaced with RPMI 1640–10% FBS, and the cells were incubated overnight. The remaining virus was then removed and replaced with RPMI 1640–10% FBS. Culture supernatant was collected immediately after addition of effectors (day 0) and on days 3, 5, and 7 postinfection.

Transwell macrophage suppression assay. Monocytes isolated 7 days prior to infection were plated at 5 X 10^5 cells per well on flat-bottomed 24-well plates in macrophage differentiation medium for 7 days at 37°C. After spinoculation with HIV_HAD (2 h at 1,200 x g and 37°C; 500 ng/10^6 cells), CD4+ and CD8+ effectors were isolated from fresh PBMCs and added to the monocyte–derived macrophages at a 1:1 ratio either directly or in a transwell (Corning transwell permeable supports). Supernatant was collected immediately after addition of effectors (day 0) and on days 3 to 7 postinfection.

Viral output and effector cell infection. Viral production in the culture supernatants was determined via p24 enzyme-linked immunosorbent assay (ELISA; PerkinElmer) per the manufacturer’s instructions. For fluorescence-activated cell sorting, T cell effectors were stained with the extracellular markers CD3-Pacific Blue and CD8-allophycocyanin-H7 (BD Biosciences). Cells were permeabilized with Cytotox/Cytoperm (BD Biosciences) and then stained with Coulter clone K57-RD1 (HIV-1 core antigen, FL-2 channel). Fluorescence data were collected on a BD FACScanto II flow cytometer and analyzed with FlowJo software.

Cytotoxicity assay. Macrophages were differentiated and infected with HIV_HAD as described above for the suppression assay. PBMCs were acquired on the day of macrophage infection and stimulated with Gag peptides (10 μg/ml) and 10 units/ml of IL-2 for 1 week. After 7 days of culture after HIV_HAD infection, the macrophage culture medium was changed. Stimulated and unstimulated primary CD4 and CD8 effector cells were added at a 1:1 effector cell/target cell ratio. Supernatant was harvested from the cultures at 24 h after addition of the effectors and analyzed for lactate dehydrogenase (LDH) release using a Cytotoxicity-96 nonradioactive cytotoxicity assay (Promega) per the manufacturer’s instructions (34). Infected macrophages without any effectors were used as a spontaneous target cell death control, and effectors cultured alone were used as a spontaneous effector cell death control. Maximum LDH release was determined by treating macrophages with 0.5% Triton-X for maximum cell death. Percent cytotoxicity was calculated as follows: 100% · (experimental LDH release – spontaneous target cell LDH release – spontaneous effector cell LDH release)/(maximum LDH release – spontaneous target cell LDH release).

Statistical analysis. Statistical analyses were performed using GraphPad Prism (version 6) and Microsoft Excel software. Unpaired, two-tailed Student’s t tests were used for comparing ES and HAART effector responses in the macrophage suppression and cytotoxicity assays. A paired, two-tailed Student’s t test was used to examine directly applied and transwell effector responses. Linear analyses used Pearson correlations.

RESULTS

In order to examine the T cell response to macrophage infection in HIV-positive individuals, we first examined the susceptibility of monocyte-derived macrophages (MDMs) from 12 ESs, 11 CPs on suppressive HAART regimen, and 19 HDs to HIV-1 infection. Using spinoculation and a replication-competent virus (HIV_HAD), we found that there was no difference in the amount of virus produced by MDMs from ESs, CPs, and HDs by day 7 postinfection (Fig. 1A and B). To determine whether there were subtle differences in the susceptibility to infection that were masked by spinoculation, we performed the infectivity assay without spinoculation in macrophages from randomly selected subsets of ESs and HDs (n = 4 for each; Fig. 1C). We found that, under these conditions, HIV_HAD was similarly capable of infecting MDMs from both ESs and HDs. Taken together, these data suggest that there is no significant difference in the susceptibility of MDMs from ESs, CPs, and HDs to HIV-1 infection.

Strong CD8+ T cell responses to HIV-1-infected CD4+ T cells have been documented in many ESs; thus, we examined the ability of unstimulated primary ES CD8+ T cells to suppress viral replication in MDMs using a variation of a previously described inhibition assay (4, 35). The responses elicited by the CD8+ effectors were divided into three categories on the basis of the distribution of the degree of inhibition observed in all the patients studied. T cells from some patients mediated either (i) a low degree of inhibition (defined as less than 35% inhibition), (ii) an intermediate degree of inhibition (defined as between 35 and 65% inhibition),
or (iii) a high degree of inhibition (defined as greater than 65% inhibition). The ES CD8$^+$ T cells mediated a high degree of inhibition on days 5 and 7. CD8$^+$ T cells from CPs 1 to 4 (Table 1) were also found to mediate high levels of inhibition, but the ES CD8$^+$ T cell response was found to be significantly superior on day 7 ($P < 0.02$; Fig. 2A). While coincubation of infected macrophages with CD8$^+$ T cells from ESs resulted in high levels of inhibition in all patients except ES5, the responses seen in CPs were much more

TABLE 1 Clinical characteristics of study patients

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<th>Patient</th>
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<th>Date (yr) HIV infection diagnosed</th>
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<th>Current viral load (no. of copies/ml)</th>
<th>Date (yr) treatment initiated</th>
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$^a$ ART, antiretroviral therapy.

$^b$ The patient is HLA-B*08/44 positive.

$^c$ The patient usually has a viral load of <75 copies/ml in the branched DNA assay.

$^d$ ND, not determined.

$^e$ NA, not available.
distribution of the inhibitory responses in each subset on day 7; (C) CD8-mediated inhibition of virus production by CD4 T cells (Fig. 3A). The distribution of the degree of inhibition was nearly identical (Fig. 2D). Taken together, our data demonstrate that negative selection and negative selection resulted in similar levels of inhibition as positive and negative selection are equal (Fig. 3B). In contrast, no significant difference in the inhibitory responses mediated by CD4+ and CD8+ T cells was seen in CPs or HDs.

In order to clarify the mechanisms of inhibition mediated by the two different effector cells, we performed a variation of the inhibition assay where we used the transwell system to separate effector cells from infected target cells. In ESs, some inhibition of viral replication was still seen when effector CD4+ T cells were not in direct contact with MDMs (Fig. 5A and B), suggesting that soluble factors were contributing to the control of viral replication. In contrast, no inhibition was seen when ES CD8+ T cell effectors were physically separated from the infected target cells, suggesting that direct cell-to-cell contact was critical for the control of viral replication (Fig. 5C and D; P = 0.015). Similar results were obtained for CPs (data not shown).

Using a cytotoxicity assay, we sought to verify that the CD8+ response against infected macrophages was indeed a result of cytolytic CD8+ T cells. While there was a modest response in ES6 for unstimulated CD4+ effectors as well as CD4+ and CD8+ T cell effectors that had been stimulated for 7 days with overlapping Gag peptides, the only strong cytotoxic responses were seen with Gag peptide-stimulated CD8+ effectors for both ESs (n = 6) and CPs (n = 5) at 24 h after effector addition (Fig. 6A). The transwell system was again used to determine if this response was dependent upon cell-cell contact, and we found that Gag peptide-stimulated CD8+ T cell effectors were incapable of killing infected macrophages when they were separated by transwells (Fig. 6B). For ES6, we also demonstrated that antibodies to class I blocked the CD8+ T cell-mediated inhibitory responses (P < 0.007; Fig. 4). In contrast, no significant difference in the inhibitory responses mediated by CD4+ and CD8+ T cells was seen in CPs or HDs.

Although CD4+ T cells are typically viewed as the targets of HIV infection, these cells can also act as effector cells and have been shown to be capable of suppressing infection in macrophages in the macaque model of elite suppression (30). Thus, we examined whether or not human ES CD4+ T cells could mediate inhibition of viral replication in macrophages. On day 5 after infection, high levels of inhibition were seen in ESs 4, 6, 8, and 31 as well as CPs 2, 5, 7, and 9, while an intermediate level of inhibition was seen in ESs 22 to 24 and CPs 1, 4, 6, and 8, making the distribution virtually identical (Fig. 2C). Taken together, our data demonstrate that ES CD8+ T cells effectively inhibit viral replication in MDMs, and this inhibition appears to be a correlate of protective immunity.

We next compared the magnitude of the CD4+ and CD8+ T cell-mediated effector responses to target MDMs. The CD8+ T cell-mediated inhibitory responses in ESs were significantly stronger than the CD4+ T cell-mediated inhibitory responses (P < 0.007; Fig. 4). In contrast, no significant difference in the inhibitory responses mediated by CD4+ and CD8+ T cells was seen in CPs or HDs.
T cell-mediated killing (data not shown). Together, our results suggest that the vast majority of CD4\(^+\) T cell effectors in the ESs and CPs that we studied were not able to kill infected macrophages after 24 h of coculture even when those cells were prestimulated with Gag peptides (Fig. 6B).

Because effector CD4\(^+\) T cells are susceptible to infection, we hypothesized that they were infected with HIV-1 during the course of experimentation. Therefore, we determined what percentage of effector cells were infected by staining for intracellular Gag using flow cytometric analysis. On day 7 after infection, the CD4\(^+\) T cell effectors from ESs, CPs, and HDs were found to be infected to similar degrees, with no significant difference seen at three different effector cell-to-target cell ratios (Fig. 7A). Furthermore, in ESs, a high level of infection of CD4\(^+\) T cell effectors occurred by day 3 postinfection (Fig. 7B), which could potentially explain the lower level of inhibition seen at later time points in some patients (Fig. 3A). Infection of effector CD4\(^+\) T cells did not correlate with their ability to inhibit viral replication in macrophages in our combined cohort of ESs and CPs (Fig. 7C). While this lack of correlation held true for ESs when they were examined separately (Fig. 7D), there was a significant correlation between the degree of viral inhibition and the percentage of infected CD4\(^+\) T cell effectors in CPs (\(R^2 = 0.82, P = 0.034\); Fig. 7E).

**DISCUSSION**

Many lines of evidence suggest that the cause of elite suppression in some patients is a result of an efficient CD8\(^+\) T cell response that prevents ongoing viral replication. This idea has long been supported by both functional studies examining the response and quality of ES CD8\(^+\) T cells (2, 3, 5, 10,36–40) and genome-wide association studies identifying major histocompatibility complex class I alleles (such as HLA-B*57 and HLA-B*27) (41–46). In addition, these protective HLA alleles have been shown to be overrepresented in multiple ES cohorts (5, 41, 47–52). While many studies have focused on the control of viral replication in CD4\(^+\) T cells, a few studies have looked at the ability of human T cell clones to kill macrophages. As with CD8\(^+\) cytotoxic T cell clones and monocyte and dendritic cell targets (53), Nef-specific cytotoxic CD4\(^+\) T cells were found to be capable of efficiently killing both CD4\(^+\) T cell and macrophage targets (28, 29). Similarly, in the macaque model of elite suppression, CD4\(^+\) T cell clones were capable of suppressing the infection of macrophages (30). Given the importance of macrophages in the pathology of HIV infection (54), determining whether or not effector T cells can control viral replication in these cells is essential for the rational design of a vaccine. In this study, we demonstrate that unstimulated, primary CD8\(^+\) T cell effectors from ESs are capable of effectively suppressing viral replication in macrophages.

Despite previous evidence to the contrary (55), macrophages from ESs seem to be able to be infected to the same degree as macrophages from patients on HAART and healthy donors. Similar results were obtained with and without spinoculation using the same replication-competent virus that was used in the prior study. While residual intracellular antiretroviral drugs may have

![FIG 4](http://jvi.asm.org/)

**FIG 4** CD8\(^+\) T cells from ESs are more effective than CD4\(^+\) T cells at inhibiting viral replication in macrophages. MDMs were infected with 500 ng HIV\(_{\text{Hut}}\) per 10\(^6\) cells and cultured with CD4\(^-\) or CD8\(^+\) T cells in a 1:2 ratio. Infection was measured by p24 ELISA for ES (n = 9), CP (n = 10), and HD (n = 18) MDMs. ***, P < 0.01; n.s., nonsignificant.
resulted in low-level inhibition of HIV-1 replication in CP MDMs, this would not explain the lack of a significant difference in the replication kinetics between ES and HD MDMs. Macrophages in different activation states have been known to be differentially susceptible to infection (56, 57), so the disparity between our results and previous findings may be a result of the methods used to induce the differentiation of monocytes into macrophages.

In contrast to the macaque model of elite suppression, where SIV-specific CD8$^+$ T cell effectors were ineffective at inhibiting viral replication in macrophages (31), we show here that primary CD8$^+$ T cells from ESs efficiently inhibited virus production in MDMs. This suppression was cell contact mediated and probably the result of cytotoxic responses, as previously described (2, 5, 10, 58). The CD8$^+$ effector inhibitory response to HIV-1-infected macrophages was significantly more potent in ESs than HAART patients. However, a few individuals on HAART had primary, inhibitory CD8$^+$ T cell responses that were comparable to the responses seen in ESs. In contrast, there was a marked difference in the killing of infected macrophages between Gag peptide-stimulated CD8$^+$ T cells from ESs and stimulated CD8$^+$ T cells from patients on HAART. This difference is similar to the differences in the capacity of CD8$^+$ T cells from ESs versus those from CPs to eliminate HIV-infected CD4$^+$ T cells seen previously (5). Interestingly, CD8$^+$ T cells from some healthy donors induced a low

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**FIG 5** CD4$^+$ T cell-mediated inhibition of viral production is partially soluble factor mediated, while CD8$^+$ T cell-mediated inhibition is contact dependent. MDMs infected with HIV$_{\text{BaL}}$ were cocultured with CD4$^+$ or CD8$^+$ T cells in a 1:1 effector cell/target cell ratio either directly or with separation in transwells. Viral production was measured by p24 ELISA. (A) CD4$^+$ effector-mediated inhibition of virus production by two ES individuals, with the CD4$^+$ response shown; (B) CD4$^+$ effector-mediated inhibition ($n = 5$); (C) CD8$^+$ effector-mediated inhibition of virus production by two ES individuals, with the CD4$^+$ response shown; (D) CD8$^+$ effector-mediated inhibition ($n = 5$).
level of inhibition of viral replication in autologous macrophages. This is contrast to the findings of our prior studies, where we saw no inhibition of viral replication in CD4<sup>+</sup> T cells by CD8<sup>+</sup> T cells from healthy donors (11, 35, 59). It is possible that this inhibition may represent an innate immune response or the development of an adaptive response during the 7-day period of coculture of infected macrophages and CD8<sup>+</sup> T cells. Taken together, while ESs clearly have a superior inhibitory CD8<sup>+</sup> T cell response against HIV-1-infected macrophages, on average, it is possible that this phenotype alone is not sufficient to explain elite control in all patients.

In contrast to the responses seen with CD8<sup>+</sup> T cell effectors, there was no significant difference in the ability of primary CD4<sup>+</sup> T cell effectors from ESs and CPs to inhibit viral replication in macrophages. Within the ES group, the CD4<sup>+</sup> inhibitory response was also consistently inferior to the response mediated by CD8<sup>+</sup> effectors. Interestingly, some ESs and CPs had strong CD4<sup>+</sup> T cell inhibitory responses. This inhibition appeared to be mediated by both soluble factors and a cell contact-dependent mechanism. Potential soluble factors involved in the response include RANTES and macrophage inflammatory protein-1-alpha/beta, which inhibit the entry of CCR5-tropic viruses and have been associated with HIV-specific CD4<sup>+</sup> T cell responses (12, 60). With regard to the cell contact-mediated suppression, prior studies have shown that some CD4<sup>+</sup> effectors may have cytotoxic activity against HIV-1-infected CD4<sup>+</sup> T cells (15, 27, 29, 30, 61–64). However, we
saw very little CD4<sup>+</sup>-mediated killing of infected macrophages over a 24-h period in this study. It is possible that CD4<sup>+</sup> T cells are capable of killing over a longer time frame, but, in general, the CD4<sup>+</sup> T cell effector response to macrophages does not appear to be a correlate of immunity in our cohort of patients. Eight out of nine of the ESs studied here have the protective HLA B*27 and/or B*57 class I alleles (Table 1). In contrast, some ESs in other larger cohorts do not have these protective alleles or strong HIV-specific CD8<sup>+</sup> T cell responses (8, 49, 51, 65). It would be interesting to determine whether suppressive CD4<sup>+</sup> responses play a role in the control of viral replication in these patients. With that said, we show here that CD4<sup>+</sup> effector cells are susceptible to infection, and this may limit the effectiveness of CD4<sup>+</sup> cytotoxic T cells. We observed a negative correlation between the susceptibility to infection and the suppressive capacity of effector CD4<sup>+</sup> T cells in ESs and CPs. The results are consistent with those of a prior study (5). From the observation that ESs and CPs. The results are consistent with those of a prior study (5).

Our data are limited by the relatively low number of patients studied and the fact that we did not include viremic CPs in our analysis. The numbers of effector CD8<sup>+</sup> T cells decline over time in patients on HAART (67), so it possible that the lower level of CD8<sup>+</sup> T cell-mediated inhibition in CPs was due to the lower number of effector CD8<sup>+</sup> T cells in these patients. We tried to address this issue by stimulating CD8<sup>+</sup> T cells with Gag peptide prior to doing a cytotoxic T lymphocyte assay, but we still saw a marked difference in the killing of infected cells by effector cells in ESs and CPs. The results are consistent with those of a prior study that showed ES CD8<sup>+</sup> T cells are more efficient than CP CD8<sup>+</sup> T cells at killing infected CD4<sup>+</sup> T cells on a cell-per-cell basis (5).

In conclusion, primary, unstimulated CD8<sup>+</sup> T cells from ESs are capable of suppressing the macrophage production of replication-competent HIV-1 in a cell contact-mediated manner that is superior to the suppression mediated by CD8<sup>+</sup> T cells in patients on HAART. Macrophages are resistant to viral cytopathic effects and are the primary target cell in the central nervous system (53, 68). Thus, the induction of CD8<sup>+</sup> T cells that efficiently kill infected macrophages may be an important feature of an HIV-1 vaccine.

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