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

Michael D. McElroy,²,* Mohamed Elrefaei,²,* Norman Jones,* Francis Ssali,† Peter Mugyenyi,† Banson Barugahare,† and Huyen Cao³*

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.

C hronic parasitic coinfection has been postulated to increase the rate of progression to AIDS in sub-Saharan Africa (1–3), although other studies have disputed this observation (4, 5). Uganda is one such country in which HIV has spread rapidly and where a high seroprevalence of schistosomiasis has been documented (6). However, little is known about the effect of Schistosoma mansoni coinfection on HIV pathogenesis, and no study to date has reported changes in the HIV-specific immune responses in coinfected individuals. Postulated mechanisms by which S. mansoni could accelerate HIV disease have included enhanced rates of HIV replication in Th2 cells (7), increased immune activation (8, 9), or enhanced selective pressure to evolve to virulent variant strains (10, 11).

Complications from chronic schistosomiasis arise from the persistent inflammatory responses associated with the granulomatous disease (12, 13). The immune response to chronic S. mansoni infection is reported to be skewed toward a Th2 phenotype (14–17). Coinfection with S. mansoni has previously been associated with an altered pattern of viral-specific responses associated with a Th1 to Th2 shift. Individuals with chronic viral infection such as hepatitis C virus (HCV)⁴ and S. mansoni demonstrated decreased HCV-specific CD4⁺ T cell proliferative responses compared with individuals with HCV alone (18). Evidence for impaired development and maintenance of memory CD8⁺ T cell responses has also been reported (19). To our knowledge, the impact of S. mansoni coinfection on HIV-specific immune responses has not been fully assessed.

We characterized the Gag-specific CD8⁺ T cell responses in HIV-1-infected Ugandan adults with or without S. mansoni coinfection. We hypothesize that S. mansoni coinfection leads to distinct dysregulation of HIV-specific responses that may contribute to the pathogenesis of HIV infection in Uganda.

Materials and Methods

Study population

HIV-1-infected Ugandan adults visiting the HIV clinic at the Joint Clinical Research Centre (JCRC) in Kampala were enrolled in a cross-sectional study. Demographic and clinical information was obtained at the time of enrollment and blood draw. Exclusion criteria included age <18 years, pregnancy, active tuberculosis, or moribund status. Institutional Review Board approvals were obtained from the California Department of Health Services and the Joint Clinical Research Center, Kampala, and all study participants gave written informed consent. Absolute numbers of CD4⁺ T cells were determined by BD TruCount (BD Biosciences), and HIV-1 RNA level was determined from plasma using the Roche Amplicor 1.5 (Roche Diagnostics) according to the manufacturer’s recommendations.

Cell preparation

Isolation of PBMC was performed by Ficoll-Hypaque (Amershams Biosciences) density centrifugation. Cryopreserved PBMC and plasma were stored and shipped to the United States in liquid nitrogen.

Antigens

Peptides corresponding to the sequences of the clade A and D consensus sequences for HIV-1 for Gag 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 μg/ml per peptide. Consensus sequences were obtained from the Los Alamos database ([http://www.hiv.lanl.gov/content/hiv-db/CONSENSUS/M_GROUP/Consensus.html](http://www.hiv.lanl.gov/content/hiv-db/CONSENSUS/M_GROUP/Consensus.html)). A single pool of overlapping peptides, corresponding to the amino acid sequence of the PP65 protein (BD Biosciences) was used to detect human CMV (HCMV)-specific response (19, 20).

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⁴ Abbreviations used in this paper: HCV, hepatitis C virus; HCMV, human CMV; SEA, S. mansoni egg Ag; IQR, interquartile range; ARV, antiretroviral treatment.

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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 allophycocyanin 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 CD3⁺ 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) naive. Twelve ART naïve SEA-positive individuals were available for this study and were clinically matched with 23 randomly selected SEA-negative vol-

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>S. mansoni Negative (n = 23)</th>
<th>S. mansoni Positive (n = 12)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>37 (23–44)</td>
<td>36 (22–73)</td>
<td>0.77</td>
</tr>
<tr>
<td>CD4⁺ (cell/mm³)</td>
<td>212 (9–671)</td>
<td>230 (40–995)</td>
<td>0.98</td>
</tr>
<tr>
<td>HIV RNA (copies/ml × 10¹⁰)</td>
<td>97.2 (2.1–750)</td>
<td>126.1 (8.7–750)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

a No history of ARV treatment.

b Median values.

Table I. Clinical characteristics of study participants

untene. No volunteers reported recent (<6 mo) treatment for parasitic infections, and the frequency of gastrointestinal symptoms did not differ between the two groups (p > 0.05, data not shown).

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 CD107ab expression

Dysregulation of HCV-specific CD8⁺ T cell responses has been observed with S. mansoni coinfection (19). We assessed the presence of Gag-specific cytolytic 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 coinfection 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 without coinfection (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 *S. mansoni* co-infection 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 *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 *S. mansoni* co-infection (Fig. 3).

**Discussion**

The role of *S. mansoni* co-infection in HIV disease pathogenesis is not yet understood. Chronic *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 *S. mansoni* coinfection also targets HIV-specific immune responses. We evaluated HIV-specific CD8⁺/H11001 T cell responses in HIV-1 positive Ugandan volunteers and found no significant differences in HIV-specific responses as measured by IFN-γ production between individuals with or without *S. mansoni* coinfection. Surprisingly, concomitant *S. mansoni* infection was associated with a significant decrease in CD8⁺/H11001 T cell responses.
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 cell 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 cytotoxic 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 coinfection does not alter the cytokine profile in HIV-1 infection 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 diagnostic 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 coinfection. Conflicting data exist on whether S. mansoni coinfection 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 immunodysfunction. 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.

References


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