Pubertal Development Predicts Resistance to Infection and Reinfection with *Schistosoma japonicum*


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(See the editorial commentary by Olds on pages 1699–701)

**Background.** In communities where *Schistosoma* species are endemic, the prevalence and intensity of schistosomiasis is disproportionately high among children, compared with adults. This epidemiologic pattern is consistent with either the slow development of resistance or the requirement of host developmental changes for the expression of resistance.

**Methods.** We enrolled 87 individuals aged 7–18 years who did not have *Schistosoma japonicum* infection and 641 individuals aged 7–30 years with *S. japonicum* infection, all of whom reside in 3 villages in Leyte, Philippines. At baseline, *S. japonicum* infection was assessed by Kato-Katz thick-smear stool examination, and the levels of the pubertal hormone dehydroepiandrosterone sulfate (DHEA-S) in serum were determined. Individuals with *S. japonicum* infection were treated with praziquantel, after which stool examination and DHEA-S level measurement were performed every 3 months for 18 months.

**Results.** In cross-sectional analyses, the intensity of infection among individuals with high DHEA-S levels was 43% lower (28 eggs per g, \(n = 243\)), compared with individuals with low DHEA-S levels (50 eggs per g, \(n = 242\)), even after adjusting for age, sex, and village (\(P = .01\)). Following praziquantel treatment, increased DHEA-S levels were associated with resistance to reinfection (\(P = .006\)). The intensity of reinfection among individuals with high DHEA-S levels was 42% lower, compared with individuals with low DHEA-S levels, even after adjusting for age, baseline intensity of *S. japonicum* infection, village, sex and water contact (\(P < .001\)).

**Conclusions.** Increased DHEA-S levels in serum, a marker for adrenal development, is associated with reduced *S. japonicum* infection and reinfection, even after adjusting for age and, by proxy, cumulative exposure. These data suggest that an intrinsic property of host pubertal development mediates, in part, the resistance to infection observed in older individuals.

Human schistosomiasis, caused by 3 species of diecious trematodes (*Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*), currently affects ~200 million individuals worldwide and produces significant morbidity in >20 million individuals [1]. Individuals living in regions of endemicity display a characteristic right-skewed distribution of prevalence and intensity of infection by age for the 3 major schistosome species [2]. Similar age-associated patterns of reinfection intensity have been observed following chemotherapeutic cure of current infections, even after accounting for differential exposure to infective cercariae [3, 4]. This epidemiologic pattern is widely interpreted as evidence of the slow acquisition of protective immune responses following years of exposure to parasite antigens [5].

Recently, observations in a schistosome infection-naïve community acutely exposed to schistosome infections have challenged this interpretation [6].
community, all age groups had experienced ~3 years of intense cumulative exposure; yet, older adolescents and adults had strikingly lower intensities of infection than children. Because of the similar duration of exposure for all age groups, this community allowed the decoupling of age and cumulative exposure. The relationship between age and intensity in this acutely exposed community replicated observations made in chronically exposed communities, which suggests that an intrinsic attribute of age, not cumulative exposure, per se, is responsible for the lower infection intensities observed in adults. These data support an alternative hypothesis that developmental changes occurring during adolescence, not cumulative exposure, are necessary for the expression of maximal resistance to schistosome infection [7].

We evaluated the impact of host pubertal development on the prevalence and intensity of *S. japonicum* infection in a cross-sectional sample (*n* = 728) and intensity of reinfection following Praziquantel (PZQ) treatment in a longitudinal cohort (*n* = 616). We tested the hypothesis that pubertal development, as assessed by the level of dehydroepiandrosterone sulfate (DHEA-S) in serum (hereafter, referred to as DHEA-S level), a marker of adrenarche, would predict decreased prevalence and intensity of *S. japonicum* infection and reinfection, after controlling for age and, by proxy, for cumulative parasite exposure.

**MATERIALS AND METHODS**

**Study area and population.** This study was conducted in 3 *S. japonicum*-endemic rice-farming villages (Macanip, Pitogo, and Buri) in Leyte, Philippines. Malaria is not endemic in this study area.

In total, 74.3% (1262 of 1699) of individuals aged 7–30 years residing in 3 villages were screened for the presence of *S. japonicum* infection by duplicate Kato-Katz examination of 3 stool specimens obtained from each study participant. The prevalence of infection with *S. japonicum* in this age range was 60.0%.

For the cross-sectional study, 641 individuals with *S. japonicum* infection met eligibility requirements: they provided blood and stool samples, lived primarily in a study village, were aged 7–30 years, were not pregnant or lactating, and provided both child assent and parental consent. In addition, 87 individuals participating in a study of the impact of *S. japonicum* infection on cognitive function were recruited as control subjects [8]. These controls subjects were 7–18 years old and were not infected with *S. japonicum*, making the cross-sectional sample size 728 participants. Twenty-five subjects with *S. japonicum* infection who had severe hepatomegaly or fibrosis on ultrasound examination, severe anemia, or severe wasting were excluded from participation in the longitudinal cohort and were referred for medical treatment. This exclusion resulted in a sample size of 616 participants for the longitudinal cohort.

Detailed description of this study population has been published elsewhere [9].

For the longitudinal study, the 616 eligible *S. japonicum*-infected subjects from the cross-sectional study, living in 331 households, were enrolled in 2 separate cohorts in October 2002 (Macanip) and April 2003 (Buri and Pitogo). After blood samples were collected and physical examinations were performed, all participants were treated with a split dose of 60 mg/kg of PZQ. Subsequently, participants were followed up at ∼1, 3, 6, 9, 12, 15, and 18 months after treatment. At each time point, stool and blood samples were collected and a physical examination was performed.

Only successfully treated participants were included in our longitudinal analyses. Treatment success was defined as having a first *S. japonicum*-negative stool sample at 4 weeks after treatment (primary success) or otherwise at 3 or 6 months after treatment (delayed success). Individuals who were persistently infected because of treatment failure or rapid reinfection were defined as having had no *S. japonicum*-negative stool sample up to 6 months after treatment.

**Stool examination.** Stool examination was performed for the cross-sectional [9] and longitudinal studies, as described elsewhere [10]. Briefly, parasite burden was determined by examination of 3 stool specimens obtained from each study participant. Each of the 3 stool specimens was examined in duplicate for *S. japonicum*, *Ascaris lumbricoides*, *Trichuris trichuria*, and hookworm using the Kato-Katz thick-smear stool examination. For each of the stool specimens, the average number of eggs per g of the duplicate test was determined, and the overall mean eggs per g were derived by averaging the parasite burden of the 3 individual specimens.

**Blood sample collection and processing.** Venipuncture was performed at baseline (time point, 0) for all subjects in the cross-sectional and longitudinal studies, as described elsewhere [9]. Venipuncture was also performed at 1, 3, 6, 9, 12, 15, and 18 months after treatment (time point, 2–8) for participants in the longitudinal cohort, as described elsewhere [10]. Serum samples obtained from individuals prior to the initiation of treatment (time point, 0) and after treatment (time point, 2–8) were analyzed for DHEA-S levels as part of a multiplex bead-based assay, as described elsewhere [11] (figure 1).

**Water contact observation.** In the longitudinal cohort, observed water contact was assessed as a proximate marker of exposure to infection and included in multivariate models as a dichotomous variable, as described elsewhere [10]. Briefly, each participant was scheduled for water contact observations on 12 separate occasions, with ~2 observations before every stool examination. On each occasion, the participant was followed from 7 a.m. to 4 p.m., and the duration of each water contact (other than household water) and percentage of body in contact with water were recorded. For each separate water
contact observation, a water contact score was calculated by multiplying the duration of contact by the percentage of body contact. For each time point, a dichotomous variable representing minimal water contact (defined as the lower 2.5% of the overall distribution of water contact) or greater contact was constructed on the basis of the mean of the relevant water contact scores.

**Statistical analyses.** Analyses were performed with SAS software, version 8.02 (SAS Institute). *S. japonicum* egg counts were log-normally distributed and the data were log-transformed [\( \ln(\text{value} + 1) \)].

The goal of our analyses was to measure the relationship between DHEA-S levels and infection and/or reinfection with *S. japonicum* after adjusting for the known effects of age and other explanatory variables. For cross-sectional analyses, we modeled the relationship between DHEA-S levels and intensity of *S. japonicum* infection, after adjusting for age, village, and sex, using linear regression.

For the longitudinal cohort, we modeled the relationship between DHEA-S levels (measured at each time point) and intensity of *S. japonicum* reinfection (beginning 3 months after treatment), after adjusting for age, village, sex, baseline intensity of *S. japonicum* infection, water contact, and nonindependence of repeated measures in individuals, using a linear mixed effects model. *S. japonicum* reinfection intensity was modeled as a polynomial function of time (time-squared). Within-person correlation was modeled by specifying random intercepts and slopes for each individual.

Because of the multilevel nature of our sample (individuals grouped in households) and, as a result, violation of the assumption of independence of observations, we adjusted both the cross-sectional (figure 2) and longitudinal (figure 3) analyses for clustering of observations in households. This was done by including household as a random effect in all of the models mentioned above. The \( P \) values and CIs reported are based on empirical (robust) SEs, used to protect against misspecification of correlation matrices. \( P \) values \(<.05 \) were considered to be statistically significant.

Because reliable age-based reference ranges for DHEA-S levels during adolescence are unavailable for similar study populations, we examined tertiles of DHEA-S level calculated from the distribution of DHEA-S levels obtained for each relevant time point (figures 2 and 3). Least-squares mean values represent the group mean *S. japonicum* egg count at baseline (figure 2) and at 18 months (figure 3) for tertiles of DHEA-S level, adjusted for confounding variables.

**Ethical clearance.** This study complied with the ethical standards of the Helsinki Declaration of 1975, as modified in 1983. It was approved by the Brown University and the Research Institute of Tropical Medicine institutional review boards.

**RESULTS**

We screened 1262 individuals in the study area who were aged 7–30 years, of whom 757 (60%) had *S. japonicum* infection. A total of 728 people were eligible to participate in the cross-sectional analysis, and 616 individuals were eligible to participate in the longitudinal cohort and were treated with PZQ at baseline. Overall, 576 (93.5%) individuals experienced suc-
DHEA-S and Resistance to *S. japonicum*

Figure 2. Intensity of *Schistosoma japonicum* infection at baseline in 728 individuals is associated with dehydroepiandrosterone sulfate (DHEA-S) level. *Shaded bars,* back-transformed, least-squares mean *S. japonicum* eggs per g of stool for tertiles of DHEA-S levels, after adjusting for age, village, sex, and clustering of individuals within households, using multilevel linear regression. *Error bars,* SEs.

cessful treatment; 512 (83.1%) had an *S. japonicum*–negative stool sample 4 weeks after treatment, 58 (9.4%) had a first *S. japonicum*–negative stool sample 3 months after treatment, and 6 (0.97%) had a first *S. japonicum*–negative stool sample 6 months after treatment. Treatment was unsuccessful for 20 individuals (3.2%), and it was not possible to assess treatment response for 20 individuals (3.2%) because of missing data from the stool examinations. In longitudinal analysis, at least 461 individuals (80.0%) contributed to each time point, with 521 individuals (90.4%) present at ≥3 follow-up examinations.

In the cross-sectional sample, the mean age at baseline was 14.6 years (95% CI, 14.2–15.1 years), and 276 individuals (37.9%) were female. In the longitudinal cohort, the mean age at baseline was 15.0 years (95% CI, 14.5–15.5 years), and 228 individuals (37.0%) were female. DHEA-S levels were available for all 728 individuals in the cross-sectional sample and for 455–599 individuals at each follow-up time point in the longitudinal cohort. Mean DHEA-S levels by age group and time point are presented in figure 1.

In the cross-sectional sample, DHEA-S level was associated with a significantly lower intensity of *S. japonicum* infection, even after accounting for age, village, sex, and clustering within households (β = −0.22; P = .002). Individuals whose DHEA-S levels were in the highest tertile had a 43.2% lower intensity of infection than individuals whose DHEA-S levels were in the lowest tertile (P = .01; figure 2).

In the longitudinal cohort, DHEA-S was associated with significantly decreased intensity of *S. japonicum* reinfection, even after accounting for age, baseline intensity of *S. japonicum* infection, village, sex, water contact, and clustering within households (β = −0.17; P = .006). Individuals whose DHEA-S levels were in the highest tertile had a 41.9% lower intensity of infection than individuals whose DHEA-S levels were in the lowest tertile (P < .001; figure 3).

**DISCUSSION**

The disproportionately high intensity and prevalence of schistosome infection in children, compared with adults, has been documented for decades [5], and understanding the mechanisms of this naturally occurring protection may guide efforts to develop a vaccine for schistosomiasis. Recently, a new endemic focus of schistosomiasis in Senegal has allowed the decoupling of host age from cumulative parasite exposure. When assessed 3 years after initial exposure, this population displayed the same epidemiologic pattern of age-related decrease in intensity of infection observed in communities of endemicity [6]. These data support the hypothesis that host developmental changes, including increases in the levels of the adrenal hormone DHEA-S, may be responsible for the consistent and dramatic reduction in susceptibility to schistosome infection that occurs near the time of puberty [7].

Dehydroepiandrosterone (DHEA) and its biologically active metabolite DHEA-S are the most abundant steroids in adolescent humans and circulate in the blood primarily bound to albumin [12]. DHEA is transported largely in its sulfated form (DHEA-S), which is metabolized to DHEA by cell surface sulfatas. Lipophylic DHEA diffuses across the cell membrane and binds to a cytoplasmic receptor that is translocated to the nucleus [13] and influences gene transcription [14]. DHEA and
DHEA-S interconvert, and both DHEA and DHEA-S can be metabolized in peripheral tissues to androstenedione, testosterone, dihydrotestosterone, and estrogens. DHEA and DHEA-S circulate at low levels in prepubescent children. The concentration of these steroids increases during puberty, plateaus during early adulthood, and decreases during senescence.

Much evidence points to a causal relationship between increasing pubertal development, DHEA-S levels, and resistance to schistosome infection: (1) in mice, exogenous administration of DHEA-S leads to decreased schistosome worm burdens after challenge infection [15]; (2) DHEA-S kills larval and adult parasites in culture at physiologic concentrations [16]; (3) in 2 cross-sectional studies (including the present study), increased DHEA-S levels are associated with decreased intensity of infection in humans [17]; and (4) in the present study, increased DHEA-S levels are associated with decreased intensity of reinfection after treatment with PZQ.

In a murine model of S. mansoni, female mice treated with DHEA-S had significantly fewer schistosome worms recovered after challenge infection, compared with controls. This resistance was positively associated with levels of DHEA-S and its metabolite, testosterone [15]. In this model, schistosome infection did not alter host DHEA-S levels, and exogenous DHEA-S did not significantly alter measured antiparasite immune responses. In an in vitro study of the paraciticidal effect of host hormones on S. mansoni, DHEA-S at physiologic doses was able to kill cercaria, schistosomula, and, to a lesser degree, adult worms in culture [16]. Together, these data indicate that DHEA-S is causally related to resistance to schistosome infection.

In a cross-sectional subanalysis of 135 individuals living in an area of Ethiopia where S. mansoni is endemic, increased DHEA-S levels were associated with decreased intensity of infection, after adjusting for age [17]. DHEA-S levels accounted for 15.2% of the variability in egg counts, even after accounting for the effect of age. This study did not assess differential water contact, sex, or household level clustering and suffered from bias in infection intensity introduced during the subsampling for blood extraction.

We conducted both a cross-sectional and a longitudinal study to examine the relationship between DHEA-S levels and resistance to infection and reinfection with S. japonicum. In communities where S. japonicum is endemic, host age is highly colinear with cumulative exposure. By adjusting for host age, and, by proxy, for cumulative exposure in our analyses, we evaluated whether the residual heterogeneity in DHEA-S levels, caused by variation in the onset and duration of puberty, was associated with decreased parasite burden. In our cross-sectional analysis (n = 728), individuals with high DHEA-S levels had a 43% lower intensity of infection than individuals with low DHEA-S levels (P = .01), even after accounting for village, sex, clustering within households, age, and, by proxy, years of cumulative exposure.

We treated the 616 eligible S. japonicum–infected individuals with PZQ and quantified reinfection and DHEA-S levels every 3 months for 18 months. As in our cross-sectional analyses,
individuals with high DHEA-S levels had a 42% lower intensity of reinfection than individuals with low DHEA-S levels (P < 0.001), even after accounting for baseline intensity of *S. japonicum* infection, village, sex, water contact, clustering within households, age, and, by proxy, years of cumulative exposure. These longitudinal data provide compelling evidence that high DHEA-S levels are associated with resistance to reinfection with schistosomes.

Potential limitations of our observational study included the following: (1) in the cross-sectional study, the age range of our uninfected control group was 7–18 years, and the age range in the infected group was 7–30 years; (2) there was residual confounding of the relationship between DHEA-S and *S. japonicum* infection intensity by age; and (3) there was reverse causality due to suppression of DHEA-S levels by current infection. The age range of our control group represents a potential design weakness of our cross-sectional study and was, in part, the motivation for conducting the longitudinal study. We note that the age range of the control subjects encompasses the majority of the variance in our predictor of interest—DHEA-S—and the uninfected control group was both younger and had lower DHEA-S levels than the infected group; therefore, any potential selection bias would be a type II error, biasing away from our hypothesis that an increased DHEA-S level is associated with a low infection intensity. We addressed the potential for residual confounding by controlling for both age and age² in our models. The age² term was not significant and did not influence the significance or magnitude of the relationship between DHEA-S and *S. japonicum* (data not shown); thus, residual confounding by age is unlikely. Reverse causality is unlikely for several reasons: (1) in mice, *S. mansoni* infection does not suppress endogenous DHEA-S levels [15]; (2) in baboons, second infections, modeling chronic infection of humans living in areas of endemicity, do not suppress endogenous DHEA-S levels [18]; (3) in our longitudinal cohort, assessment of the DHEA-S and *S. japonicum* reinfection relationship commenced when individuals had been uninfected for several months; and (4) in infected individuals, we did not detect any increase in DHEA-S levels 1 month after treatment (data not shown).

DHEA-S is known to have potent immunomodulatory activities, including up-regulation of Th2-driven antibody isotypes [19] and down-regulation of proinflammatory cytokines [20]. Whether DHEA-S mediates resistance in humans through a direct antiparasite effect or via innate or acquired immune mechanisms remains a major unanswered question. If DHEA-S mediates resistance through a direct antiparasite effect or via innate immune mechanisms, such as host skin thickness or fat deposition, then capitalizing on these mechanisms for vaccine development will be difficult. If, however, DHEA-S mediates resistance via enhancement of acquired protective immune responses, then vaccine strategies designed to induce and augment these protective acquired immune responses, including hormonal adjuvants [21], may be promising.

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