Impairment of the *Schistosoma mansoni*–Specific Immune Responses Elicted by Treatment with Praziquantel in Ugandans with HIV-1 Coinfection

Sarah Joseph,1 Frances M. Jones,1 Maureen E. Laidlaw,1 Gamal Mohamed,1 Patrice A. Mawa,2 Proscovia B. Namujju,2 Moses Kizza,2 Christine Watera,2 James A. G. Whitworth,2 David W. Dunne,1 and Alison M. Elliott2

1Department of Pathology, University of Cambridge, Cambridge, United Kingdom; 2Uganda Virus Research Institute, Entebbe, Uganda

We show that Ugandan adults coinfected with *Schistosoma mansoni* and human immunodeficiency virus type 1 (HIV-1) are able to mount *S. mansoni*–specific immune responses but that few such responses increase after treatment with praziquantel (PZQ). Levels of soluble worm antigen (SWA)–specific immunoglobulin (Ig) G1, IgG2, IgG3, IgG4, interleukin (IL)–4, and IL-5 increased significantly in HIV-negative participants after treatment with PZQ, whereas most soluble egg antigen–specific antibody responses and levels of interferon-γ were unaltered. Only levels of SWA–specific IL-5 increased in HIV-1–coinfected participants after treatment. These deficiencies in immune responses may account for the previously reported increased susceptibility to infection and reinfection with *S. mansoni* in individuals coinfected with HIV-1.

The progression of HIV-1 in rural Africa is similar to that in the developed world, despite a marked difference in the prevalence of helminth infection [1]. Our previous study [2] and other studies [3] failed to provide evidence that coinfection with *Schistosoma mansoni* has a deleterious effect on HIV-1 load or CD4+ T cell counts. However, certain helminth-specific cellular immune responses were impaired in Kenyan car washers coinfected with HIV-1 and *S. mansoni* [4], and studies in vitro suggest that activated lymphocytes [5] producing Th2 cytokines [6, 7] and mast cells [8] might be particularly susceptible to viral entry and destruction during HIV-1 infection, suggesting that Th2-mediated immunity to helminths might be particularly vulnerable in HIV-1–coinfected individuals.

Treatment with praziquantel (PZQ) kills *S. mansoni* worms and eggs in vivo, and levels of antibody (Ab) [9] and Th2 cytokines increase in response to *S. mansoni* worm antigen (Ags) after treatment with PZQ [10, 11]. Eosinophilia and schistosome-specific IgE, IL-4, and IL-5 [12] correlate with low levels of reinfection after treatment, and Th2 responses are thought to be protective in humans. Karanja et al. [13] showed that coinfected individuals with the lowest CD4+ T cell counts were the most susceptible to reinfection with *S. mansoni* after treatment. We measured the effects of HIV-1 coinfection on *S. mansoni*–specific humoral and cytokine responses in coinfected Ugandans before and 5 weeks after treatment, to assess how HIV-1 coinfection affects (1) *S. mansoni*–specific immune responses and (2) changes in these responses elicited by treatment with PZQ.

**Materials and methods.** The present study was based at the Uganda Virus Research Institute (UVRI) and the AIDS Support Organisation (Entebbe, Uganda). Ethics approval was given by the UVRI Science and Ethics Committee and the Uganda National Council for Science and Technology. Adult HIV-1–positive participants were members of a cohort described elsewhere [2, 14]. None of the participants was known to be taking antiviral drugs. HIV-1–negative participants were recruited from adults attending the voluntary HIV-1 counseling and testing clinic at UVRI. *S. mansoni* infection was treated with 2 doses of PZQ (20 mg/kg), 4 h apart. Blood samples were obtained from participants immediately before treatment and again 5 weeks later; whole-blood assays (WBAs) were set up within ~4 h. At enrollment, we compared cytokine and Ab responses of HIV-1–positive/*S. mansoni*–negative participants with those of HIV-1–positive/*S. mansoni*–positive and HIV-1–negative/*S. mansoni*–positive participants. After treatment, cytokine and Ab responses of HIV-1–positive/*S. mansoni*–positive participants were compared with those of HIV-1–negative/*S. mansoni*–positive participants.

CD4+ T cells were counted by use of FACScount (Becton Dickinson). HIV-1 serologic testing was conducted by use of a rapid test (Capillus HIV-1/2; Cambridge Diagnostics) and was confirmed by use of an indirect EIA (Recombigen [env and gag] HIV-1-1 EIA; Cambridge Biotech) and a com-
petitive ELISA (Wellcozyme HIV-1 recombinant; Murex Diagnostics). Serum samples for which results were discrepant were examined by use of HIV-1-1 Western blot (Cambridge Biotech). Plasma was stored at −80°C before testing in an HIV-1 load assay (Amplicor HIV-1-1 Monitor; version 1.5; Cobas).

Stools were examined for S. mansoni ova by use of the Kato-Katz method: 2 slides were made and were read twice, 24 h apart [15]. Serum levels of circulating anodic Ag (CAA) of S. mansoni [16] were measured. S. mansoni infection was diagnosed if ova were detected or if the serum level of CAA was >4 ng/mL. Seven HIV-1–positive participants had CAA levels >4 ng/mL, with no detectable S. mansoni ova, and the HIV-1–negative participants were selected on the basis of a stool sample containing S. mansoni ova. Three HIV-1–positive participants had CAA levels <4 ng/mL and stool samples that contained S. mansoni ova. Soluble worm Ag (SWA), soluble egg Ag (SEA), and Plasmodium falciparum schizont (PISE) Ag for use in WBAs and Ab ELISAs were prepared as described elsewhere [17, 18].

Cytokine responses were assessed by use of WBA [2]. Unseparated, heparinized blood was diluted with RPMI 1640 medium supplemented with penicillin, streptomycin, and glutamine, to a final concentration of 1:4, was plated in 96-well plates, and was stimulated with Ag or phytohemagglutinin (PHA; Sigma; final concentration, 10 µg/mL) or was left unstimulated. Supernatants were harvested on day 1 for IL-2 and IL-4 and on day 6 for IFN-γ and IL-5. Cytokines were measured by use of ELISA (PharMingen). The sensitivity of the assays was 8–1800 pg/mL. Cytokines produced in the absence of Ag were subtracted from those in stimulated wells. Low levels of spontaneous IL-2, IL-4, and IL-5 (median, 8 pg/mL) were detected in ~20% of WBAs from all groups. IFN-γ was detected in ~50% of cultures, and, although there were no significant differences in levels between the HIV-1–negative/S. mansoni–positive group (median, 56 pg/mL) and the HIV-1–positive/S. mansoni–positive group (median, 24 pg/mL), cultures from the latter group contained significantly more spontaneous IFN-γ (P = .045) than did those from HIV-1–positive/S. mansoni–negative participants (median, 8 pg/mL).

IgG1, IgG2, IgG3, IgG4, and IgE specific for SWA and SEA were measured by use of ELISA, as described elsewhere [18], using human isotype–specific reagents from Unipath, except mouse anti–human IgE (Calbiochem) and streptavidin-biotinylated horseradish peroxidase (Amersham).

Data were analyzed by use of SPSS software (version 10; SPSS). Intergroup comparisons were made by use of the Mann-Whitney U test, and intragroup comparisons were made by use of the Wilcoxon test. Spearman’s rank correlations were used to examine the relationships between CD4+ T cell count and immune responses.

Results. The groups were as follows: HIV-1 positive/S. mansoni negative (n = 71; mean age, 35 years; 21% male; median CD4+ T cell count, 297 cells/µL [range, 7–1618 cells/µL]; median time in the cohort, 2.5 years); HIV-1 negative/S. mansoni positive (n = 10; age, not recorded; 80% male; CD4+ T cell count, 971 cells/µL [range, 670–1073 cells/µL]; and HIV-1 positive/S. mansoni positive (n = 26; mean age, 34 years; 39% male; CD4+ T cell count, 377 cells/µL [range 5–1278 cells/µL]; median time in the cohort, 2.1 years). At enrollment, the median CD4+ T cell counts of the HIV-1–positive/S. mansoni–positive group and the HIV-1–positive/S. mansoni–negative group were both significantly lower (P < .001) than that of the HIV-1–negative/S. mansoni–positive group.

At enrollment, median levels of S. mansoni SEA- and SWA-specific Abs were lower in the HIV-1–positive/S. mansoni–positive group than in the HIV-1–negative/S. mansoni–positive group, although this difference was not statistically significant. Median levels of all S. mansoni–specific Abs were significantly higher in the HIV-1–positive/S. mansoni–positive group than in the HIV-1–positive/S. mansoni–negative group (P < .01; figure 1A, i–x). All were positive for PISE-specific IgG1, and there was no significant difference in median levels between any of the groups (data not shown).

Median levels of PHA- and SWA-induced IL-2, IL-4, IL-5, and IFN-γ were higher in the HIV-1–negative/S. mansoni–positive group than in the HIV-1–positive/S. mansoni–positive group (figure 2A, i–viii), and the differences were statistically significant for all cytokines except IL-4. There were no significant differences in the median levels of PHA-induced IL-2, IL-4, IL-5, or IFN-γ between the HIV-1–positive/S. mansoni–positive and HIV-1–positive/S. mansoni–negative groups (figure 2A, v–vii), whereas the levels of SWA-specific IL-5 (P < .01; figure 2A, iii) and IFN-γ (P < .01; figure 2A, iv) were significantly higher in the HIV-1–positive/S. mansoni–positive group than in the HIV-1–positive/S. mansoni–negative group. Levels of SWA-specific IL-2 and IL-4 showed the same trend, but the difference was not statistically significant (figure 2A, i–ii).

Pretreatment levels of PHA-induced IL-2, IL-4, IL-5, and IFN-γ were positively correlated with CD4+ T cell counts in the HIV-1–positive/S. mansoni–positive and HIV-1–positive/S. mansoni–negative groups (P = .4–.5; figure 3A). Levels of SWA-specific IL-2, IL-4, and IFN-γ positively correlated with CD4+ T cell counts in both the HIV-1–positive/S. mansoni–positive and HIV-1–positive/S. mansoni–negative groups (P = .3–.6; and P = .05, for all associations except with IFN-γ in the HIV-1–positive/S. mansoni–positive group [P = .2] and IFN-γ in the HIV-1–positive/S. mansoni–negative group (P = .237), although the median levels of these cytokines in the HIV-1–positive/S. mansoni–negative group were significantly lower than those in the HIV-1–positive/S. mansoni–positive group (figure 2A, i, iii, iv). There were no correlations between CD4+ T cell counts and cytokine levels in the HIV-1–negative/S. mansoni–positive group.

In the HIV-1–negative/S. mansoni–positive group, median
Figure 1. **A**, Box-plots showing *Schistosoma mansoni*–specific antibody (Ab) responses at enrollment, in optical density (OD) units. Boxes show the 25th percentile–75th percentile, with the median indicated and outliers shown separately. The HIV-1–positive (*HIV*+) *S. mansoni*–positive (*Sch*+) group (*n* = 26) was compared with the HIV-1–negative (*HIV*−) *S. mansoni*–positive group (*n* = 71), and the HIV-1–negative (*HIV*−) *S. mansoni*–negative (*Sch*/ negative) group (*n* = 10) was compared with the HIV-1–positive group, by use of the Mann-Whitney *U* test, and significant differences are indicated. **B**, Changes in the Ab responses of the HIV-1–negative group (*n* = 26), compared with those of the HIV-1–positive group (*n* = 9) 5 weeks after treatment. Baseline responses were subtracted from those 5 weeks after treatment for each individual, and the changes in the relative amounts of each isotype of each specificity are shown in OD units. Changes within groups were determined by use of the Wilcoxon test for paired samples. **P** < .01; **P** < .05. SEA, soluble egg antigen; SWA, soluble worm antigen.

Levels of SWA-specific IgG1, IgG2, IgG3 and IgG4 increased significantly after treatment (*P* < .05), and SWA-specific IgE showed a similar trend (*P* = .08), with 8 of 9 participants responding. In the HIV-1–negative/*S. mansoni*–positive group, levels of SEA-specific IgG2 and IgE increased (*P* < .05; figure 1B, i–x). Levels of PfSE-specific IgG1 did not change significantly in any group (data not shown). In the HIV-1–positive/*S. mansoni*–positive group, levels of SWA-specific IgG4 increased significantly. No other *S. mansoni*–specific humoral immune response increased significantly in HIV-1–positive/*S. mansoni*–positive participants (figure 1B, i–x).

In the HIV-1–negative/*S. mansoni*–positive group, median
Figure 2. A. Production of phytohemagglutinin (PHA)–induced and soluble worm antigen (SWA)–specific interleukin (IL)–2, IL-4, IL-5, and interferon (IFN)–γ, as determined in whole-blood assays using blood samples obtained at enrollment from HIV-1–negative (HIV–) Schistosoma mansoni–positive (Sch+) (n = 10), HIV-1–positive (HIV+) Sch+ (n = 23), and HIV– S. mansoni–negative (Sch−) (n = 61) participants. Boxes show the 25th percentile–75th percentile, with the median marked and outliers indicated. The HIV+ Sch+ group was compared with the HIV− Sch+ group, and the HIV+ Sch+ group was compared with the HIV+ Sch− group. All cytokines were measured in picograms per milliliter, and median levels were compared by use of the Mann-Whitney U test. B. Changes in levels of PHA-induced and SWA-specific IL-2, IL-4, IL-5, and IFN-γ (in picograms per milliliter) from HIV− Sch+ (n = 22) and HIV− Sch− (n = 10) participants between 0 and 5 weeks after treatment with praziquantel. Pretreatment responses were subtracted from those at 5 weeks. Changes within groups were assessed by use of the Wilcoxon test for paired samples. **P < .01; *P < .05.
levels of SWA-specific IL-2, IL-4, and IL-5 increased significantly after treatment ($P \leq .01$; figure 2B, i–iii). In the HIV–1–negative/S. mansoni–positive group, median levels of PHA-induced IL-4 increased significantly after treatment (figure 2B, vi; $P \leq .01$). PHA-induced IL-2 and IL-5 showed a similar trend, but the increases were not statistically significant (figure 2B, v, vii); in the case of PHA-induced IL-5, this might have been because levels were close to the sensitivity of the assay. In both the HIV–1–negative/S. mansoni–positive and HIV–1–positive/S. mansoni–negative groups, median levels of SWA-specific, PHA-induced IFN-γ were unchanged after treatment, but pretreatment levels of PHA-induced IFN-γ were near the upper limit of sensitivity of the assay (figure 2B, iv, vii). In the HIV–1–positive S. mansoni–positive group, SWA-specific IL-5 was the only cytokine response that increased significantly after treatment with PZQ (figure 2B, iii), but posttreatment levels of this cytokine were still significantly lower than those in the HIV–1–negative/S. mansoni–positive group (data not shown).

Discussion. We have assessed the effect of HIV-1 on S. mansoni–specific humoral and cellular immune responses before and after treatment with PZQ. Pretreatment results suggested that the ability to respond to S. mansoni was somewhat preserved in HIV–1–infected participants: levels of circulating S. mansoni–specific Abs were not significantly decreased, and, although production of cytokines was much reduced, levels of SWA-specific IL-5 and IFN-γ were significantly higher in HIV–1–positive/S. mansoni–positive participants than in HIV–1–positive/S. mansoni–negative participants. Levels of spontaneously produced IFN-γ were also higher in coinfected participants, suggesting, perhaps, the presence of increased numbers of activated cells. The same was not true for the boost in responses to S. mansoni after treatment. Treatment of HIV–1–negative/S. mansoni–positive participants with PZQ resulted in increases in particular immune responses akin to those previously described in a cohort of 187 Ugandans heavily exposed to infection with S. mansoni [11]. Levels of SWA-specific IgG1, IgG2, IgG3, and IgG4 increased, whereas all SEA-specific responses except IgG2 and IgE were unchanged. The only Ab response that increased in HIV–1–positive/S. mansoni–positive participants was SWA-specific IgG4, but levels were low. Levels of PISE–specific IgG1 were not significantly altered after treatment, suggesting that the increases were S. mansoni specific and occurred after damage of adult worms and the release of Ag [19]. In HIV–1–negative participants, levels of SWA-specific IL-4, IL-5, and IL-2 increased after treatment, whereas, in HIV–1–positive participants, only levels of SWA-specific IL-5, perhaps derived from non-T cells, increased significantly [20]. In their study of HIV–1–coinfected car washers, Mwinzi et al. [4] reported that some Th2 responses were reduced by HIV–1, whereas levels of IL-5 were not significantly affected. The failure to boost worm-specific IL-2 and IL-4 responses probably contributed to the deficiencies in Ab responses, because these cytokines are known to be required for maintenance of production of Abs by B cells. Levels of PHA-induced IL-2, IL-4, and IL-5 were significantly decreased by HIV–1 and, along with levels of SWA-specific IL-2, IL-5, and IFN-γ, were positively correlated with the number of CD4+ T cells, suggesting that such T cells and their cytokines were limiting, as has been suggested elsewhere [4].

HIV–1–associated CD4+ T cell immunodeficiency can be associated with reduced fecal excretion of S. mansoni ova [21], with reduced efficacy of PZQ, and with increased susceptibility to reinfection with S. mansoni after treatment [13]. Despite possessing circulating S. mansoni–specific B cells, HIV–1–positive/S. mansoni–positive participants failed to mount the humoral and cellular immune responses that have been shown to correlate with resistance to reinfection after treatment with PZQ, suggesting the existence of a mechanism by which their immunity to the parasite might be impaired [13].

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