

HIV-Specific IL-10-Positive CD8⁺ T Cells Are Increased in Advanced Disease and Are Associated with Decreased HIV-Specific Cytotoxicity¹

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IL-10-producing T cells have been shown to inhibit Ag-specific CD8⁺ T cell responses, and may play a role in the immune dysregulation observed in HIV-1 infection. We characterized the Gag-specific IL-10 responses by CD8⁺ T cells in HIV-1-positive volunteers from Uganda. HIV-specific IL-10 responses were detected in 32 of 61 (52.4%) antiretroviral naive and 2 of 15 (13.3%) volunteers with a complete virologic response on antiretroviral therapy (< 400 copies/ml). The frequency of HIV-specific IL-10-positive cells was significantly higher in volunteers with advanced disease (CD4⁺ T cell count <200 cells/mm³; $p = 0.0004$), and correlated positively with plasma HIV RNA ($r = 0.43$, $p = 0.0004$). Interestingly, the frequency of Gag-specific CD107a/b-, but not IFN- γ -, positive cells was significantly lower in individuals with detectable IL-10-positive CD8⁺ T cells ($p = 0.004$). Gag-specific IL-10-positive CD8⁺ T cells demonstrated a pattern of surface memory marker expression that is distinct compared with CD107a/b- and IFN- γ -positive CD8⁺ T cell populations ($p < 0.0001$). Our study describes a distinct population of IL-10-positive CD8⁺ T cells that may play a role in HIV-associated immune dysfunction. *The Journal of Immunology*, 2006, 176: 1274–1280.

The appearance of HIV-specific cytotoxic CD8⁺ T lymphocytes (CTL) is associated with the drop in plasma virus levels during acute infection and the control of set-point viremia during chronic infection (1, 2). CTL are able to lyse infected cells before progeny virions are produced (3), and therefore, have the potential to limit viral load and slow disease progression. Nevertheless, virus-specific CTL responses ultimately fail to prevent HIV disease progression (4). The causes for the failure of cellular immunity to contain HIV infection remain poorly understood, but may be associated with the observed progressive functional defects in HIV-specific effector T cell function in chronic infection (4, 5). Immune dysfunction in HIV infection has been shown to be associated with defects in cytokine production (6), perforin expression (7–9), alterations in phenotypic maturation (10, 11), as well as decreased proliferative ability of virus-specific CD8⁺ T cells (9, 12).

IL-10 is one of the key molecules involved with immunosuppression (reviewed in Ref. 13). The effect of IL-10 on CD8⁺ T cell function is not fully understood (14–17), but may include regulatory properties (18). Both CD4⁺ and CD8⁺ T cells have been shown to express high IL-10 levels in HIV infection (19, 20). IL-10-producing HIV-specific CD25⁺CD4⁺ regulatory T cells have previously been shown to suppress both CD4⁺ and CD8⁺ HIV-specific T cell function in vitro (21, 22). In addition, a higher frequency of CD4⁺ T cells producing IL-10 was observed in HIV-infected individuals with progressive or active HIV replication compared with nonprogressors (23). To our knowledge, the rela-

tionship between IL-10 production by CD8⁺ T cells and CD4 count, plasma HIV RNA, and HIV-specific function has not previously been examined. We describe a population of HIV-specific IL-10-positive T cells in HIV-1-infected volunteers from Uganda. The presence of these IL-10-positive CD8⁺ T cells was associated with advanced HIV disease and correlated positively with plasma HIV RNA. Our data suggest that these IL-10-positive CD8⁺ T cells may have an important regulatory role in the immune dysfunction observed in HIV infection.

Materials and Methods

Study subjects and samples

HIV-1-positive volunteers ($n = 76$) were recruited from the HIV clinic at the Joint Clinical Research Centre (JCRC; Kampala, Uganda). Sixty-one were antiretroviral (ARV)³ naive and 15 volunteers had a complete virologic response on ARV therapy (<400 copies/ml). Demographic information was obtained at the time of enrollment and blood draw. Clinical characteristics including the prevalence of coinfections in this population were previously described (24, 25). Exclusion criteria included age <18 years, pregnancy, active tuberculosis, *Schistosoma mansoni* coinfection, or moribund status. A summary of the patient clinical data is shown in Table I. Institutional Review Board approvals were obtained from the California Department of Health Services and the JCRC, and all study participants gave written informed consent. Absolute numbers of CD4⁺ T cells were determined by BD TruCount (BD Biosciences) and the HIV-1 RNA level was determined from plasma using the Roche Amplicor 1.5 (Roche), as per manufacturer's recommendations, and the lower limit of detection was 400 copies/ml (26). Isolation of PBMC was performed by Ficoll-Hypaque (Amersham Biosciences) density centrifugation. Cryopreserved PBMC and plasma were stored and shipped to the U.S. in liquid nitrogen.

Antigens

Peptides corresponding to the sequences of clade A and D consensus sequences of HIV-1 for Gag (www.hiv.lanl.gov/content/hiv-db/CONSENSUS/M_GROUP/Consensus.html) were synthesized as 15 aa overlapping by 11 aa (Mitochor Mimotopes). Gag synthetic peptides used for all T cell assays were pooled into one single pool of peptides (total 123) for each consensus clade A or D, with a final concentration of 1 μ g/ml per peptide. A single pool of overlapping peptides, corresponding to the amino acid

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³ Abbreviations used in this paper: ARV, antiretroviral; HCMV, human CMV; RA, receptor antagonist.

Table I. Summary of clinical characteristics of study participants^a

HIV Positive	Age ^b (years)	CD4 Count ^b	HIV Plasma RNA ^b (copies × 10 ³ /ml)
ARV naive (n = 61)	37 (23–55)	198.5 (4–723)	98.3 (<0.4–750)
On ARV (n = 15) ^c	39 (28–61)	294 (73–444)	<0.4
p =	0.25	0.28	<0.0001

^a Clinical characteristics at time of enrollment and blood draw.

^b Median values and ranges.

^c All volunteers have achieved complete virologic response.

sequence of the PP65 protein (BD Biosciences) was used to detect the human CMV (HCMV)-specific response (27, 28)

Flow-based intracellular cytokine staining

Detection of Gag-specific IFN- γ production was performed using PBMC (1×10^6) incubated with Gag peptide pools for 2 h at 37°C in 5% CO₂ in the presence of costimulatory anti-CD49d and anti-CD28 (1 μ g/ml; BD Biosciences), followed by brefeldin A for 4 h as previously described (29, 30). Detection of HIV-specific IL-10 production was performed using a similar protocol except Golgi stop (BD Pharmingen) was substituted for brefeldin A, and incubation time was extended to 12–14 h (25). PHA (10 μ g/ml; Sigma-Aldrich) and LPS (1 ng/ml; Sigma-Aldrich) were used as positive controls in the IFN- γ and IL-10 assays, respectively. Media alone without Ag stimulation was used in negative controls. Cells were then stained with different combinations of the following Abs: IFN- γ FITC, CD45RA FITC, IL-10 PE, CD45 RA PE, CCR7 PE CY7, CD3 PerCP Cy5.5, CD8 allophycocyanin Cy7, CD4 Pacific Blue (BD Pharmingen). A minimum of 30,000 CD3⁺ cells/sample were acquired using a six-color flow cytometer (LSRII; BD Biosciences) and analysis was performed by FLOWJO software (Tree Star). Results were expressed as: the percentage of IFN- γ or IL-10-positive CD8⁺ T cells (percent positive = percent Ag-specific – percent negative control). Responses $\geq 0.1\%$ and two times the background were considered positive. All volunteers demonstrated significant IFN- γ and IL-10 production following PHA and LPS stimulation, respectively. Background expression was <0.05%.

CD107a/b degranulation assay

Degranulation assay was performed as previously described (31). Cytotoxic CD8⁺ T cell function has been shown to correlate directly with T cell degranulation which is a prerequisite process of perforin-granzyme-mediated lytic function (32), and can be measured by increased expression of surface CD107 (31, 33). Briefly, PBMC (0.5×10^6) were incubated with 1 μ g/ml anti-CD49d and anti-CD28 (BD Biosciences), anti-CD107a and anti-CD107b FITC (CD107a/b; BD Biosciences), and Gag peptide pools, in a 0.2 ml final volume for 6 h. *Staphylococcus* enterotoxin B (1 μ g/ml; Sigma-Aldrich) and media alone were used as positive and negative controls, respectively. Cells were subsequently stained with anti-CD45 RA PE, anti-CCR7 PE CY7, anti-CD3 PerCP Cy5.5, and anti-CD8 allophycocyanin Cy7, CD4 Pacific Blue (BD Pharmingen). A minimum of 30,000 CD8⁺ events/sample was acquired by flow cytometry. Percentages of CD3⁺CD8⁺ T cells expressing CD107a/b were determined after subtraction of background activity using preset gating. Results were expressed as: the percentage of CD107a/b-positive CD8⁺ T cells (percent positive = percent Ag-specific – percent negative control). Responses $\geq 0.1\%$ and two times the background were considered positive. All volunteers demonstrated significant CD107a/b expression following *Staphylococcus* enterotoxin B stimulation. Background expression was <0.1%.

S. mansoni ELISA

Serologic evidence for schistosomiasis was determined as previously described (25) from frozen plasma using anti-*S. mansoni* egg Ag ELISA (34) per manufacturer's instructions (IVD Research). We have previously demonstrated that *S. mansoni* coinfection was associated with increased frequency of IL-10-positive HIV-specific CD8⁺ T cell responses (25). Therefore, only individuals without serologic evidence of *S. mansoni* infection were evaluated in the current study to exclude schistosomiasis as a contributing factor in HIV-specific IL-10 production.

Cytokine ELISA

Plasma and PBMC (2×10^6 /ml) cocultured with Gag peptides for 48 h were tested for the presence of IL-10 by ELISA (R&D Systems) according

to the manufacturer's protocol. The lower limit of detection for IL-10 was 7.8 pg/ml per test sample. Results were expressed as the level of IL-10 in picograms per 100 μ l of plasma or supernatant. All samples with below detectable IL-10 levels were assigned a value of 7.8.

Statistical analysis

Groups were compared using the Mann-Whitney *U* test or the paired *t* test, and analysis was performed with PRISM software version 4.02 (Graph-Pad). The Spearman correlation coefficient was used to determine the correlation between two variables, and the linear least-square regression model was used in multivariate analysis. Statistical significance was defined as $p < 0.05$.

Results

Relationship between Gag-specific IL-10 expression by CD8⁺ T cells and CD4 T cell count

An inverse correlation between CD4⁺ T cell count and viral load has been reported (35), and was also demonstrated in the current study participants ($r = -0.52$; $p < 0.0001$, data not shown). However, the relationship between the CD4⁺ T cell count and HIV-1-specific CD8⁺ T cell IL-10 responses is unknown. We first determined the frequency of Gag-specific IL-10-positive CD8⁺ T cells in 61 ARV naive HIV-1-infected volunteers (25). Degranulation and IFN- γ production by Gag-specific CD8⁺ T cells were also determined concurrently. All volunteers demonstrated significant responses to at least one of the three markers examined. Representative plots of Gag-specific IL-10, degranulation, and IFN- γ -positive CD8⁺ T cell responses from two volunteers are shown in Fig. 1. Gag-specific IL-10-positive CD8⁺ T cells were detected in 32 of 61 (52.4%) volunteers (Table II). Interestingly, the IL-10-positive group had lower CD4⁺ T cell count compared with the IL-10-negative group ($p = 0.001$). Gag-specific IL-10-positive CD8⁺ T cells were detected in 23 of 31 (74.1%) volunteers with a CD4⁺ T cell count <200 cells/mm³, and 9 of 30 (30%) volunteers with a CD4⁺ T cell count >200 cells/mm³. The frequency of Gag-specific IL-10-positive CD8⁺ T cells was significantly higher in volunteers with a CD4⁺ T cell count <200 cells/mm³ ($p = 0.0004$; Fig. 2).

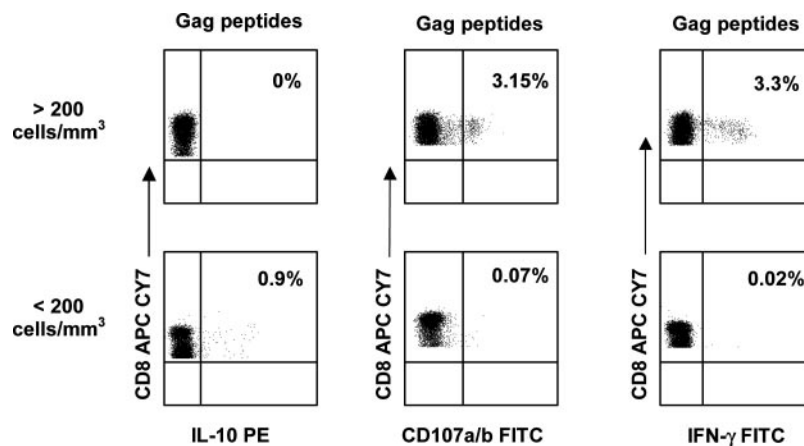
To determine whether the presence of IL-10-positive HIV-specific CD8⁺ T cells was associated with increased plasma IL-10 levels, we measured the plasma IL-10 levels and observed no significant differences between HIV-1-positive volunteers with or without Gag-specific IL-10-positive CD8⁺ T cell responses ($p = 0.28$; data not shown). In addition, we were unable to detect IL-10 in the supernatant of PBMC following stimulation with Gag peptides (data not shown).

Relationship between Gag-specific IL-10 expression by CD8⁺ T cells and plasma HIV RNA

An inverse correlation between HIV-specific CTL responses and viral load has been reported (36–39). However, the lack of viral control in the presence of high frequencies of HIV-1-specific IFN- γ -positive T cell responses has also been described (33, 40–42). We found a significant positive correlation between the frequency of Gag-specific IL-10-positive CD8⁺ T cells and plasma HIV RNA ($r = 0.43$, $p = 0.0004$; Fig. 3A). This correlation remained significant in a multivariate least square regression analysis model after controlling for CD4⁺ T cell count ($R^2 = 0.11$; $p = 0.03$, data not shown). In contrast, there was no correlation between the frequency of CD107a/b-positive CD8⁺ T cells ($p = 0.88$; Fig. 3B), or Gag-specific IFN- γ ($p = 0.89$; Fig. 3C), and plasma HIV RNA.

Early ARV therapy has been shown to reduce IL-10 production by CD4⁺ T cells (23). To determine the effect of ARV therapy on the frequency of Gag-specific IL-10-positive CD8⁺ T cells, we

FIGURE 1. IL-10-positive and effector Gag-specific CD8⁺ T cell populations are distinct. PBMC were stimulated with Gag peptides, then stained with either IL-10 PE, or anti-CD107a/b FITC, IFN- γ FITC, and anti-CD3 PerCP CY5.5, anti-CD8 allophycocyanin CY7, and anti-CD4 Pacific Blue. Samples were first gated on the CD3⁺/CD8⁺ lymphocyte population then the percent of IL-10, CD107a/b, and IFN- γ -positive cells was determined. Representative plots of the frequencies of the Gag-specific CD8⁺ T cells expressing IL-10, CD107a/b, or IFN- γ from two volunteers with CD4 T cell count more than, or less than, 200 cell/mm³. Results were expressed as the percentage of Gag-specific CD8⁺ T cells expressing IL-10, CD107a/b, or IFN- γ .



measured Gag-specific CD8⁺ T cell responses in 15 HIV-1-infected volunteers who achieved complete virologic response on ARV therapy (HIV RNA <400 copies/ml). There were no significant differences in age ($p = 0.25$) or CD4 T cell count ($p = 0.28$) between the ARV naive and ARV receiving volunteers (Table I), and 6 of 15 (40%) volunteers on ARV had a CD4⁺ T cell count <200 cells/mm³. Interestingly, Gag-specific IL-10-positive CD8⁺ T cells were detected in only 2 of 15 (13.3%) volunteers on ARV compared with 52.4% of ARV naive volunteers (Fig. 4). Significantly higher frequencies of Gag-specific IL-10-positive CD8⁺ T cells were observed in ARV naive individuals (median = 0.11%; range = 0.0–1.3%) compared with those on ARV (median = 0.0%; range = 0.0–0.2%; $p < 0.0001$). Gag-specific IFN- γ , and CD107a/b-positive CD8⁺ T cells were detected in 54 of 61 (88.5%) ARV naive volunteers, compared with 8 of 15 (53.3%), and 6 of 15 (40%) volunteers on ARV, respectively ($p = 0.0004$ and $p < 0.0001$ for IFN- γ and CD107a/b, respectively; data not shown).

Effect of Gag-specific IL-10 production by CD8⁺ T cells on effector function

We next examined the association between IL-10 production and cytotoxicity (as measured by CD107a/b) (31), or IFN- γ production by Gag-specific CD8⁺ T cells. The frequency of CD107a/b-positive CD8⁺ T cells was significantly higher in volunteers with no evidence of IL-10 responses compared with IL-10-positive volunteers ($p = 0.004$; Fig. 5). In addition, there was a negative correlation between the frequency of Gag-specific IL-10-positive CD8⁺ T cells and the frequency of CD107a/b-positive CD8⁺ T cells ($r = -0.33$, $p = 0.009$; data not shown). No significant difference in the frequency of IFN- γ -positive CD8⁺ T cells between the IL-10-negative and -positive groups was observed ($p = 0.2$; Fig. 5).

Table II. Summary of clinical characteristics of ARV naive volunteers^a

IL-10 Positive CD8 ⁺ T Cells	Age (years) ^b	CD4 Count ^b	HIV Plasma RNA (copies $\times 10^3$ /ml) ^b
Undetected ($n = 29$)	36.5 (23–47)	297 (9–723)	49.5 (2.1–750)
Detected ($n = 32$)	38 (24–55)	144.5 (4–687)	219.5 (16.8–750)
$p =$	0.17	0.001^c	0.01

^a Clinical characteristics at time of enrollment and blood draw.

^b Median values and range.

^c Values in bold are statistically significant ($p < 0.05$).

To determine whether the presence of IL-10-positive HIV-specific CD8⁺ T cells was associated with altered responses against non-HIV Ags, we analyzed HCMV-specific responses in the same study population. PP65-specific CD8⁺ T cell responses were detected in 54 of 61 study participants. No significant differences in the frequency of PP65-specific CD107a/b and IFN- γ -positive CD8⁺ T cells were observed between volunteers with or without Gag-specific IL-10-positive HIV-specific CD8⁺ T cell responses ($p = 0.77$, and $p = 0.33$, respectively; data not shown). In addition, PP65-specific IL-10-positive CD8⁺ T cell responses were not detected in most volunteers irrespective of the presence of Gag-specific IL-10-positive CD8⁺ T cell responses.

Expression of surface memory markers by IL-10-positive Gag-specific CD8⁺ T cells

Specific CD8⁺ T cell functions have been ascribed to distinct populations defined by immunophenotyping, including expression of the homing receptor CCR7, and different isoforms of CD45R (reviewed in Ref. 43). Representative plots of the phenotype of the HIV-specific IL-10, CD107a/b- and IFN- γ -positive CD8⁺ T cells

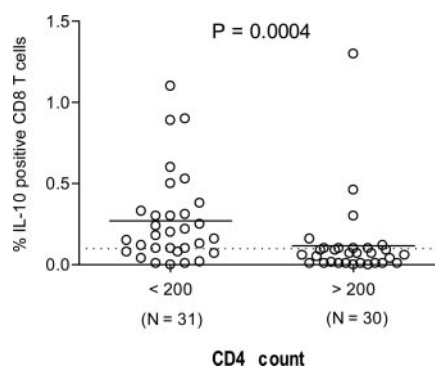


FIGURE 2. Higher Gag-specific IL-10-positive CD8⁺ T cell frequency is observed in volunteers with a CD4 T cell count <200 cell/mm³. PBMC were stimulated with Gag peptides, then stained with either IL-10 PE, or anti-CD107a/b FITC, IFN- γ FITC, and anti-CD3 PerCP CY5.5, anti-CD8 allophycocyanin CY7, and anti-CD4 Pacific Blue. Samples were first gated on the CD3⁺/CD8⁺ lymphocyte population then the percentage of IL-10-positive cells was determined. Results were expressed as the percentage of Gag-specific CD8⁺ T cells expressing IL-10. Bars represent median values. Dashed line represents the cutoff for significant IL-10 expression. Frequencies of Gag-specific IL-10-positive cells were compared in volunteers with CD4 T cell counts <200 ($n = 31$) and >200 ($n = 30$). Differences in IL-10 expression between the two groups were statistically significant ($p = 0.0004$).

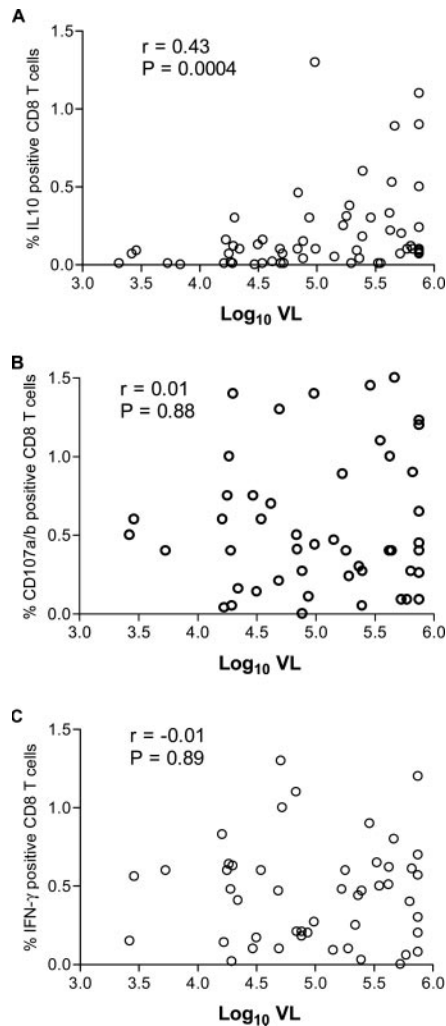


FIGURE 3. Positive correlation between the frequency of HIV-specific IL-10-positive CD8⁺ T cells and HIV plasma RNA. The percentage of Gag-specific (A) IL-10, (B) CD107a/b, or (C) IFN- γ -positive CD8⁺ T cells is plotted against the HIV plasma RNA (\log_{10} viral load (VL)). A significant correlation between the two parameters in A was observed ($r = 0.43$, $p = 0.004$). No correlation between the two parameters in B and C is observed ($r = 0.01$, $p = 0.88$; and $r = -0.01$, $p = 0.89$, respectively).

are shown in Fig. 6A. IL-10-positive CD8⁺ T cells demonstrated a heterogeneous phenotype expression of CCR7, and were mainly CD45 RA-negative (Fig. 6B). Differences in the pattern of CCR7, but not in CD45 RA, expression between the IL-10 and CD107a/b-positive Gag-specific CD8⁺ T cells were statistically significant ($p < 0.0001$ and $p = 0.13$, respectively). Similar phenotypic differences were also observed between the IL-10- and IFN- γ -positive Gag-specific CD8⁺ T cells ($p < 0.0001$ and $p = 0.42$, respectively).

Representative plots of mutually exclusive expression of Gag-specific IL-10-, CD107a/b-, and IFN- γ -positive CD8⁺ T cells from two volunteers are shown in Fig. 1. IL-10-positive, but not CD107a/b and IFN- γ -positive, cells were detected in the volunteer with a CD4 T cell count < 200 cells/mm³. In contrast, only CD107a/b- and IFN- γ -positive cells were detected in the volunteer with a CD4 T cell count > 200 cells/mm³. These results suggest that the Gag-specific IL-10-positive CD8⁺ T cell population may be distinct from the effector CD8⁺ T cell populations, as determined by CD107a/b and IFN- γ expression, and display a heterogeneous pattern of CCR7 expression.

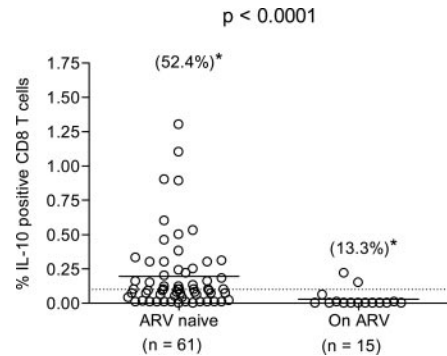


FIGURE 4. Antiretroviral treatment is associated with decreased frequency of Gag-specific IL-10-positive CD8⁺ T cells. PBMC were stimulated with Gag peptides, then stained with either IL-10 PE, or anti-CD107a/b FITC, IFN- γ FITC, and anti-CD3 PerCP CY5.5, anti-CD8 allophycocyanin CY7, and anti-CD4 Pacific Blue. Samples were first gated on the CD3⁺/CD8⁺ lymphocyte population then the percentage of IL-10-positive cells was determined. Results were expressed as the percentage of Gag-specific CD8⁺ T cells expressing IL-10. Bars represent median values. Dashed line represents the cutoff for significant IL-10 expression. *, Percentage values represent the fraction of ARV naive (32 of 61), or volunteers on ARV (2 of 15) that express Gag-specific IL-10-positive CD8⁺ T cells over the total number of ARV naive, or volunteers on ARV (equivalent to 100%). Differences in IL-10 expression between HIV-positive ARV naive and volunteers on ARV were statistically significant ($p < 0.0001$).

Effect of rIL-10 on CD107a/b, and IFN- γ expression by the Gag-specific CD8⁺ T cells

IL-10 has been shown to modulate CD8⁺ T cell responses in vitro (14–17). Next, we examined the effect of IL-10 on the HIV-specific effector function in vitro. PBMC ($n = 10$) were stimulated with Gag peptides in the presence of 1 ng/ml rIL-10, and the frequency of CD8⁺ T cells expressing IFN- γ or CD107a/b was assessed. Addition of rIL-10 was associated with reduced frequency of CD107a/b-positive cells in 8 of 10 volunteers ($p = 0.01$; Fig. 7), but had no significant effect on the frequency of IFN- γ -positive

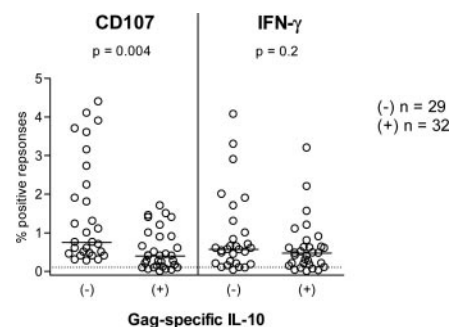


FIGURE 5. Decreased frequency of Gag-specific CD107a/b-positive CD8⁺ T cells in IL-10-positive compared with IL-10-negative volunteers. PBMC were stimulated with Gag peptides, then stained with IL-10 PE, or, either anti-CD107a/b FITC, IFN- γ FITC, and anti-CD3 PerCP CY5.5, anti-CD8 allophycocyanin CY7, and anti-CD4 Pacific Blue. Samples were first gated on the CD3⁺/CD8⁺ lymphocyte population then the percentage of IL-10, CD107a/b, and IFN- γ -positive cells was determined. Frequencies of Gag-specific IFN- γ and CD107a/b-positive cells were compared in IL-10-positive and IL-10-negative volunteers. Results were expressed as the percentage of Gag-specific CD8⁺ T cells expressing IFN- γ or CD107a/b. Bars represent median values. Dashed line represents the cutoff for significant IFN- γ and CD107a/b expression. Differences in CD107a/b, but not IFN- γ , expression between IL-10-negative and IL-10-positive volunteers were statistically significant ($p = 0.004$, $p = 0.2$, respectively).

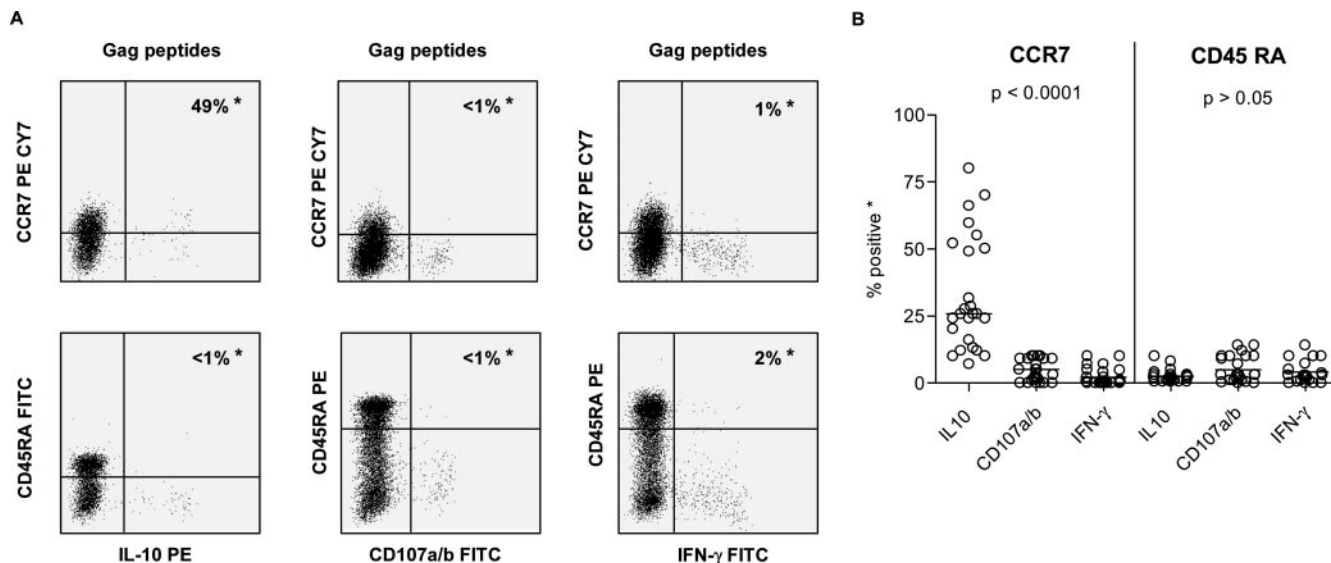


FIGURE 6. Distinct surface memory markers expression by IL-10-positive Gag-specific CD8⁺ T cells. PBMC were stimulated with Gag peptides, then stained for various memory markers and the percentage of IL-10, CD107a/b, or IFN- γ -positive CD8⁺ T cells was determined by flow cytometry. Samples were first gated on the CD3⁺/CD8⁺ lymphocyte population then the percentage of IL-10, CD107a/b, and IFN- γ -positive cells was determined and the extent of CCR7 and CD45 RA expression was also examined. **A**, Representative plots of the phenotype of the Gag-specific CD8⁺ T cells expressing IL-10, CD107a/b, or IFN- γ . *, Percentage values represent the fraction of IL-10, CD107a/b, or IFN- γ -positive cells that express CCR7, or CD45 RA over the total number of IL-10, CD107a/b, or IFN- γ -positive cells (equivalent to 100%). **B**, Data are only from individuals with significant expression of all three markers examined ($n = 25$). Bars represent median values. Differences in CCR7 expression between IL-10 and either CD107a/b or IFN- γ were statistically significant ($p < 0.0001$), while differences in CD45 RA expression were not statistically significant ($p = 0.13$, and $p = 0.42$ between IL-10 and either CD107a/b or IFN- γ , respectively).

cells ($p = 0.12$; data not shown). The addition of anti-IL-10 abrogated the inhibitory effect of rIL-10 on the frequency of CD107a/b-positive cells ($p = 0.31$; data not shown).

Discussion

HIV infection is associated with increased IL-10 production by PBMC, constitutively, and after mitogen stimulation (44–47). Disease progression has been attributed to complex changes in cytokine expression (19, 45, 48–50), including a Th1 to Th2 cy-

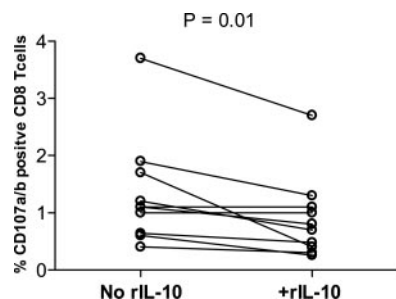


FIGURE 7. Exogenous IL-10 is associated with decreased CD107a/b expression. PBMC ($n = 10$) were stimulated with Gag peptides for 6 h in the presence of anti-CD107a/b FITC, and either rIL-10 (1 ng/ml) alone or rIL-10 + anti-IL-10, then stained with anti-IFN- γ PE, anti-CD3 PerCP CY5.5, anti-CD8 allophycocyanin CY7, and anti-CD4 Pacific Blue and analyzed by flow cytometry. Samples were first gated on the CD3⁺/CD8⁺ lymphocyte population then the percentage of CD107a/b, and IFN- γ -positive cells was determined. Results were expressed as the percentage of Gag-specific CD8⁺ T cells expressing IFN- γ or CD107a/b without rIL-10 added or in the presence of rIL-10. The two dots joined by a line represent the values obtained from the same individual and analysis was performed by paired t test. Significant differences were observed in the percentage of Gag-specific CD8⁺ T cells expressing CD107a/b ($p = 0.01$) between the two groups.

tokine phenotype switch (46, 47, 51, 52). However, the role of increased HIV-specific IL-10 expression by CD8⁺ T cells in modulating T cell dysfunction in chronic HIV infection has not previously been addressed. In this study, the frequency of the HIV-specific IL-10-positive CD8⁺ T cells was significantly higher in volunteers with AIDS (CD4 T cell count <200 mm³), and increased IL-10 expression by HIV-specific CD8⁺ T cells may represent a marker for T cell dysfunction and disease progression.

HIV-specific IL-10-positive CD8⁺ T cells were positively correlated with plasma HIV RNA. This correlation remained significant after controlling for CD4 T cell count in a multivariate regression analysis. In addition, a complete virologic suppression following ARV therapy was associated with a significant reduction in HIV-1-specific IL-10-positive CD8⁺ T cells. These results suggest that the generation of HIV-1-specific IL-10-positive CD8⁺ T cells may be Ag-dependent. Chronic antigenic stimulation in vivo has been shown to result in the generation of IL-10-producing CD4⁺ T cells that have regulatory properties (53). We postulate that chronic HIV antigenic stimulation may also drive the activation and expansion of Ag-driven IL-10-producing regulatory CD8⁺ T cells. Alternatively, the presence of IL-10-positive CD8⁺ T cells may simply reflect on the state of immune dysfunction associated with increased plasma HIV RNA. Decreased IL-10 on ARV therapy may therefore indicate an improved immune functional state as a result of immune reconstitution.

The presence of HIV-specific IL-10-positive CD8⁺ T cells was associated with significantly reduced HIV-1-specific cytotoxicity in our study. This finding leads us to postulate that HIV-specific IL-10-positive CD8⁺ T cells may exert an inhibitory effect on distinct populations of HIV-specific effector CD8⁺ T cells. However, a similar association between HIV-specific IL-10-positive cells and HCMV-specific cytotoxicity was not observed, and may reflect on distinct differences in viral immunopathogenesis. Whether the effect of HIV-specific IL-10-positive cells is Ag specific and if they

are associated with altered responses against other non-HIV recall Ags remains to be determined.

Analysis of the surface memory marker expression of the IL-10-positive Gag-specific CD8⁺ T cells demonstrated a significant difference in the pattern of memory marker expression compared with other effector CD8⁺ T cells. However, the IL-10-positive CD8⁺ T cells displayed a heterogeneous pattern of surface immunophenotypes and perhaps a better identification of this T cell population will require functional characterization such as IL-10 production.

Distinct T cell populations with regulatory functions produce IL-10 (54–57). CD25⁺CD4⁺ regulatory T cells (58–63) are able to inhibit immune responses both in vitro and in vivo (58, 64–66). Suppression seems to be mediated by cell contact-dependent mechanisms (58, 67–69) and cytokines such as IL-10 (60, 61, 70). More recently, CD8⁺ T cells with regulatory properties have also been described (71–75). However, the role of IL-10-producing CD8⁺ T cells in modulating HIV-specific T cell responses has not been previously addressed. Whether Gag-specific IL-10-positive CD8⁺ T cells display similar suppressive properties described in other IL-10-producing regulatory T cells remains to be determined. Previous studies have found that proliferation of CD4⁺ T cells from HIV-infected individuals was inversely associated with the levels of IL-10, and that responses were partially restored by anti-IL-10 Abs (76, 77). In this study, the addition of rIL-10 suppressed in vitro expression of HIV-specific CD107a/b. These results suggest that IL-10 and IL-10-positive CD8⁺ T cells may exert a direct inhibitory effect on cytolytic function in vitro. The function of the IL-10-positive CD8⁺ T cells in vivo remains unknown, and direct cell-cell contact may also be required for the IL-10-positive CD8⁺ T cells exert their maximum inhibitory effect. In summary, our study demonstrates the presence of an HIV-specific IL-10-positive CD8⁺ T cell population in advanced disease that may have regulatory properties. These findings warrant further investigation of the respective contribution of these IL-10-positive CD8⁺ T cells to HIV immunopathogenesis and disease progression.

Disclosures

The authors have no financial conflict of interest.

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