SCHISTOSOMA MANSONI, NEMATODE INFECTIONS, AND PROGRESSION TO ACTIVE TUBERCULOSIS AMONG HIV-1–INFECTED UGANDANS

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Abstract. Rates of tuberculosis (TB) in Africa are highest among people infected with HIV. Searching for additional risk factors in a cohort of HIV-infected Ugandan adults, we previously found that a type 2 cytokine bias and eosinophilia were associated with progression to active TB. A possible role for helminth infection was assessed in this study. We analyzed TB incidence in 462 members of this cohort who were screened for filarial infections, gastrointestinal nematodes, and schistosomiasis. Progression to TB was not associated with gastrointestinal nematodes (rate ratio [RR], 1.18; confidence intervals [CIs], 0.66–2.10) or *Mansonella perstans* (RR, 0.42; CI, 0.13–1.34). A weak association between *Schistosoma mansoni* infection and TB was found (RR, 1.42; CI, 0.86–2.34); after adjusting for potential explanatory variables and using more stringent diagnostic criteria, the association was strengthened (RR, 2.31; 1.00–5.33). This analysis suggests an effect of *S. mansoni* infection on progression to active TB among HIV-1–infected Ugandans.

INTRODUCTION

One major consequence of the HIV epidemic has been the increased rate of tuberculosis (TB) in HIV-infected subjects.[1] Reactivation of latent TB occurs faster in HIV infection, rapidly progressive TB disease is more common, and case fatality rates are higher than in HIV-uninfected subjects. It is the major cause of death in HIV-infected Africans.[2] Furthermore, the effect of TB on viral replication may aggravate the underlying immunosuppression and accelerate HIV disease progression in infected subjects.[3,4] The World Health Organization recommends isoniazid preventive therapy for all HIV-infected subjects with evidence of latent TB infection[5]; however, there are significant difficulties in identifying these subjects, the duration of efficacy may be short, and the cost-effectiveness of this strategy is debatable.[6,7] Establishing other risk factors for progression to active TB among HIV-infected subjects may lead to better preventive strategies in this high-risk population, for whom antiretroviral therapy has not yet become widely available. Attempts to characterize the immunologic profile of susceptibility to disease have considered both the type 1/type 2 model of immune responses to infection and the role of immunoregulatory cytokines in the response to mycobacteria. We previously showed, in a cohort of HIV-infected adults in Uganda, that eosinophilia, associated with a type 2 cytokine profile, was the strongest predictor of subsequent progression to active TB.[8] Helminth infections are well-recognized inducers of a type 2 immune response and were associated with eosinophilia in this cohort.[9,10] As part of a larger study of the impact of helminth infection on HIV disease progression that has already been reported,[9] we assessed the role of helminth infection as a risk factor for progression to active TB in HIV-infected adults in Uganda.

MATERIALS AND METHODS

Study subjects. Study subjects, who attend either The AIDS Support Organization (TASO) clinic or the Uganda Virus Research Institute (UVRI) clinic in Entebbe, were members of the Entebbe cohort (EC). This prospective cohort of HIV-infected adults was established by the UK Medical Research Council (MRC) program on AIDS in Uganda in 1995,[11] and recruitment to the cohort is ongoing. Participants (~2,300 between October 1995 and March 2002) attend routinely every 6 months and at additional interim visits if they fall sick. Antiretroviral (ARV) drugs did not become widely available to study participants until February 2003.

Worm study enrollment. All patients attending their routine EC appointment in February to July 2001 and February to March 2002 were counseled and enrolled into this study after providing written informed consent. A questionnaire was completed, stool and blood samples were collected, and albendazole 400 mg was provided to all subjects. Subjects identified from their enrollment specimens as having *Schistosoma mansoni* were treated with praziquantel 40 mg/kg 1 month after enrollment.

Laboratory tests. Duplicate 41.7-mg Kato-Katz smears,[12] modified formol-ether concentration, and charcoal culture for *Strongyloides* were performed on all fecal specimens where possible. Enzyme-linked immunosorbent assay (ELISA) for circulating anodic antigen (CAA) of *S. mansoni*[13] was performed on serum samples to boost the limited diagnostic yield of a single stool sample and also to avoid the possible confounding effect of immunosuppression on fecal egg excretion.[14] Subjects were diagnosed as infected with *S. mansoni* if either Kato-Katz method or CAA ELISA was positive. Microfilaria were diagnosed using a modified Knott concentration method described for hyperproteinemic patients.[15] The above techniques are described in detail elsewhere.[9,16]

Blood samples were examined for $CD4^+$ T-lymphocyte count by FACScount (Becton Dickinson, Erembodegen, Belgium).

Diagnosis of TB and analysis of TB incidence rates. Data on TB diagnosis and treatment have been collected systematically within the cohort from August 1997. An operational study assessed the implementation of isoniazid prophylaxis in this cohort between October 1998 and November 1999[6]; tuberculin skin tests (TSTs; 5 IU of purified protein derivative [PPD] given intradermally using the Mantoux technique) were performed on 1,597 subjects recruited since 1995, of

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whom 74 subjects received 6 months of isoniazid therapy. The National TB Control Program did not consider the results of this study to be sufficient to recommend ongoing implementation of isoniazid preventive therapy (IPT) in this population, so IPT has not been provided since 1999, but tuberculin skin testing on new EC recruits has continued.

Figure 1 shows the time-course for analysis of TB incidence in this cohort. Subjects recruited into EC before August 1997 (of whom some may have developed TB before this date) were excluded from the study. Subjects with a history of TB before recruitment to the EC were also excluded. An assumption was made that those diagnosed as having helminths at enrollment into this study had helminth infection over the previous period that they had been in the EC.[9]

During follow-up, participants with evidence of active TB were studied and treated. Active TB was classified as follows: 1) confirmed, positive culture for *Mycobacterium* spp.; 2) probable, symptoms and signs of TB, with either clinical response and weight gain $\geq$ 2 kg after treatment of TB, or microscopy positive for acid-fast bacilli, but without a positive culture result; 3) possible, symptoms and signs of TB but no information regarding response to treatment; and 4) TB diagnosed elsewhere, details of studies not available. In subsequent analyses, unless otherwise stated, TB was defined as any case classified above as 1), 2), or 3).

Follow-up times (from EC recruitment until 18 months after Worm study enrollment) were censored if the participant developed TB, died, defaulted, started isoniazid preventive therapy or ARV therapy, or were treated for the helminth species in question. As different helminth species were treated (or not) at different time-points, some analyses were based on shorter follow-up periods, depending on the helminth under scrutiny (see below). Crude and adjusted rate ratios (RRs) and confidence intervals (CIs) were estimated using Cox proportional hazard regression. *P* values for adjusted RRs were estimated using the likelihood ratio test. Information on socio-economic factors and risk factors for helminth exposure was collected in the Worm study.[9] These variables were also tested against TB incidence by univariate analyses. Stepwise regression analysis identified variables that were independently associated (*P* < 0.20) with both helminth status and TB incidence; these were included in the final model, along with age, sex, CD4^{+} count at EC recruitment, other helminths, and Bacille Calmette-Avérin (BCG) status (recorded as presence of a BCG scar).

Ethical approval. Ethical clearance for the study was granted by the Uganda National Council for Science and Technology; the science and ethics committee for UVRI; and the ethics committee of the London School of Hygiene and Tropical Medicine.

RESULTS

Tuberculosis in the EC. A total of 1,168 ARV-naïve subjects were recruited to the EC between August 1997 and March 2002. Median age at recruitment was 31 years (range, 17–66 years); 70% were female. CD4^{+} T-lymphocyte count at recruitment was $\leq$ 200 cells/mm^3 in 586 (48%) subjects, $\geq$ 500 cells/mm^3 in 214 (18%) subjects, and missing in 8 (< 1%) subjects. Forty-two percent were in World Health Organization clinical stage 1 or 2.

One hundred forty-nine subjects (13%) had been treated for TB before EC recruitment. These were excluded from subsequent analyses of TB incidence. The isoniazid preventive therapy study between 1998 and 1999 included 45 subjects who joined the EC in or after August 1997 who received isoniazid. These subjects were censored at the date of starting isoniazid.

TB diagnosis rates were analyzed between recruitment to EC and 18 months after enrollment in the Worm study (September 2003). One hundred twenty-six subjects developed TB during this time, with an incidence of 70 per 1,000 person-years (CI, 59–84 per 1,000 person-years). TB incidence was inversely associated with CD4^{+} count at EC recruitment (compared with CD4^{+} $\leq$ 200 cells/mm^3: RR, 0.92; CI, 0.62–1.37 for CD4^{+} 200–499 cells/mm^3, *P* = 0.70; RR, 0.48; CI, 0.28–0.81 for CD4^{+} $\geq$ 500 cells/mm^3, *P* = 0.006) and with World Health Organization clinical stage (RR, 1.8; CI, 1.38–2.35 per advancing stage, *P* < 0.001).

A positive TST ($\geq$ 5 mm) was present in 22% subjects at recruitment and was strongly associated with progression to TB (RR adjusted for CD4^{+} count = 3.7; CI, 2.51–5.40; *P* < 0.001).

Mortality was lower in subjects diagnosed with TB during this follow-up period (RR adjusted for age, sex, and CD4^{+} count = 0.74; CI, 0.57–0.96; *P* = 0.02).

Tuberculosis in Entebbe cohort subjects enrolled in the Worm study. Four hundred sixty-two subjects were enrolled in the Worm study between February 2001 and March 2002. Of these, 39 had an incomplete screen for helminth infection; after excluding these, 423 were included in subsequent analyses. Seventy percent of the subjects were women. Only EC

Enrolment into Entebbe Cohort (n=1119):

Helminth screening plus mass albendazole (n=376):

S.mansoni –infected subjects given praziquantel (n=126):

Follow-up visit 18 months after helminth screening:

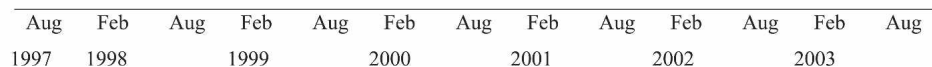| Aug	Feb	Aug	Feb	Aug	Feb	Aug	Feb	Aug	Feb	Aug	Feb	Aug
1997	1998		1999		2000		2001		2002		2003	

FIGURE 1. Time-course for TB incidence analysis in the Entebbe Cohort (numbers exclude those with a previous history of TB).

participants who had survived until 2001–2002 could be enrolled in the Worm study, so median CD4$^+$ count at EC recruitment was higher in those in the Worm study than in those not enrolled (346 versus 137 cells/mm^3; $P < 0.0001$).

Helminth prevalence in the Worm study was 54%, with *S. mansoni*, hookworm, *S. stercoralis,* and *M. perstans* being the principal species in this cohort. Details on diagnosis of, and risk factors associated with, different helminths are provided elsewhere.[9,16]

Forty-seven of these 423 subjects had a diagnosis of TB before EC recruitment (Table 1). Of the remaining 376, 66 developed TB during the follow-up period (incidence 69 per 1,000 person-years; CI, 55–84 per 1,000 person-years).

S. mansoni and TB. Of 140 subjects with *S. mansoni* infections, 14 had a diagnosis of TB before EC recruitment (10% versus 12% without *S. mansoni*, $P = 0.61$).

There was no association between *S. mansoni* infection and TST positivity (20% with *S. mansoni* versus 24% without *S. mansoni*, $P = 0.40$).

Antischistosomal treatment was given to infected subjects 1 month after Worm study enrollment; all subjects were censored at this date. Sixty-six subjects developed TB between EC recruitment and this date (incidence 139 cases per 1,000 person-years; CI, 109–177 per 1,000 person-years). TB incidence was slightly greater among *S. mansoni*–infected subjects (RR, 1.42; $P = 0.17$; Table 1). The RR was greater using a stricter case definition of confirmed or probable TB diagnoses only (RR, 1.82; CI, 1.06–3.10; $P = 0.03$), and was greater still if including only confirmed TB diagnoses (RR, 2.51; CI, 1.15–5.51; $P = 0.02$).

The association was only slightly weakened by adjustment for socio-economic factors and other potential explanatory variables. Stepwise regression analysis identified only proximity to Lake Victoria as independently associated with both *S. mansoni* status and (inversely) with TB incidence; this was included in the final model, along with age, sex, CD4$^+$ count at EC recruitment, other helminths, and BCG status. Using this model, the association between *S. mansoni* and TB inci-

dence was only slightly weakened (adjusted RR, 1.35; CI, 0.80–2.30; $P = 0.27$; Figure 2A; for confirmed or probable TB diagnoses only: RR, 1.68; CI, 0.96–2.96; $P = 0.07$; for confirmed TB diagnoses: RR, 2.31; CI, 1.00–5.33; $P = 0.05$; Figure 2B).

Incidence rates were slightly but not statistically higher in those with the highest intensity *S. mansoni* infections (223 per 1,000 person-years in those with >100 eggs per gram of feces [epg] versus 171 per 1,000 person-years in *S. mansoni*–infected subjects with <100 epg; RR, 1.41; $P = 0.47$).

M. perstans and TB. Of 34 subjects with *M. perstans* infections, only 1 had a diagnosis of TB before EC recruitment (3% versus 12% without *M. perstans*, $P = 0.11$).

M. perstans infection was associated with a reduced prevalence of positive TST responses (6% versus 25%, $P = 0.02$).

No effective treatment was given to subjects infected with *M. perstans*, so associations with TB incidence were analyzed through to 18 months after Worm study enrollment. TB incidence was slightly lower in *M. perstans*–infected subjects (31 per 1,000 person-years versus 74 per 1,000 person-years; RR, 0.42; $P = 0.14$; Table 1). Multivariate analysis, including (for this helminth) electricity supply and proximity to the lake, along with age, sex, CD4$^+$ count at EC recruitment, other helminths, and BCG status, did not substantially change the RR (adjusted RR, 0.41; $P = 0.14$). Restricting the analysis to probable or confirmed TB cases gave an adjusted RR of 0.32 (CI, 0.08–1.33; $P = 0.12$) and to confirmed cases gave an adjusted RR of 0.37 (CI, 0.05–2.80; $P = 0.34$).

Gastrointestinal nematodes and TB. Of 132 subjects with gastrointestinal (GI) nematode infections, 15 had a diagnosis of TB before EC recruitment (11% versus 11% without GI nematodes, $P = 0.91$). There was no association between GI nematode infection and positive TST responses (23% versus 23%, $P = 0.91$).

Albendazole was given to all subjects at Worm study enrollment, so for the purposes of this analysis, all subjects were censored at this time-point. Because some subjects were recruited to the EC and enrolled in the Worm study at the same

TABLE 1
Baseline characteristics and TB incidence rate ratios according to helminth infection

	S. mansoni		GI nematodes		*M. perstans*		No helminths*
	No.	P†	No.	P†	No.	P†	No.
Total	140		132		34		168
Previous TB	14		15		1		23
Total analysed‡	126		83		33		139
Median CD4$^+$ count at EC recruitment (interquartile range)	371 (179–512)	0.92	365 (203–615)	0.96	436 (237–649)	0.31	362 (195–574)
WHO clinical stage 3 or 4	58 (46%)	0.10	30 (36%)	0.92	11 (33%)	0.40	45 (32%)
BCG scar (missing values)	77 (62%)	0.56	42 (53%)	0.60	20 (63%)	0.68	75 (54%)
			3		1		1
TST ≥ 5 mm (missing values)	24 (20%)	0.40	19 (23%)	0.91	2 (6%)	0.02	35 (36%)
	8		1		1		7
TB cases	25		17		3		20
Person-years	138		129		98		216
Incidence per 1000 person-years (CIs)	181 (123–168)		132 (82–213)		31 (10–95)		93 (60–144)
Rate ratio (RR) (CIs)	1.42 (0.86–2.34)		1.18 (0.66–2.10)		0.42 (0.13–1.34)		–
Adjusted RR§ (CIs)	1.35 (0.80–2.30)	0.27	0.95 (0.50–1.80)	0.88	0.41 (0.13–1.33)	0.14	–
Confirmed TB cases	13		7		1		7
Adjusted RR (CIs)	2.31 (1.00–5.33)	0.05	0.83 (0.32–2.18)	0.34	0.37 (0.05–2.80)	0.71	–

* No helminth group presented for comparison, but significance tests and rate ratios are given for individual helminth species vs subjects without that particular helminth species; note that many subjects were infected with multiple helminths.
† χ^2 for comparison of proportions, Wilcoxon rank-sum test for comparison of medians, and likelihood ratio test for rate ratios.
‡ For no helminth group and for GI nematode group, no. of subjects excludes those recruited to Entebbe cohort (EC) and enrolled in Worm study at the same visit.
§ Adjusted for age, sex, BCG status, recruitment log$_{10}$ CD4$^+$ count, other helminth infections, and socioeconomic factors: for *S. mansoni*, walking distance from Lake Victoria; for GI nematodes, electricity supply at home; for *M. perstans*, both.
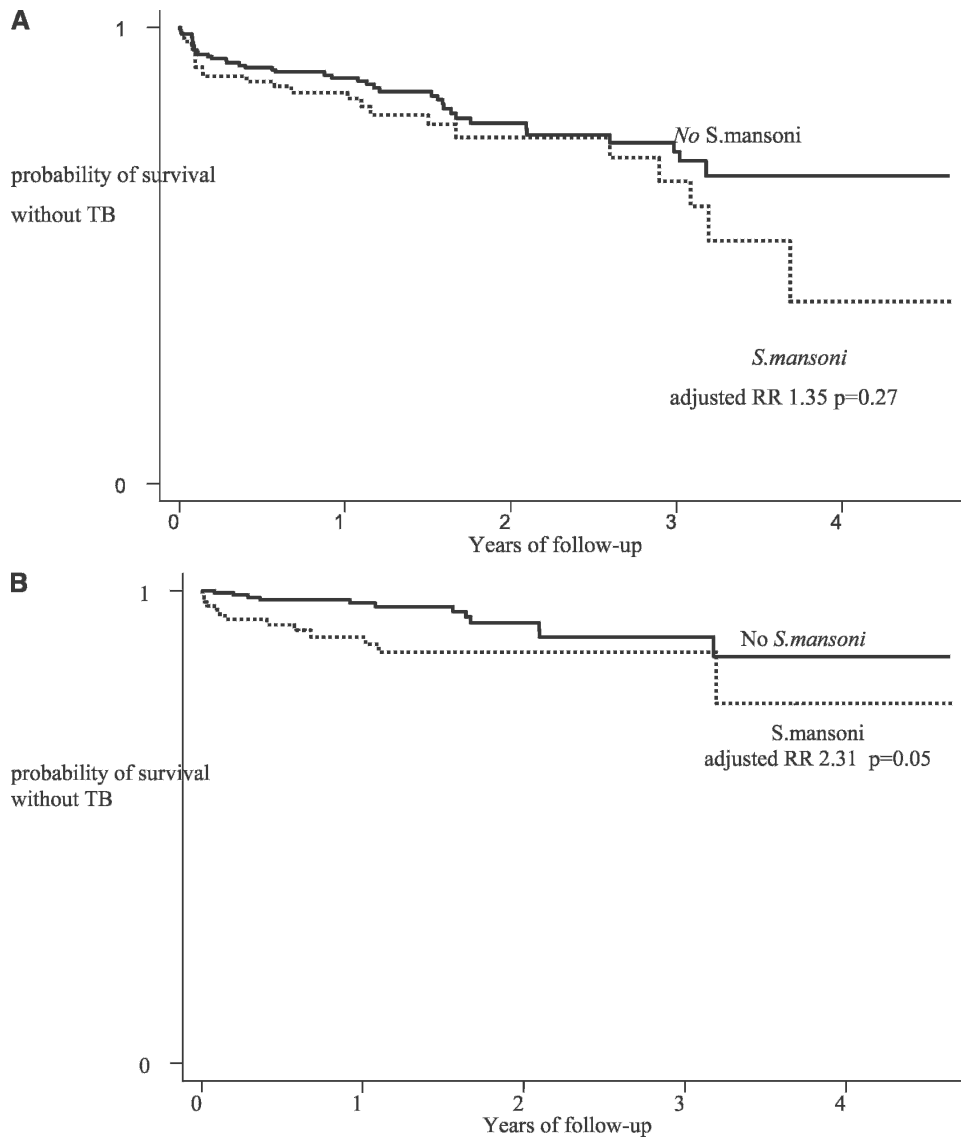
FIGURE 2.   (**A**) Survival to active TB for individuals with and without *S. mansoni* infection. See Table 1 for statistical tests and explanatory variables. (**B**) Survival to active TB for individuals with and without *S. mansoni* infection—confirmed TB cases only ($N = 13$). See Table 1 for statistical tests and explanatory variables.

visit, subsequent analyses were restricted to 294 subjects who were recruited to EC at least 6 months before Worm study enrollment (and without a diagnosis of TB before EC recruitment). There was no association between TB incidence and GI nematode infection (132 per 1,000 person-years versus 109 per 1,000 person-years; RR, 1.18; $P = 0.59$; Table 1). Multivariate analysis, including (for this helminth group) electricity supply, along with age, sex, $CD4^+$ count at EC recruitment, other helminths, and BCG status, did not substantially change the RR (adjusted RR, 0.95; $P = 0.88$). Restricting the analysis to probable or confirmed TB cases gave an adjusted RR of 0.99 (CI, 0.50–1.95; $P = 0.97$) and to confirmed cases gave an adjusted RR of 0.83 (CI, 0.32–2.18; $P = 0.71$). No association was seen between number of concurrent helminth infections and TB incidence (data not shown).

DISCUSSION

In a cohort with a high incidence of TB, and prospective data on TB incidence rates, we were unable to show a clear

association between helminth infection and progression to active TB. Analysis according to helminth species (grouped by phyla or superfamilies sharing similar life cycles or host tissue locations) revealed some differences. For GI nematodes, a weak association seen in crude univariate analyses was further weakened after adjusting for potential explanatory variables such as socio-economic factors and $CD4^+$ T-lymphocyte count. However, there was evidence for an association between *S. mansoni* infection and TB incidence; although not statistically significant on crude analysis (the study had > 75% power to detect a 2-fold difference in TB incidence between those with and without *S. mansoni*), the association became stronger and statistically significant when stricter criteria for TB diagnosis were used and only slightly weaker after adjusting for potential explanatory variables. Despite the limitations on assessing *S. mansoni* infection intensity on the basis of a single stool sample, slightly higher TB rates were seen in the subjects with the heaviest infections.

Studies in animal models of co-infection, as well as human

studies *in vitro* and *in vivo*, have indicated the suppression of anti-mycobacterial immune responses in the presence of helminth infection.[17–24] Despite these data, evidence supporting a role for helminths in accelerating progression to active TB has not been subjected to systematic epidemiologic study. A small hospital-based case-control study from Brazil found an association between intestinal nematodes and tuberculosis, but was subject to the potential biases inherent in such a study design.[25] We previously showed a strong association between eosinophilia and progression to TB in this cohort. In subgroup analyses, we also found an association between anti-mycobacterial interleukin (IL)-5 responses and progression to TB (in participants with a BCG scar) and IL-10 responses and progression to TB (in subjects with a positive tuberculin skin test response). Retrospective testing of stored sera for *Schistosoma*-circulating antigen failed to implicate *S. mansoni*.[8,26] With more extensive screening for *Schistosoma* infections in this study, we found evidence of a relationship. Screening for intestinal nematodes and circulating filarial infections, which are also strongly associated with eosinophil count in this cohort,[9] did not implicate these helminths in this association.

Ethical considerations have generally prevented prospective studies of the effects of untreated helminth co-infection. The current Worm study has attempted to deal with this problem with the assumption that infections diagnosed at enrollment had been present when patients were initially recruited to the EC. The (adult) age distribution of the cohort would support this assumption because the well-established age-prevalence distributions of the more common infections in this population suggest that subjects are likely to have been infected in childhood. Anti-schistosomal treatment was not widely available before this study, and we already showed supportive evidence for the chronicity of the other infections.[8,9] We can also be confident that this assumption holds for *M. perstans*, which was the least likely to have been treated and for which the most follow-up time was available (the majority of *M. perstans*–infected subjects remained infected when re-screened 6 months after Worm study enrollment[9]). This helminth was also most strongly associated with eosinophilia.[9] There was a weak inverse association between this infection and TB incidence (the study had 75% power to detect the difference as statistically significant at the 5% level), despite an association with reduced tuberculin skin test reactivity. This did not seem to be caused by less mycobacterial exposure in *Mansonella*-exposed subjects; the association was, if anything, stronger after adjusting for socio-economic and demographic variables. *Mycobacterium*-specific immune responses, although skewed, were detected as frequently in *Mansonella*-infected subjects.[27,28] As we have previously shown, it seems clear that helminth species vary in their effects in co-infected subjects.[9]

We addressed the potential biases inherent in the retrospective study design. First, an excess mortality in helminth-infected subjects could lead to an underestimate of a true association by reducing the number of helminth-infected subjects surviving to enrollment in the Worm study. However, our previous analyses have shown that helminth infection is not associated with more advanced HIV disease or increased mortality in this cohort.[9] Nor was diagnosis of TB in the cohort as a whole associated with an increased mortality rate; in fact, mortality was lower in subjects who were diagnosed with TB (this could be explained by misclassification of deaths from undiagnosed TB in the cohort—those with most advanced disease may have succumbed to TB before a diagnosis could be made—or perhaps a protective effect of anti-tuberculous therapy against other opportunistic infections). Second, a true association between helminth infection and TB could lead to the exclusion of helminth-infected subjects from the analysis because of a history of TB before EC recruitment. However, no association was found between helminth infection and previous TB. Third, defective anti-mycobacterial immune responses in helminth-infected subjects might lead to less classic presentations, and therefore underdiagnosis, of TB in these subjects. This explanation is unlikely because TB diagnosis was not based only on smear positivity. Smear-negative and extra-pulmonary TB constituted a significant proportion of diagnosed cases, and categorization by strength of evidence for a diagnosis of TB strengthened the association with *S. mansoni* and did not affect the results for other helminths. Fourth, TB infection may be less common among helminth-infected subjects, because of differences in environmental exposures. As discussed above, however, adjusting for socio-economic variables did not unmask any associations; nor were TST responses less prevalent in helminth-infected subjects, except in the case of *M. perstans*. Finally, there was no association between BCG immunization (as measured by the presence of a BCG scar) and helminth infection,[27] and inclusion of BCG status in the multivariate analyses did not affect the RRs.

Several mechanisms could explain the association between *S. mansoni* and TB progression, independent of type 2 cytokine bias and eosinophilia. Enhanced production of cytokines such as transforming growth factor-β and IL-10, which have been shown in human *Schistosoma* infection in our cohort and others, has been associated with suppression of anti-mycobacterial immunity.[10,27,29–32] Immune activation is well described in African subjects, is associated with active TB, and may itself be driven by concurrent infections including helminths.[4,33–38] However, any effects of helminths on TB progression may be mediated as much by influencing HIV-induced immunopathology as by direct effects on anti-mycobacterial immunity. The preserved immune status shown in subjects with hookworm and *Mansonella* infections in our cohort would support this argument.[9,34,39]

Helminth-induced modulation of anti-mycobacterial immune responses may be quite different in other settings. Although TB incidence is lower in HIV-negative populations, in such patients, the cellular mechanisms by which helminths could affect anti-mycobacterial immune responses are not abrogated by CD4+ depletion and may be more easily shown. Timing is important in polarization of the immune response,[40] which may be set early, perhaps *in utero*.[22] Current studies are underway to establish the effect of maternal and early childhood helminth infection on anti-mycobacterial immune responses and subsequent incidence of TB to address this issue. Finally, this analysis, added to published evidence from this cohort and from other settings, questions the assumption that all asymptomatic helminth infections are detrimental in HIV-infected subjects.[9,10,41] Placebo-controlled studies of treatment of mild-moderate helminth infection and prospective follow-up of TB incidence and HIV progression should be encouraged.

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