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Coinfection with Schistosoma mansoni Is Associated with Decreased HIV-Specific Cytolysis and Increased IL-10 Production

Michael D. McElroy, Mohamed Elrefaei, Norman Jones, Francis Ssali, Peter Mugyenyi, Banson Barugahare, and Huyen Cao

Impaired virus-specific immune responses have previously been observed with Schistosoma mansoni coinfection. We characterized Gag-specific responses in HIV-1-positive Ugandans with and without S. mansoni coinfection. We observed no significant difference in the frequency of IFN-γ CD8+ T cells between the two groups. Interestingly, expression of CD107, a marker for cytolytic activity, was significantly lower in volunteers with S. mansoni coinfection compared with those with HIV-1 infection alone (p = 0.002). In contrast, the frequency of IL-10-positive Gag-specific CD8+ T cell responses was higher in volunteers with S. mansoni coinfection (p = 0.004). Analysis of human CMV-specific CD8+ T cell responses in the same individuals failed to reveal a similar pattern of altered CD107 and IL-10 expression. Our results suggest that S. mansoni coinfection is associated with decreased Gag-specific CD8+ cytolytic T cell responses and increased number of Gag-specific IL-10 positive CD8+ T cells. Our findings may have important implications toward the implementation of HIV preventive and therapeutic programs in Africa. The Journal of Immunology, 2005, 174: 5119–5123.
Flow-based intracellular cytokine staining

HIV-specific intracellular IFN-γ production was detected 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-CD28 and anti-CD49d (1 µg/ml; BD Biosciences), followed by brefeldin A for 4 h as previously described (20, 21). Detection of HIV-specific intracellular IL-10 production was performed using a similar protocol except Golgi Stop (BD Pharmingen) was substituted for brefeldin A, and incubation time extended to 12–14 h.PHA (10 µg/ml; Sigma-Aldrich) and LPS (1 ng/ml; Sigma-Aldrich) were used as positive controls in the IFN-γ and IL-10 assays, respectively. Medium alone without Ag stimulation was used in negative controls. Cells were stained with anti-IFN-γ FITC or anti-IL-10 PE, anti-CD3 PerCP-Cy5.5, and anti-CD8 aliphosphocyanin Cy7 (BD Pharmingen). A minimum of 30,000 CD3⁺ cells per sample was acquired using a six-color flow cytometer (LSRII; BD Biosciences) and analysis was performed by FLOWJO software (Tree Star). Results were expressed as: percentage of IFN-γ⁺ or IL-10-positive CD8⁺ T cells (percent positive = percent Ag-specific – percent negative control). Responses ≥0.1% and two times the background were considered positive. All study participants demonstrated significant IFN-γ and IL-10 production following PHA and LPS stimulation, respectively. Background expression was <0.05%.

CD107 degranulation assay

Degranulation assay was performed as previously described (22). Briefly, PBMC (0.5 × 10^6) were incubated with 1 µg/ml anti-CD28 and anti-CD49d (BD Biosciences), FITC-conjugated anti-CD107a and anti-CD107b (CD107a/b) Abs (BD Biosciences), and Gag peptide pools, in a 0.2-ml final volume. *Staphylococcus* enterotoxin B (1 µg/ml; Sigma-Aldrich), and media alone were used as positive and negative controls, respectively. Cells were incubated for 1 h at 37°C in 5% CO₂, followed by an additional 5 h in the presence of the secretion inhibitor Golgi Stop (BD Pharmingen). A minimum of 30,000 CD8⁺ events per sample were 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: percentage of CD107a/b-positive CD8⁺ T cells (percent positive = percent Ag-specific – percent negative control). Responses greater than or equal to 0.1% and two times the background were considered positive. All study participants demonstrated significant CD107 expression following *Staphylococcus* enterotoxin B stimulation. Background expression was <0.1%.

*S. mansoni* ELISA

Serologic evidence for schistosomiasis was determined from frozen plasma using anti-*S. mansoni* egg Ag (SEA) ELISA (23) per manufacturer’s instructions (IVD Research).

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 µl of plasma or supernatant. All samples with below detectable IL-10 levels were assigned a value of 7.8.

Statistical analysis

Statistical analysis was performed with PRISM software version 4.02 (GraphPad Software). Data are presented as median values (± interquartile range (IQR)) and were compared using the Mann-Whitney U test. Statistical significance was defined as p < 0.05.

Results

Clinical characteristics

A total of 35 individuals was evaluated in this study with 12 of 35 demonstrating SEA seropositivity. Positive SEA serostatus was established in 27 of 204 volunteers from our study population, yielding a prevalence estimate of 13.2% (data not shown). To minimize the potential confounding effect of HIV clinical status, volunteers with and without *S. mansoni* coinfection were matched in age, CD4 cell count, and HIV RNA level (Table I). All volunteers were antiretroviral treatment (ART) naïve. Twelve ART naïve SEA-positive individuals were available for this study and were clinically matched with 23 randomly selected SEA-negative vol-

**Table I. Clinical characteristics of study participants**

<table>
<thead>
<tr>
<th></th>
<th>S. mansoni</th>
<th>S. mansoni</th>
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<tr>
<td></td>
<td>Negative (n = 23)</td>
<td>Positive (n = 12)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37 (23–44)</td>
<td>36 (22–73)</td>
</tr>
<tr>
<td>CD4⁰ (cell/µl)</td>
<td>212 (9–671)</td>
<td>230 (40–995)</td>
</tr>
<tr>
<td>HIV RNA³ (copies/ml)</td>
<td>97.2 (2.1–750)</td>
<td>126.1 (8.7–750)</td>
</tr>
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</table>

⁰ No history of ARV treatment.
₁ Median values.
₂ Numbers in parentheses represent the range.

Effect of *S. mansoni* coinfection on Gag-specific IFN-γ-positive CD8⁺ T cells

*S. mansoni* coinfection has been associated with lower HCV-specific IFN-γ production (18). To determine whether *S. mansoni* coinfection alters HIV-specific responses, we first assessed the frequency of Gag-specific IFN-γ-positive CD8⁺ T cell responses in our study population (Fig. 1, A and B). No significant differences were observed between volunteers with HIV-1 infection alone (median = 0.60%; IQR, 0.27–1.2%) and those with *S. mansoni* coinfection (median = 0.40%; IQR, 0.21–1.7%; Fig. 1B, p = 0.7).

Effect of *S. mansoni* coinfection on Gag-specific CD107a/b expression

Disregulation of HCV-specific CD8⁺ T cell responses has been observed with *S. mansoni* coinfection (19). We assessed the presence of Gag-specific cytotoxic activity in HIV-positive volunteers with or without *S. mansoni* coinfection (representative plots shown in Fig. 1A). Cytotoxic CD8⁺ T cell function has been shown to correlate directly with T cell degranulation and can be measured by increased expression of surface CD107 (22, 24). HIV-positive volunteers with *S. mansoni* coinfection demonstrated significantly lower CD107 expression in response to Gag (median = 0.24%; IQR, 0.05–0.55%) compared with those without coinfection (median = 1.10%; IQR, 0.42–2.2%; Fig. 1C, p = 0.002). Gag-specific CD107 up-regulation was observed in all HIV-positive volunteers without *S. mansoni* coinfection (Fig. 1C).

Effect of *S. mansoni* infection on Gag-specific IL-10-positive CD8⁺ T cells

Higher frequencies of CD4⁺ T cells that constitutively produce IL-10 are found in chronic progressors with active viral replication compared with nonprogressors in HIV infection (25). The role of IL-10-positive CD8⁺ T cells in HIV immune dysregulation has not previously been evaluated. We examined the effect of *S. mansoni* coinfection on the frequency of Gag-specific IL-10-positive CD8⁺ T cells (representative plots shown in Fig. 2A). Significantly higher frequencies of Gag-specific IL-10-positive CD8⁺ T cells were observed in individuals with *S. mansoni* coinfection (median = 0.55%; IQR, 0.24–0.83%) compared with those with HIV-1 infection alone (median = 0.09%; IQR, 0.0–0.31%; p = 0.004) (Fig. 2B). All volunteers with *S. mansoni* coinfection had detectable IL-10 production in response to Gag (Fig. 2B).

Plasma IL-10 levels were also tested, and no significant differences were observed between HIV-1-positive volunteers with or without *S. mansoni* co-infection (p = 0.4, 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).
To determine whether the \textit{S. mansoni} coinfection targets other Ag-specific responses, we performed similar analysis on the HCMV-specific responses on the same study population. PP65-specific CD8\(^+\)/H11001 T cell responses were detected in 29 of 35 study participants. No significant differences were noted between HIV-1-positive volunteers with or without \textit{S. mansoni} co-infection (\(p = 0.05\); Fig. 3). In addition, HCMV-specific IL-10-positive CD8\(^+\)/H11001 T cell responses were not detected in most volunteers with or without \textit{S. mansoni} coinfection (Fig. 3).

**Discussion**

The role of \textit{S. mansoni} co-infection in HIV disease pathogenesis is not yet understood. Chronic \textit{S. mansoni} infection has previously been associated with impaired viral-specific responses (19, 26) and T cell hyporesponsiveness (reviewed in Ref. 27). Decreased cytolytic functions and immature T cell phenotypes have also been observed in HIV infection (28, 29). Thus, we hypothesized that \textit{S. mansoni} coinfection also targets HIV-specific immune responses.

We evaluated HIV-specific CD8\(^+\) T cell responses in HIV-1 positive Ugandan volunteers and found no significant differences in HIV-specific responses as measured by IFN-\(\gamma\)/H9253 production between individuals with or without \textit{S. mansoni} coinfection. Surprisingly, concomitant \textit{S. mansoni} infection was associated with a significant decrease in Gag-specific cytolytic responses. To our

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**FIGURE 1.** Frequency of Gag-specific IFN-\(\gamma\) and CD107a/b-positive CD8\(^+\) T cells. PBMC were stimulated with Gag peptides, then stained with either CD107a/b FITC or IFN-\(\gamma\) FITC, along with CD3 PerCP CY5.5 and CD8 allophycocyanin CY7. Samples were first gated on the CD3\(^+\)/CD8\(^+\) lymphocyte population then the percentage of IFN-\(\gamma\) and CD107a/b-positive cells was determined. A, Representative plots of the percentage of Gag-specific CD8\(^+\) T cells expressing IFN-\(\gamma\) and CD107a/b. Results were expressed as percentage of Gag-specific CD8\(^+\) T cells expressing IFN-\(\gamma\) (B) or CD107a/b (C) in HIV-positive volunteers with and without \textit{S. mansoni} coinfection. Bars represent median values. Dashed line represents the cutoff for significant IFN-\(\gamma\) expression. Differences between the two groups in B were statistically significant (\(p = 0.004\)).

**FIGURE 2.** Frequency of Gag-specific IL-10-positive CD8\(^+\) T cells. PBMC were stimulated with Gag peptides, and stained with IL-10 PE, anti-CD3 PerCP CY5.5, and CD8 allophycocyanin CY7. Samples were first gated on the CD3\(^+\)/CD8\(^+\) lymphocyte population then the percentage of IL-10-positive cells was determined. A, Representative plots of the percentage of Gag-specific CD8\(^+\) T cells expressing IL-10. B, Results were expressed as percentage of Gag-specific CD8\(^+\) T cells expressing IL-10 in HIV-positive volunteers with and without \textit{S. mansoni} coinfection. Bars represent median values. Dashed line represents the cutoff for significant IL-10 expression. Differences between the two groups in B were statistically significant (\(p = 0.004\)).
knowledge, this is the first observation of altered effector functions in HIV infection that may be attributed to schistosomiasis. We did not observe similar skewing in HCMV-specific CD8+ T cells responses although this finding may reflect on distinct differences in viral immunopathogenesis.

HIV-infected individuals with S. mansoni co-infection also displayed a significantly higher number of Gag-specific IL-10-positive CD8+ T cells. Although IL-10 production by T cells has been observed in HIV infection (30–32), the detection of Gag-specific IL-10-positive CD8+ T cells have not previously been reported. This finding raises the interesting speculation that S. mansoni dysregulates the cellular immune responses in HIV infection by enhancing the activity of IL-10-producing CD8+ T cells. Whether this IL-10-positive CD8+ T cell population directly impairs the maintenance of cytolytic HIV-specific T cells remains to be elucidated. Interestingly, we observed no differences in plasma IL-10 levels between HIV-positive individuals with and without schistosomiasis. This finding, coupled with comparable levels of HIV-specific IFN-γ responses between the two studied groups, suggests that S. mansoni co-infection does not alter the cytokine profile in HIV-infected individuals as previously reported in other chronic viral infections (18).

S. mansoni is endemic in Uganda, and the infection rate is region-specific (33–36). We found a lower S. mansoni seropositivity rate in our study population compared with previous reports from Uganda (4, 6). This finding may be due to regional endemicity differences and likely also reflect on the limitation of our serologic diagnosis approach (23). Previous studies have reported decreased Schistosoma egg excretion in HIV-positive patients leading to an underestimation of parasitic infection intensities (37, 38). Interestingly, none of the participating S. mansoni-seropositive volunteers in our study reported recent treatment, and all reported symptoms attributable to parasitic infection were not significantly different between the two groups. This finding underscores the need for targeted algorithms in the diagnosis of S. mansoni infection (acute and chronic) in the HIV-positive population in Uganda.

Our study is the first to demonstrate a skewing of HIV-specific T cell response associated with S. mansoni co-infection. Conflicting data exist on whether S. mansoni co-infection leads to more rapid progression of HIV disease (2, 4, 5, 9, 39, 40). Recent studies on S. mansoni treatment in a cohort of HIV-infected Ugandans did not lead to improved CD4 cell count and viral load at 6 mo (4). However, the effect of praziquantel on extraluminal granulomatous responses, which is characteristic of chronic schistosomiasis, is not well studied (41). Aggressive anti-parasitic treatment may reverse or improve the observed T cell immunodeficiency. The cross-sectional design and the sample size of our study do not allow speculations of possible S. mansoni-associated adverse HIV clinical outcome or the effectiveness of praziquantel on the virus-specific immune responses. These questions should be the focus of larger future randomized longitudinal trials. Finally, our findings may also have important implications in the effectiveness of T cell-based HIV vaccines in regions where HIV and parasitic infections are highly endemic.

Disclosures
The authors have no financial conflict of interest.

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12. Pedras-Vasconcelos, J. A., and E. J. Pearce. 1996. Type 1 CD8+ T cell responses between the two studied groups, suggests that S. mansoni co-infection does not alter the cytokine profile in HIV-1 infection as previously reported in other chronic viral infections (18).