

## T-Helper-2 Cytokine Responses to Sj97 Predict Resistance to Reinfection with *Schistosoma japonicum*

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Received 24 August 2005/Returned for modification 6 October 2005/Accepted 18 October 2005

Although schistosomiasis is effectively treated with Praziquantel, rapid reinfection with rebound morbidity precludes effective control based on chemotherapy alone and justifies current efforts to develop vaccines for these parasites. Using a longitudinal treatment-reinfection study design with 616 participants 7 to 30 years of age, we evaluated the relationship between cytokine responses to *Schistosoma japonicum* soluble adult worm extract (SWAP), Sj97, Sj22.6, and Sj67, measured 4 weeks after treatment with Praziquantel, and resistance to reinfection in a population from Leyte, The Philippines, where *S. japonicum* is endemic. *S. japonicum* transmission was high: 54.8% and 91.1% were reinfected within 6 and 18 months, respectively. A Th2 bias in the following cytokine ratios, interleukin-4 (IL-4)/IL-12, IL-5/IL-12, IL-13/IL-12, IL-4/gamma-IFN (IFN- $\gamma$ ), IL-5/IFN- $\gamma$ , and IL-13/IFN- $\gamma$ , in response to SWAP predicted a 1.4- to 2.9-month longer time to reinfection ( $P < 0.05$ ) and a 27 to 55% lower intensity of reinfection ( $P < 0.05$ ). Similarly, a Th2 bias in response to Sj97 predicted a 1.6- to 2.2-month longer time to reinfection ( $P < 0.05$ ) and a 30 to 41% lower intensity of reinfection ( $P < 0.05$ ). Only a high IL-5/IL-10 ratio in response to Sj22.6 predicted a 3.0-month-longer time to reinfection ( $P = 0.03$ ). Cytokine responses to Sj67 were not associated with protection. In a large population-based treatment-reinfection study we found that Th2 responses to SWAP and Sj97 consistently predicted resistance to reinfection. These findings underscore Th2-type immune responses as central in human resistance to *S. japonicum* and support Sj97 as a leading vaccine candidate for this parasite.

Human schistosomiasis, caused by three species of dioecious trematodes (*Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*), currently affects ~200 million individuals worldwide and produces significant morbidity in over 20 million individuals (31). Although schistosomiasis is effectively treated with Praziquantel (PZQ), rapid reinfection with rebound morbidity precludes effective control based on chemotherapy alone and justifies current efforts to develop vaccines for these parasites (6, 12).

The feasibility of an effective anti-schistosome vaccine was demonstrated in animal models of *S. mansoni* infection in which multiple exposures to irradiation-attenuated larval parasites established a protective immunity to challenge parasites (16, 28, 30). This resistance to infection is transferable with the immunoglobulin G (IgG) fraction of immunized sera. The potential for an anti-schistosome vaccine is further corroborated by evidence of naturally acquired resistance to reinfection observed in epidemiologic studies of exposed human populations (8, 13). These community-based studies consistently demonstrate an age-related decrease in the intensity of reinfection following chemotherapeutic cure even after adjusting

for exposure to infectious water in both *S. mansoni* and *S. haematobium* (18, 19, 45).

Many potential anti-schistosome vaccine candidates have been identified in animal models and human studies, and several of these are progressing toward clinical trials (5). Unfortunately, the specific type of protective immune response generated by these antigens is frequently discordant between animal and human studies. Th1-type vaccine responses are frequently protective in murine models (33–35, 46), while Th2-type responses are associated with resistance to reinfection in humans (19, 32, 39). This divergence makes generalizability from animal models of resistance problematic and underscores the importance of well-controlled, immunoepidemiology studies of schistosomiasis in humans.

The association of Th2 responses and resistance to schistosome infection in humans has been observed for eosinophilia (24), anti-parasite IgE (17, 19, 38), and peripheral blood mononuclear cell (PBMC) production of interleukin-4 (IL-4) and IL-5 in response to crude parasite extracts (32, 39). Despite broad acceptance of the paradigm, Th2 cytokine responses to specific parasite antigens have been prospectively associated with resistance to reinfection after PZQ treatment in only a single study in *S. mansoni* (3).

We evaluated the relationship between cytokine responses to *S. japonicum* vaccine candidates and resistance to reinfection in 616 individuals living in a region of The Philippines where *S. japonicum* is endemic. Using a longitudinal treatment-reinfection study design, we assessed the relationship

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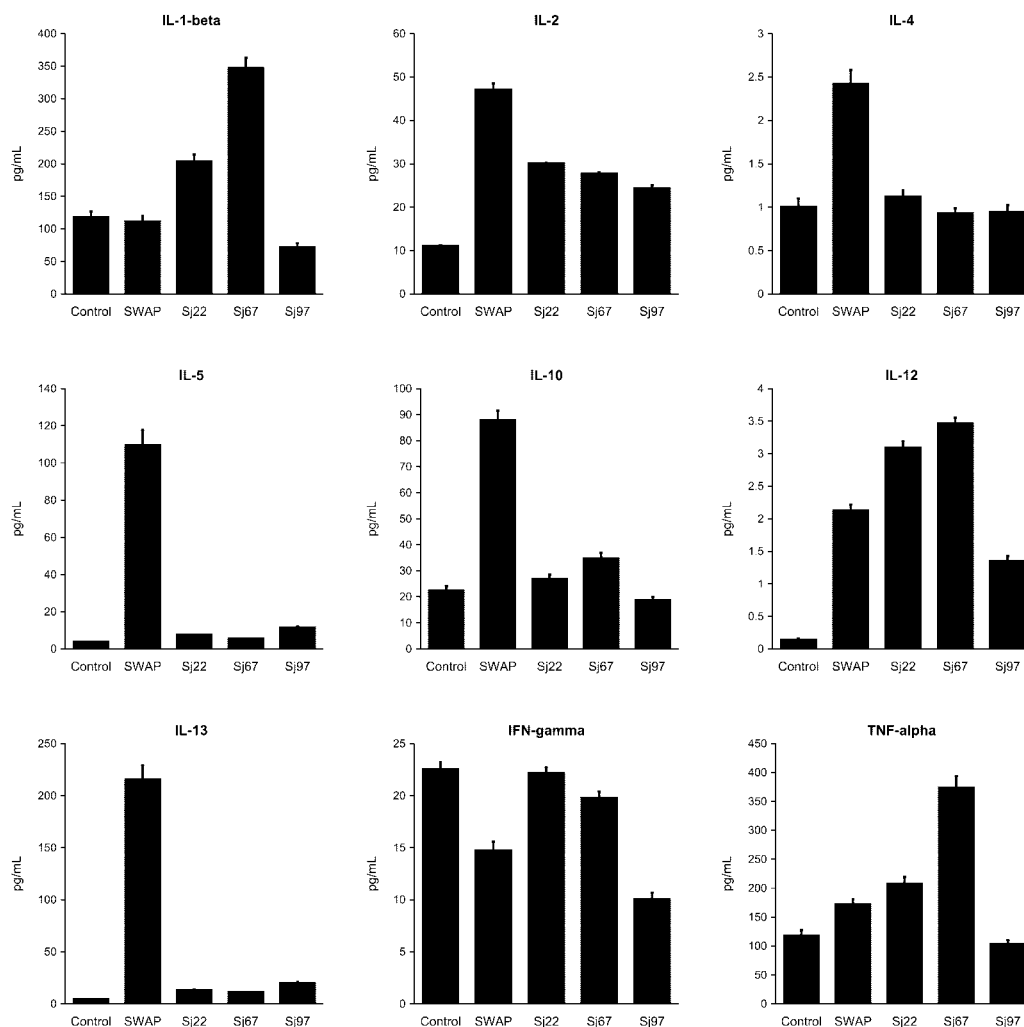


FIG. 1. Cytokine production by PBMC 4 weeks after treatment of *S. japonicum* infection with PZQ. Control medium,  $n = 545$ ; SWAP,  $n = 510$ ; Sj22.6,  $n = 535$ ; Sj67,  $n = 492$ ; Sj97,  $n = 531$ . Bars represent geometric means. Error bars represent standard errors for the geometric mean.

between cytokine responses to a soluble adult worm extract (SWAP), Sj97, Sj22.6, and Sj67, measured 4 weeks after treatment with PZQ, and resistance to reinfection over an 18-month follow-up period. Because assessing individual cytokines may oversimplify cellular immunologic phenomena in part due to potential antagonism between Th2 and Th1 cytokines (11, 15, 23), we also evaluated the relationship between antigen-specific cytokine ratios and reinfection. We tested the hypothesis that a Th2-type cytokine balance in response to these vaccine candidates would predict a longer time to reinfection and lower intensity of reinfection.

#### MATERIALS AND METHODS

**Study area and population.** This prospective treatment-reinfection study was conducted in three rice-farming villages (Macanip, Pitogo, and Buri) in Leyte, The Philippines, where *S. japonicum* is endemic. Malaria is not endemic in this study area. Government-sponsored treatment in this study area was provided by the Schistosomiasis Control Program, which conducts annual mass-treatment campaigns. Participation is reported to be low because of the absence of pre-treatment screening for the presence of infection (A. Ida, Director, Schistosomiasis Control Program, Leyte, Philippines, personal communication).

In total 74.3% (1,262 out of 1,699) of individuals between the ages of 7 and 30 years residing in three villages were screened for the presence of *S. japonicum* infection by duplicate Kato-Katz examination of three stool samples prior to enrolment. The prevalence of infection with *S. japonicum* in this age range was 60.0%. Subjects were eligible for participation if they were infected with *S. japonicum* (based on examination of three consecutive stool samples), lived primarily in a study village, were between ages 7 to 30 years, were not pregnant or lactating, and provided both child assent and parental consent or adult consent. Subjects with severe hepatomegaly or fibrosis on ultrasound examination as well as subjects with severe anemia or severe wasting were excluded from participation and referred for medical treatment.

**Enrolment, treatment, and follow-ups.** Six-hundred sixteen participants, living in 331 households, were enrolled in two separate cohorts, in October 2002 (Macanip) and April 2003 (Buri and Pitogo). After blood collection and physical examination, all participants were treated with a split dose of 60 mg PZQ/kg of body weight. Subsequently, participants were followed up at approximately 1, 3, 6, 9, 12, 15, and 18 months posttreatment. At each time point, stool and blood samples were collected and a physical examination was performed. All participants were transported to the study clinic by study staff for enrolment and follow-up visits.

**Stool examination.** At 4 weeks posttreatment a single stool sample was collected from each individual to assess treatment response. In the week prior to each subsequent follow-up, three consecutive stool samples were collected from each participant at their home. Each of the stool specimens was examined in

duplicate for *Schistosoma japonicum*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs within 24 h of collection by the Kato-Katz method. For each of the stool specimens, the average number of eggs per gram (epg) of the duplicate exam was determined. For each time point, the overall mean epg was derived by averaging the egg counts of the three individual specimens.

**Blood collection and cell stimulation.** Four weeks posttreatment, venipuncture was performed and 10 ml of blood was collected into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing heparin as anti-coagulant. Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation over Histopaque-1077 in Accuspin tubes (Sigma-Aldrich, St. Louis, MO). PBMC were suspended in RPMI 1640 at  $2 \times 10^6$ /ml. PBMC were used at 500,000 cells per well in 48-well microtiter plates. Stimulants were added to a final volume of 500  $\mu$ l. Cells were incubated for 3 days in a humidified incubator at 37°C and 5% CO<sub>2</sub>. On the third day, 300  $\mu$ l of cell-free culture supernatant was harvested from each well and stored in barcode-labeled tubes at -80°C for subsequent cytokine analyses. For each participant, PBMC stimulated with antigens (SWAP, Sj22, Sj97, and Sj67) and a media control were assayed.

***S. japonicum* antigens.** SWAP was prepared as described previously (2). Sj97 was biochemically purified from adult *S. japonicum* worms as reported previously (36). Recombinant Sj67 was expressed as a soluble thioredoxin fusion protein and purified as described previously (44). Briefly, Sj67 was purified from induced *Escherichia coli* lysate by anion-exchange and size exclusion chromatography followed by cleavage of thioredoxin with Factor Xa protease. Sj67, with native amino- and carboxy-terminal ends, was purified from thioredoxin and reaction components by hydroxyapatite chromatography and endotoxin depleted by polymyxin chromatography. Recombinant Sj22.6 with native amino- and carboxy-terminal ends was prepared in a similar fashion with hydrophobic interaction chromatography replacing the size exclusion step. All protein concentrations were determined with the Pierce bicinchoninic acid protein assay (Iselin, NJ). Antigens and media were formulated as 2 $\times$  stocks in RPMI 1640-Glutamax (Gibco-BRL, Carlsbad, CA) supplemented with 20% human type AB sera and 2 $\times$  penicillin-streptomycin in single lots, aliquoted into single-use tubes, and stored at -80°C. The final antigen concentrations used in the stimulation assays were the following: SWAP, 5  $\mu$ g/ml; Sj97, 1  $\mu$ g/ml; Sj22.6, 10  $\mu$ g/ml; and Sj67, 10  $\mu$ g/ml. Endotoxin in these preparations was below the level necessary for stimulation of cytokines from human lymphocytes as assessed by a colorimetric *Limulus* amebocyte lysate assay (BioWittaker, Walkersville, MD) (32).

**Cytokine assays.** All sample identification and pipetting was performed by a barcode-enabled, high-speed pipetting robot (Tecan, Research Triangle Park, NC). Similar to our multiplexed serum cytokine assay kits (14), we developed an 11-plex sandwich capture assay kit by coupling 500 mg of the following detection antibodies: IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, gamma interferon (IFN- $\gamma$ ), and tumor necrosis factor alpha (TNF- $\alpha$ ) (BD Pharmingen, San Diego, CA) to  $6.25 \times 10^7$  microspheres from unique bead regions, according to manufacturer's (Luminex, Austin, TX) instructions. Beads were prepared and pooled as a single lot and aliquoted into single-use tubes and stored at 4°C. Standards (BD Pharmingen) were pooled at appropriate concentrations in a single lot and were aliquoted into single-use tubes and stored at -80°C. Biotinylated detection antibodies (BD Pharmingen) were pooled into single-use aliquots and stored at -80°C. High-concentration (500 pg/ml each cytokine) and low-concentration (0 pg/ml) controls were pooled and stored in single-use aliquots at -80°C. These controls were assayed on all plates. The assay kits demonstrated <4% interanalyte interference, and the median interassay coefficient of variation for all analytes, as assessed by use of 40 replicate high-concentration controls, was 14.3%. The lower limit of detection was consistently <2.4 pg/ml for all cytokines. As expected, IL-6 and IL-8 levels to all stimuli, including control media, were above the range of quantification (>5,000 pg/ml) of our assays, likely due to the monocyte activation which accompanies PBMC preparation (25). These cytokines were therefore not examined in subsequent analyses.

**Water contact observation.** Water contact was assessed as a proximate marker of exposure to infection. Each participant was scheduled for water contact observations on 12 separate occasions, approximately two observations prior to every 3-month follow-up. Water contact observations were scheduled to allow an equivalent number of weekdays and weekend days for all participants. On each occasion the participant was followed from 7 a.m. to 4 p.m., and the duration of every water contact (other than household water) and percentage of body in contact with water were recorded. If a participant was acutely ill on the observation day, and therefore not ambulating, he or she was rescheduled for observation on another day. For each separate water contact observation, a water contact score was calculated by multiplying the duration of contact by the percentage of contact. For each water contact observation date, a total water contact score was calculated as the sum of all water contact scores for that date. For each individual, an overall mean water contact score was calculated by averaging all

TABLE 1. Time to reinfection and intensity of reinfection as predicted by age, sex, baseline *S. japonicum* egg count, water contact, and village of residence in a cohort ( $n = 576$ ) of 7- to 30-year-old individuals from Leyte, the Philippines

| Determinants                                                       | Beta  | Lower CI | Upper CI | P value |
|--------------------------------------------------------------------|-------|----------|----------|---------|
| Time to reinfection <sup>a</sup>                                   |       |          |          |         |
| Age (yrs)                                                          | -0.01 | -0.03    | 0.00     | 0.18    |
| Sex (female)                                                       | -0.25 | -0.45    | -0.05    | 0.015   |
| Baseline <i>S. japonicum</i> intensity (log-transformed eggs/gram) | 0.11  | 0.04     | 0.17     | 0.002   |
| Minimal water contact <sup>b</sup>                                 | -0.67 | -1.45    | 0.12     | 0.097   |
| Village A (compared to village B)                                  | 0.33  | 0.08     | 0.59     | 0.011   |
| Village C (compared to village B)                                  | -0.63 | -0.99    | -0.27    | 0.001   |
| Intensity of reinfection <sup>c</sup>                              |       |          |          |         |
| Age (yrs)                                                          | -0.02 | -0.03    | -0.00    | 0.033   |
| Sex (female)                                                       | -0.26 | -0.46    | -0.06    | 0.012   |
| Baseline <i>S. japonicum</i> intensity (log-transformed eggs/gram) | 0.18  | 0.10     | 0.25     | <0.001  |
| Minimal water contact <sup>b</sup>                                 | -0.26 | -0.54    | 0.03     | 0.079   |
| Village A (compared to village B)                                  | 0.53  | 0.28     | 0.79     | <0.001  |
| Village C (compared to village B)                                  | -0.45 | -0.75    | -0.14    | 0.004   |

<sup>a</sup> Betas represent the per-unit increase/decrease in log hazards of reinfection for the indicated determinant adjusted for the other determinants listed and for nonindependence of observations within households by multilevel complementary log-log regression analysis.

<sup>b</sup> Lower 2.5% compared to upper 97.5% of overall water contact distribution, included in the model as a time-varying covariate.

<sup>c</sup> Betas represent the per-unit increase/decrease in mean log *S. japonicum* eggs/gram for the indicated determinant adjusted for the other determinants listed, for within-person correlation and for nonindependence of observations within households by multilevel repeated measures linear regression analysis.

available total water contact scores. For time-varying analyses, mean water contact was calculated for the relevant time frame prior to each follow-up. The relevant time frame prior to follow-up was designated as between 5 weeks prior to the previous follow-up and 5 weeks prior to the current follow-up. The 5-week offset was chosen based on the *S. japonicum* prepatent period. Water contact data were available for 2,844 out of 3,696 (76.9%) possible observations. To facilitate the use of water contact as a covariate in multivariate analyses, missing data were imputed. Each missing observation was replaced by the individual's overall mean water contact. Imputation was possible for all but 21 individuals (3.4%), because these had no water contact observation data available for any of the dates, and these individuals were excluded from analysis.

**Data management and statistical analyses.** Data forms collected in the field were barcoded and entered using Filemaker 5.5 software (Filemaker Inc., Santa Clara, CA). Analyses were performed in SAS version 8.02 (SAS Institute, Cary, NC). *S. japonicum* egg counts and cytokine responses were log-normally distributed, and the data were Log<sub>e</sub> transformed [Ln(value + 1)]. To allow the calculation of cytokine ratios, all zero values of individual cytokines were set to 0.01 pg/ml.

Differences in time to reinfection were estimated using an interval-censored proportional hazards model (complementary log-log model implemented with Proc Genmod) (4, 26). The proportional hazards assumption was assessed with a likelihood ratio test for the interaction between time and main effect. No significant interactions with time were found in any of the time-to-reinfection models (data not shown). Differences in mean intensity of reinfection over time were estimated using a repeated-measures linear regression model (Proc Mixed with an unstructured correlation matrix for within-person correlation over time) (21). Main effects (cytokine responses) were added to base models, including the potential confounders age, sex, water contact, village of residence, and baseline intensity of infection. To allow adjustment for minimal or absent exposure to infective water during the relevant time frame prior to each follow-up, a dichotomous variable representing minimal water contact (defined as the lower 2.5% of the overall distribution of water contact) was entered into all main effects models as a time-varying covariate. Estimated survival functions (see Fig. 2 to 4) and mean time to reinfection were calculated using the complementary log-log model estimates, are adjusted for confounding, and were estimated for an average participant (male, 15.6 years old at baseline, from village B, with a baseline

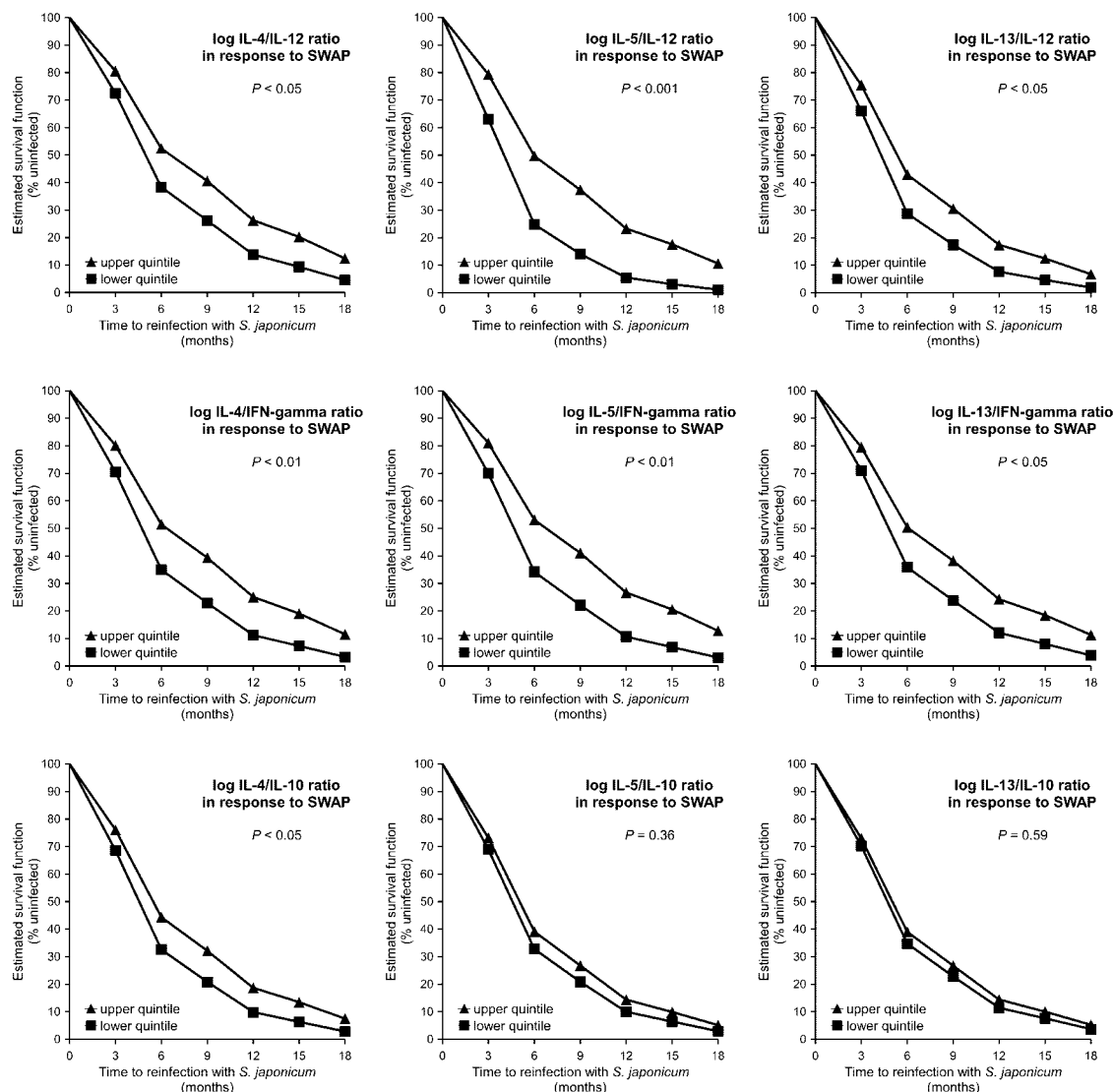


FIG. 2. Time to reinfection with *S. japonicum* after treatment with PZQ, as predicted by cytokine ratios in responses to SWAP ( $n = 493$ ). Triangles represent the upper quintile, and squares represent the lower quintile of each log cytokine-ratio distribution.  $P$  values reflect significance testing comparing the upper and lower quintile for each cytokine ratio. Estimated survival functions and  $P$  values are estimated using a complementary log-log model and are adjusted for confounders and clustering (see Materials and Methods).

infection intensity of 43.3 epg and observed water contact) (26). Least-square (LS) mean values (see Fig. 5 to 7) represent the back-transformed group mean *S. japonicum* egg count at 18 months, adjusted for confounding and estimated for an average participant (as described above). Due to the multilevel nature of our sample (individuals grouped within households) and, as a result, violation of the assumption of independence of observations, we adjusted all analyses for clustering of observations within households. This was done by including household as a random effect in all the above-mentioned models (43).  $P$  values and confidence intervals reported are based on empirical (robust) standard errors, used to protect against misspecification of correlation matrices.  $P$  values of  $<0.05$  were considered statistically significant.

Only successfully treated participants were included in our analyses. Treatment success was defined as having a first *S. japonicum* negative stool sample at 4 weeks posttreatment (primary success) or otherwise at 3 or 6 months posttreatment (delayed success). Treatment failure was defined as having had no *S. japonicum*-negative stool sample up to 6 months posttreatment.

Our approach to analysis was based on the hypothesis that Th2 cytokine responses to *S. japonicum* antigenic stimulation of PBMC predict resistance to reinfection (i.e., time to reinfection and intensity of reinfection). We first assessed the effects of individual cytokine responses, followed by the effects

of Th2 cytokine responses (IL-4, IL-5, and IL-13) relative to the concurrent Th1 (IL-12 and IFN- $\gamma$ ) and anti-inflammatory cytokine (IL-10) responses. Relative effects were assessed as log cytokine ratios, calculated by dividing each participant's raw (untransformed) Th2 cytokine measurement by the raw Th1 cytokine measurement, measured simultaneously in the same PBMC culture, and taking the natural log [ $\ln(\text{Th2}/\text{Th1})$ ]. Effects of the individual cytokines and the log cytokine ratios on reinfection were assessed as continuous variables and as 5-level categorical variables (quintiles of the distribution). These approaches gave similar results, and for illustrative purposes the upper quintile of each cytokine ratio (Th2 polarized) compared to the lower quintile (Th1 polarized) is presented.

Absolute cytokine responses to antigenic stimulation and cytokine responses in control medium were both included in multivariate models (7). This approach allows assessment of the absolute effect of a cytokine response and, after adjusting for baseline (unstimulated) response, of the *S. japonicum*-specific effect. All antigen-specific cytokine reinfection associations are presented adjusted for cytokine levels in control media.

**Ethical clearance.** This study was approved by Brown University and The Philippines Research Institute of Tropical Medicine institutional review boards.

## RESULTS

We screened 1,262 individuals in the study area aged 7 to 30 years, of whom 60% were *S. japonicum* infected. Six-hundred sixteen individuals were eligible to participate and were treated with PZQ at baseline. Overall, 93.5% of individuals were successfully treated; 512 had a negative stool sample 4 weeks posttreatment, and 45 and 19 had a first negative stool sample 3 and 6 months, respectively. Treatment was unsuccessful in 20 (3.2%) individuals, and for another 20 individuals (3.2%) it was not possible to assess treatment response due to missing stool data. Forty-one individuals (7.1%) were censored in time-to-reinfection analysis because they were lost to follow-up. In repeated measures analysis, at least 461 (80.0%) individuals contributed to each time point, with 521 (90.4%) present at three or more follow-ups.

Cytokine measures in control media and in response to SWAP, Sj22.6, Sj97, and Sj67 were available for 545, 510, 535, 531, and 492 individuals, respectively. Geometric mean cytokine responses to control medium and *S. japonicum*-specific antigens are presented in Fig. 1. Mean (95% confidence interval [CI]) age at baseline was 15.6 (15.1 to 16.1) years, and 214 (37.2%) were female.

**Time to reinfection and intensity of reinfection with *S. japonicum*.** Using crude egg count data obtained 18 months posttreatment, 144 out of 461 (31.2%) individuals were uninfected, 236 out of 461 (51.2%) were lightly infected (1 to 99 epg), 61 out of 461 (13.2%) were moderately infected (100 to 399 epg), and 20 out of 461 (4.3%) were heavily infected ( $\geq 400$  epg). The intensity of reinfection was comparable to the pretreatment intensity of infection in this cohort (22). In proportional hazards models adjusted for age, sex, baseline infection intensity, village, water contact, and household clustering, 54.8% and 91.1% were estimated to be reinfected within 6 and 18 months, respectively. Adjusted mean time to reinfection for an average participant was 8.6 months. Adjusted mean intensity of reinfection for an average participant at 18 months was 10.5 epg.

Results from our base time-to-reinfection and mean-intensity-of-reinfection models, including all the potential confounders of the association between resistance to reinfection

TABLE 2. Hazards of reinfection as predicted by cytokine responses in PBMC to *S. japonicum* antigens in a cohort of 7- to 30-year-old individuals from Leyte, the Philippines<sup>a</sup>

| Cytokine      | Response to SWAP |         | Response to Sj22 |         | Response to Sj97 |         |
|---------------|------------------|---------|------------------|---------|------------------|---------|
|               | Hazard ratio     | P value | Hazard ratio     | P value | Hazard ratio     | P value |
| IL-1 $\beta$  | 1.72             | 0.01    | 1.44             | 0.084   | 1.69             | 0.008   |
| IL-2          | 0.61             | 0.002   | 1.04             | 0.79    | 1.08             | 0.62    |
| IL-4          | 0.83             | 0.24    | 1.03             | 0.85    | 0.20             | <0.001  |
| IL-5          | 0.72             | 0.045   | 1.21             | 0.27    | 1.17             | 0.34    |
| IL-10         | 1.36             | 0.14    | 1.61             | 0.065   | 1.53             | 0.073   |
| IL-12         | 1.36             | 0.14    | 1.01             | 0.95    | 1.42             | 0.018   |
| IL-13         | 0.81             | 0.20    | 1.61             | 0.006   | 1.44             | 0.027   |
| IFN- $\gamma$ | 1.17             | 0.35    | 0.98             | 0.90    | 1.20             | 0.26    |
| TNF- $\alpha$ | 1.41             | 0.061   | 1.54             | 0.024   | 1.60             | 0.01    |

<sup>a</sup> Multilevel complementary log-log regression; hazard ratios comparing upper quintile to lower quintile of cytokine distribution, adjusted for baseline cytokine production (i.e. unstimulated control), baseline *S. japonicum* egg count, age, sex, water contact (included as a time-varying covariate), and village of residence and for nonindependence of observations within households.

TABLE 3. Difference in mean intensity of reinfection as predicted by cytokine responses in PBMC to *S. japonicum* antigens in a cohort of 7- to 30-year-old individuals from Leyte, the Philippines<sup>a</sup>

| Cytokine      | Response to SWAP    |         | Response to Sj22    |         | Response to Sj97    |         |
|---------------|---------------------|---------|---------------------|---------|---------------------|---------|
|               | Difference in means | P value | Difference in means | P value | Difference in means | P value |
| IL-1 $\beta$  | 0.61                | 0.012   | 0.29                | 0.15    | 0.38                | 0.056   |
| IL-2          | -0.49               | <0.001  | -0.12               | 0.44    | 0.06                | 0.71    |
| IL-4          | -0.24               | 0.11    | -0.04               | 0.76    | 0.01                | 0.95    |
| IL-5          | -0.36               | 0.022   | 0.30                | 0.06    | 0.35                | 0.12    |
| IL-10         | -0.02               | 0.91    | 0.31                | 0.25    | 0.38                | 0.01    |
| IL-12         | 0.51                | 0.018   | -0.01               | 0.96    | 0.21                | 0.22    |
| IL-13         | -0.41               | 0.011   | 0.20                | 0.17    | 0.37                | 0.025   |
| IFN- $\gamma$ | 0.06                | 0.71    | -0.04               | 0.77    | 0.51                | 0.006   |
| TNF- $\alpha$ | 0.23                | 0.20    | 0.40                | 0.046   | 0.38                | 0.056   |

<sup>a</sup> Multilevel repeated measures linear regression analysis; difference in mean log *S. japonicum* eggs/gram between upper quintile and lower quintile of cytokine distribution, adjusted for baseline cytokine production (unstimulated control), baseline *S. japonicum* egg count, age, sex, water contact (included as a time-varying covariate), and village of residence and for nonindependence of observations within households.

and cytokine profiles, are presented in Table 1. Older individuals had longer time to reinfection ( $P = 0.046$ ); however, this association was attenuated after adjusting for water contact ( $P = 0.18$ ; Table 1). Females had a 1.2 month longer mean time to reinfection ( $P = 0.015$ ) and a 23% lower mean intensity of reinfection ( $P = 0.012$ ) compared to males (Table 1).

**Water contact.** Nearly all individuals had some observed water contact; on only two occasions was no water contact observed. Nevertheless, there was marked variation in water contact between individuals, ranging from crossing a small body of water to working in a rice paddy for most of the day. We did not detect a linear dose-response relationship between water contact and time to reinfection or intensity of reinfection (data not shown). However, exploratory analysis revealed that a very low level of water contact (defined as the lower 2.5% of the overall distribution of water contact) did predict a longer time to reinfection (adjusted hazard ratio, 0.51 [0.23 to 1.12];  $P = 0.097$ ) and 28% lower intensity of reinfection ( $P = 0.079$ ) than higher levels of water contact (upper 97.5% of the overall distribution of water contact) (Table 1). Water contact in the lowest 2.5% corresponds roughly with a water contact of both feet for  $\leq 60$  s, or its equivalent, per day.

**Individual cytokine responses to *S. japonicum* antigens predict time to and intensity of reinfection.** PBMC production of IL-2 and IL-5 in response to SWAP predicted longer time to reinfection and decreased mean intensity of reinfection (Tables 2 and 3). Conversely, production of IL-1-beta in response to SWAP predicted decreased time to reinfection and higher mean intensity of reinfection (Tables 2 and 3). TNF- $\alpha$  produced in response to Sj22.6 predicted decreased time to reinfection and higher mean intensity of reinfection. IL-1 $\beta$ , IL-10, IL-13, and TNF- $\alpha$  produced in response to Sj97 predicted both decreased time to reinfection and increased intensity of reinfection (Tables 2 and 3). Cytokine responses to Sj67 were not associated with resistance to reinfection (data not shown).

**Th2 cytokine balance in response to *S. japonicum* antigens predicts time to and intensity of reinfection.** Although production of the Th2 cytokines IL-4 and IL-13 in response to SWAP

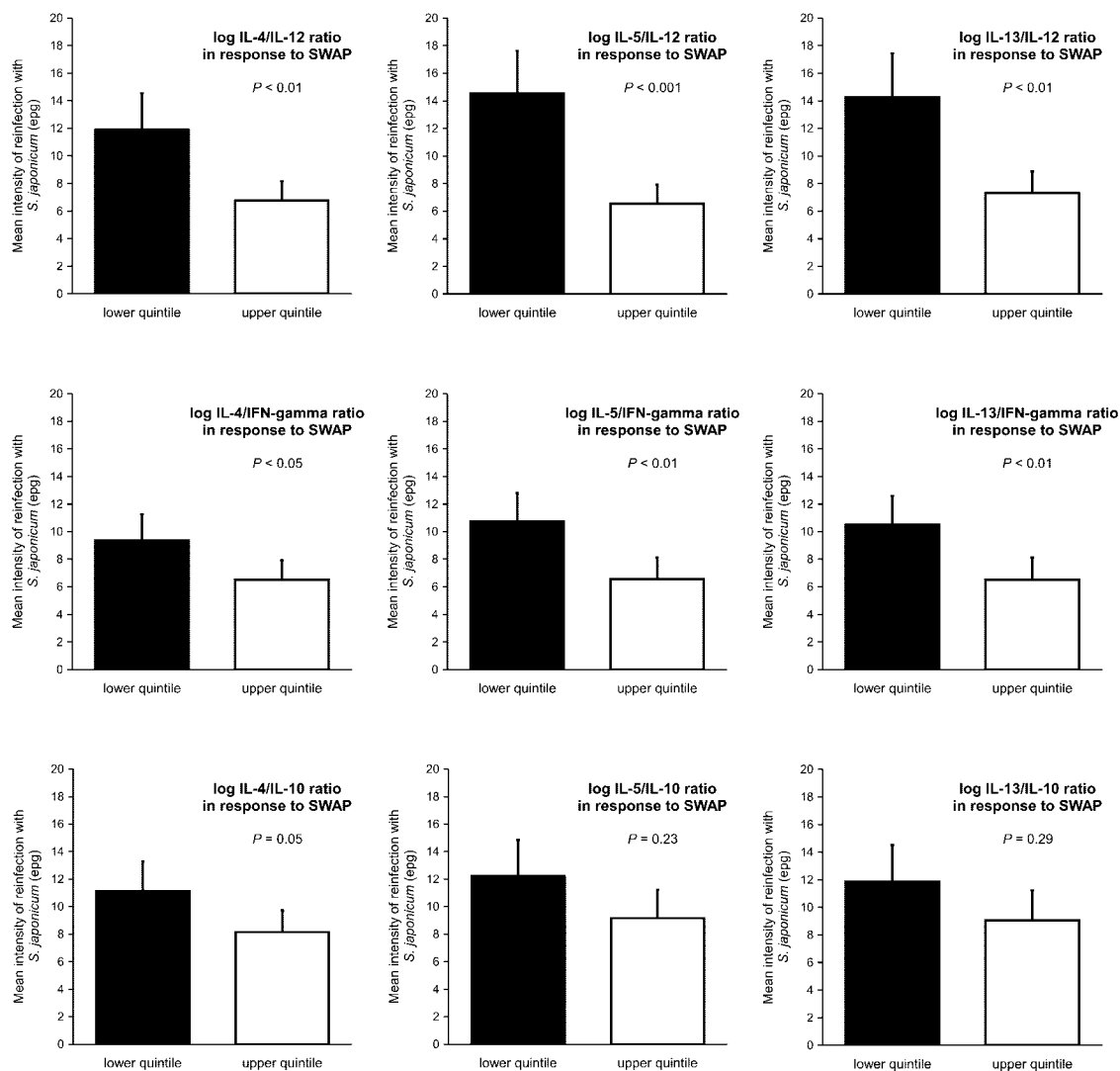


FIG. 3. Intensity of reinfection with *S. japonicum* 18 months after treatment with PZQ, as predicted by cytokine ratios in responses to SWAP ( $n = 493$ ). White and black bars represent the back-transformed LS mean intensity of reinfection for the upper and lower quintile of each log cytokine-ratio distribution, respectively. Error bars represent standard errors. LS mean estimates, standard errors, and  $P$  values for differences in means between quintiles are adjusted for confounders and clustering (see Materials and Methods).

did not significantly predict time to reinfection on their own (Table 2), when these responses and that of IL-5, were assessed relative to Th1 cytokines IL-12 and IFN- $\gamma$ , they were consistently associated with longer time to reinfection as well as lower mean intensity of reinfection (Fig. 2 and 3). Individuals with these cytokine ratios in the upper quintile had a mean time to reinfection that was 1.4 to 2.9 months longer (Fig. 2) and a mean intensity of reinfection that was 27 to 55% lower than those of individuals with ratios in the lowest quintile (Fig. 3).

Similarly, individuals with cytokine ratios in the upper quintile for IL-4/IL-12, IL-5/IL-12, IL-13/IL-12, and IL-4/IFN- $\gamma$  in response to Sj97 had longer times to reinfection (Fig. 4) compared to those of individuals in the lowest quintile. Individuals with cytokine ratios in the upper quintile for IL-5/IL-12, IL-13/IL-12, IL-4/IFN- $\gamma$ , IL-5/IFN- $\gamma$ , and IL-13/IFN- $\gamma$  in response to Sj97 had lower mean intensity of reinfection compared to in-

dividuals in the lowest quintile (Fig. 5). Individuals with these cytokine ratios in the upper quintile had a mean time to reinfection that was 1.6 to 2.2 months longer and a mean intensity of reinfection that was 30 to 41% lower than those of individuals with ratios in the lowest quintile. Individuals with an IL-5/IL-10 ratio in the upper quintile in response to Sj22.6 had a mean time to reinfection that was 3.0 months longer than those of individuals with ratios in the lowest quintile (Fig. 6), but no differences in intensity of reinfection were seen (Fig. 7). Ratios of cytokines produced in response to Sj67 were not associated with resistance to reinfection (data not shown).

## DISCUSSION

Rapid reinfection with significant morbidity despite treatment with PZQ remains the major impetus for vaccine development for schistosomiasis. While several vaccine candidates

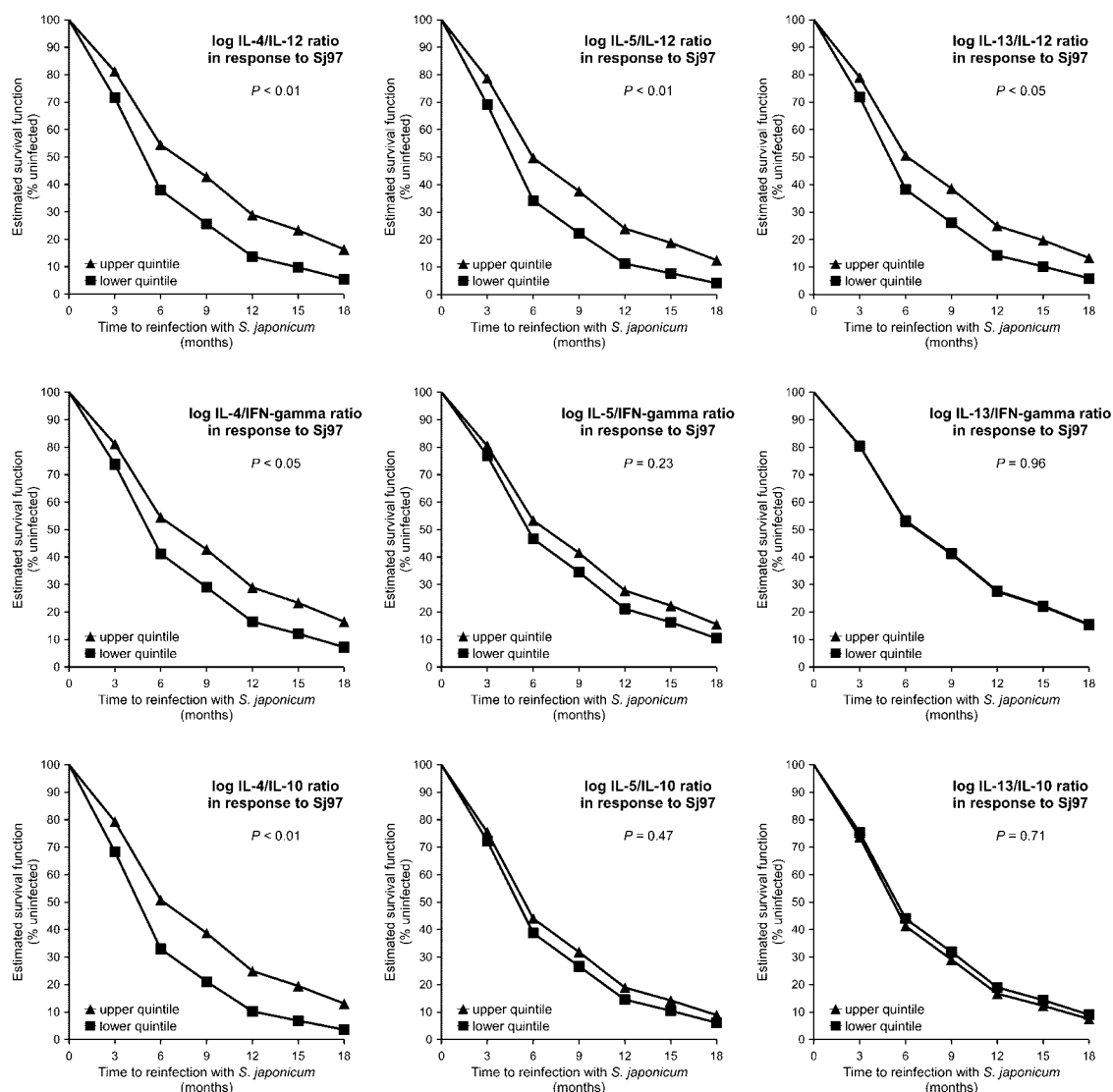


FIG. 4. Time to reinfection with *S. japonicum* after treatment with PZQ, as predicted by cytokine ratios in responses to Sj97 ( $n = 514$ ). Triangles represent the upper quintile, and squares represent the lower quintile of each log cytokine-ratio distribution.  $P$  values reflect significance testing comparing the upper and lower quintile for each cytokine ratio. Estimated survival functions and  $P$  values are estimated using a complementary log-log model and are adjusted for confounders and clustering (see Materials and Methods).

have shown promise in animal models of schistosomiasis (5), there is unfortunately limited generalizability from these models to human resistance. This situation has complicated vaccine development efforts and mandates alternative approaches for the identification and evaluation of vaccines for these parasites. We conducted a large-scale treatment-reinfection study in three villages in The Philippines where *S. japonicum* is endemic with simultaneous assessment of water contact and antigen-specific cytokine production to several vaccine candidates prior to reinfection with *S. japonicum*. In our study, we found that Th2 cytokine responses to SWAP and Sj97 were consistently associated with resistance to reinfection.

Surprisingly few rigorous studies have been published reporting cellular immune correlates of resistance to schistosome infection in humans. In an analysis of 58 *S. mansoni*-infected individuals aged 9 to 40 years, Roberts et al.

reported an association between increased IL-5 production in response to soluble egg antigen measured 3 months after treatment and decreased reinfection measured over a 21-month follow-up period (39). This relationship was attenuated after controlling for age and exposure. In an analysis of 93 individuals (49 uninfected and 44 infected) living in an area of China where *S. japonicum* is endemic, Shen et al. reported an association between increased IFN- $\gamma$  production in response to soluble egg antigen and decreased prevalence of infection at the end of a 9-month transmission season (41). This association was attenuated after adjusting for age and sex, and the authors acknowledged that the unexpectedly low infection prevalence at follow-up (15%) suggests caution in interpreting these results. In an analysis of 59 *S. haematobium*-infected children aged 9 to 15 years, Medhat et al. reported an association between IL-4 and IL-5 pro-

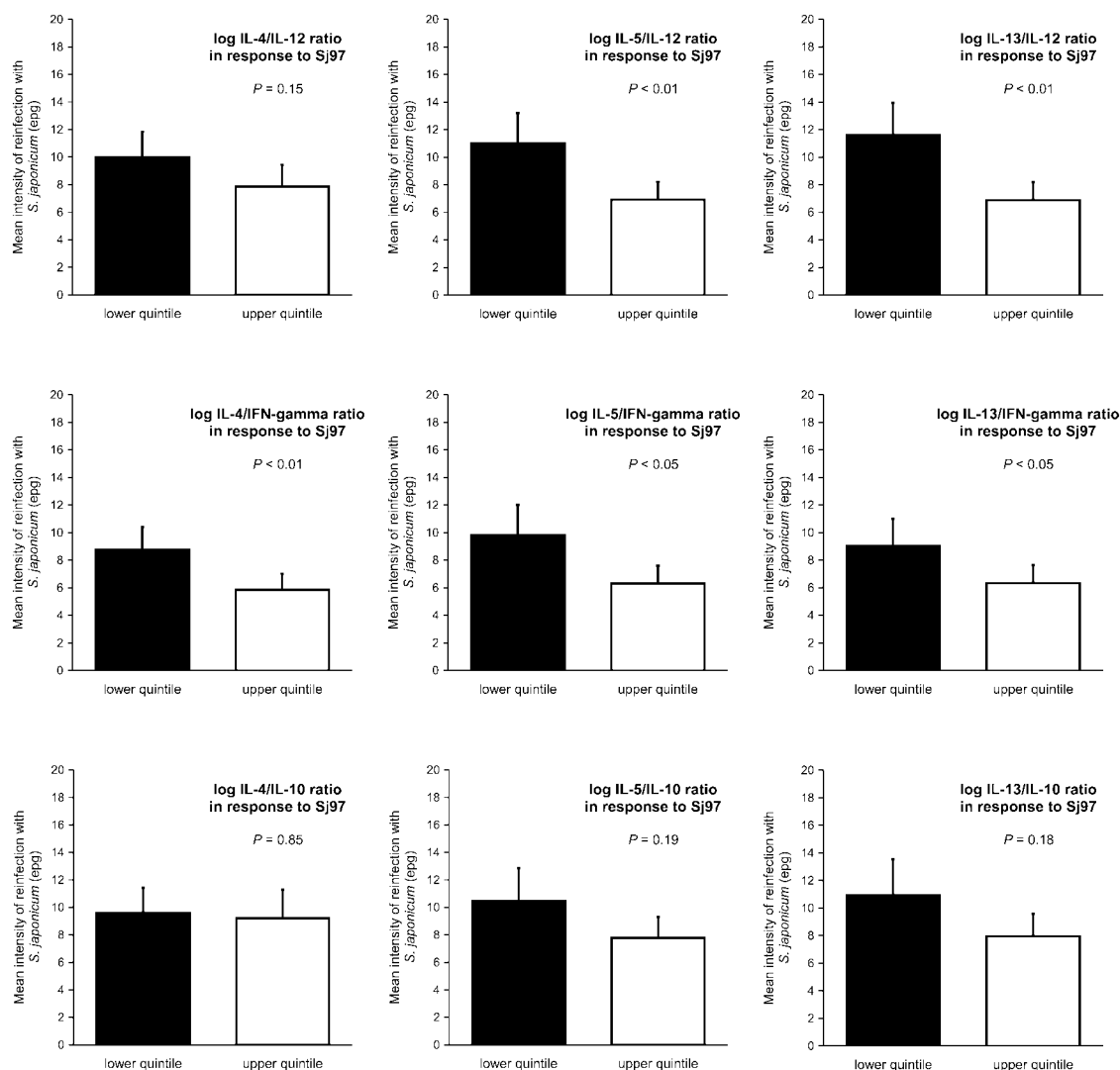


FIG. 5. Intensity of reinfection with *S. japonicum* 18 months after treatment with PZQ, as predicted by cytokine ratios in responses to Sj97 ( $n = 514$ ). White and black bars represent the back-transformed LS mean intensity of reinfection for the upper and lower quintile of each log cytokine-ratio distribution, respectively. Error bars represent standard errors. LS mean estimates, standard errors, and  $P$  values for differences in means between quintiles are adjusted for confounders and clustering (see Materials and Methods).

duction in response to SWAP measured 12 to 18 months after treatment and a defined phenotype of resistant or susceptible to reinfection (32). In this study, cytokine responses were measured after the relevant reinfection period when individuals were already heterogeneously infected, thus obscuring causal inference between cytokine levels and resistance.

Cytokine patterns of purified antigens have been associated with resistance to infection in three studies in *S. mansoni*, a single study in *S. haematobium*, and a single study in *S. japonicum*. In a cross-sectional study of 119 individuals living in an area of Brazil where *S. japonicum* is endemic, Ribeiro et al. reported that individuals who produced IL-5 ( $\geq 30$  pg/ml) in response to stimulation with MAP-3 had a decreased risk of being heavily infected ( $\geq 200$  epg) after adjusting for age (37). In sequential retrospective and longitudinal studies of 141 individuals living in an area of Egypt where *S. japonicum* is

endemic, Al-Sherbiny et al. report that IL-5 responses to Sm97 and IFN- $\gamma$  responses to Sm14-FABP were higher in individuals who (i) retrospectively did not become infected following historical treatment and (ii) prospectively remained uninfected following treatment (3). This report did not attempt to adjust for water contact, age, or other potential confounding variables. In a retrospective study of 31 *S. mansoni*-infected and 29 *S. haematobium*-infected individuals, El Ridi et al. report that the proportion of detectable IFN- $\gamma$  production in response to rSG3PDH measured 12 to 36 months following PZQ treatment was higher in individuals who were currently infected (*S. mansoni*, 15; *S. haematobium*, 19) compared to individuals who were not currently infected (*S. mansoni*, 16; *S. haematobium*, 10) (20). This report did not attempt to adjust for water contact, age, or other potential confounding variables, and cytokine responses were measured after the relevant reinfection period, complicating causal inference between cytokine levels

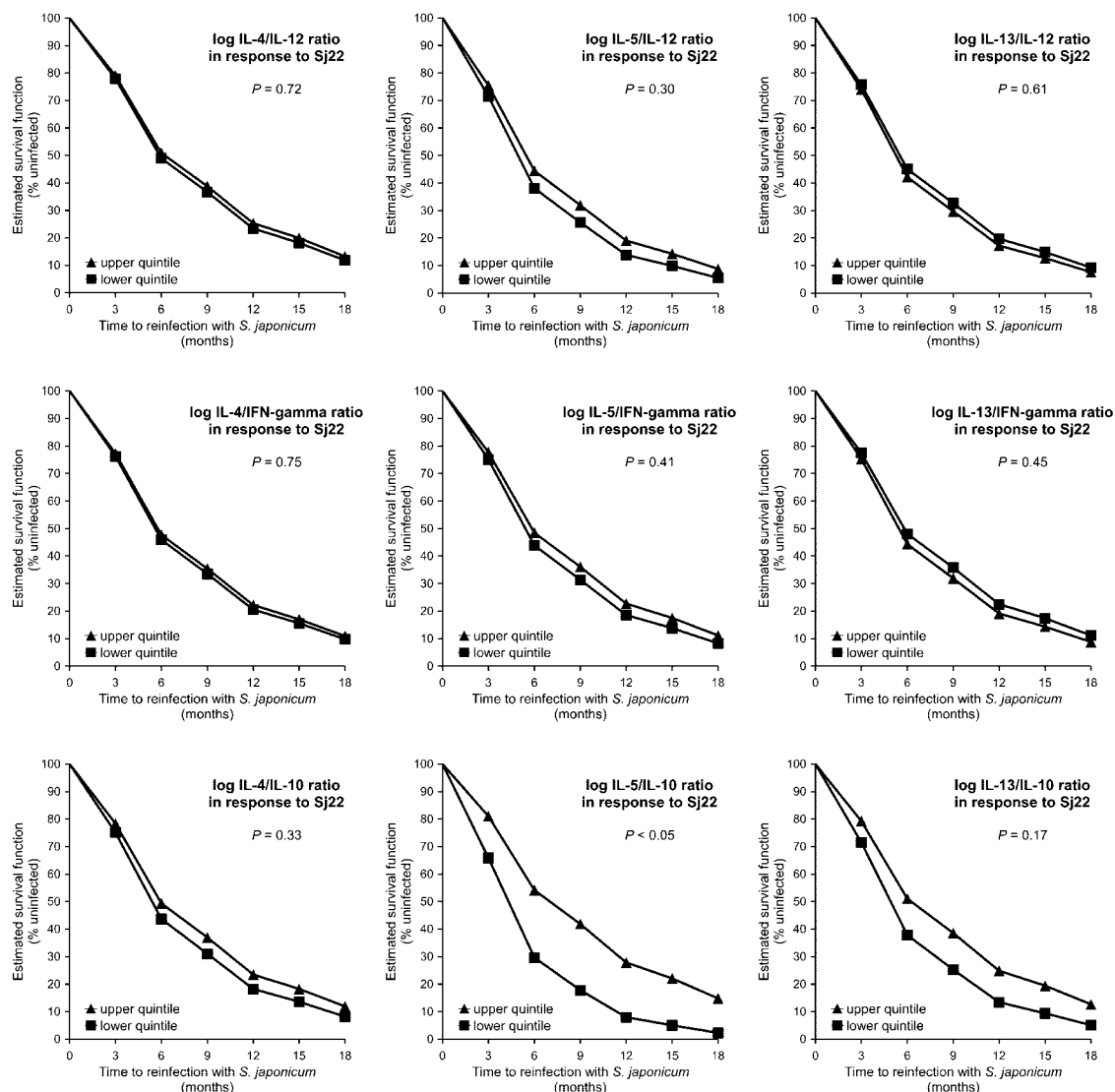


FIG. 6. Time to reinfection with *S. japonicum* after treatment with PZQ, as predicted by cytokine ratios in responses to Sj22.6 ( $n = 519$ ). Triangles represent the upper quintile, and squares represent the lower quintile of each log cytokine-ratio distribution.  $P$  values reflect significance testing comparing the upper and lower quintile for each cytokine ratio. Estimated survival functions and  $P$  values are estimated using a complementary log-log model and are adjusted for confounders and clustering (see Materials and Methods).

and resistance. In a retrospective study of individuals aged 5 to 76 years living in an area of the Philippines where *S. japonicum* is endemic, Acosta et al. reported an association between IL-5 production in response to SWAP measured 1 year after PZQ treatment and a defined phenotype of resistant or susceptible to reinfection in males (67) and individuals with age  $\geq 20$  years (64) (1, 2). This study also identified associations between IFN- $\gamma$  production in response to SWAP and recombinant Sj97 and the resistant phenotype. In this study, cytokine responses were measured after the relevant reinfection period, complicating causal inference between cytokine levels and resistance.

In our study, *S. japonicum* transmission was surprisingly high, with more than half of the participants becoming reinfected within 6 months and 91.1% reinfected by 18 months following treatment. Consistent with this high reinfection rate, virtually

every individual had observed contact with potentially infectious water during the relevant study period. Despite our attempt to quantify this exposure, we were unable to detect a continuous linear relationship between observed water contact and reinfection intensity; however, we did detect lower reinfection in individuals with very low (lower 2.5 percentile) versus higher observed water contact. As expected, we detected an inverse relationship between age and reinfection (10) as well as decreased reinfection in females compared to males (2, 9).

We quantified the production of nine cytokines in response to five stimuli in PBMCs obtained 4 weeks after PZQ treatment in 492 to 545 individuals. With the exception of lower IL-4 levels, cytokine levels in response to SWAP in our study population were comparable to those of previous reports of SWAP-stimulated PBMCs (39) and whole-blood cultures (27) in PZQ-treated *S. mansoni*-infected individuals.

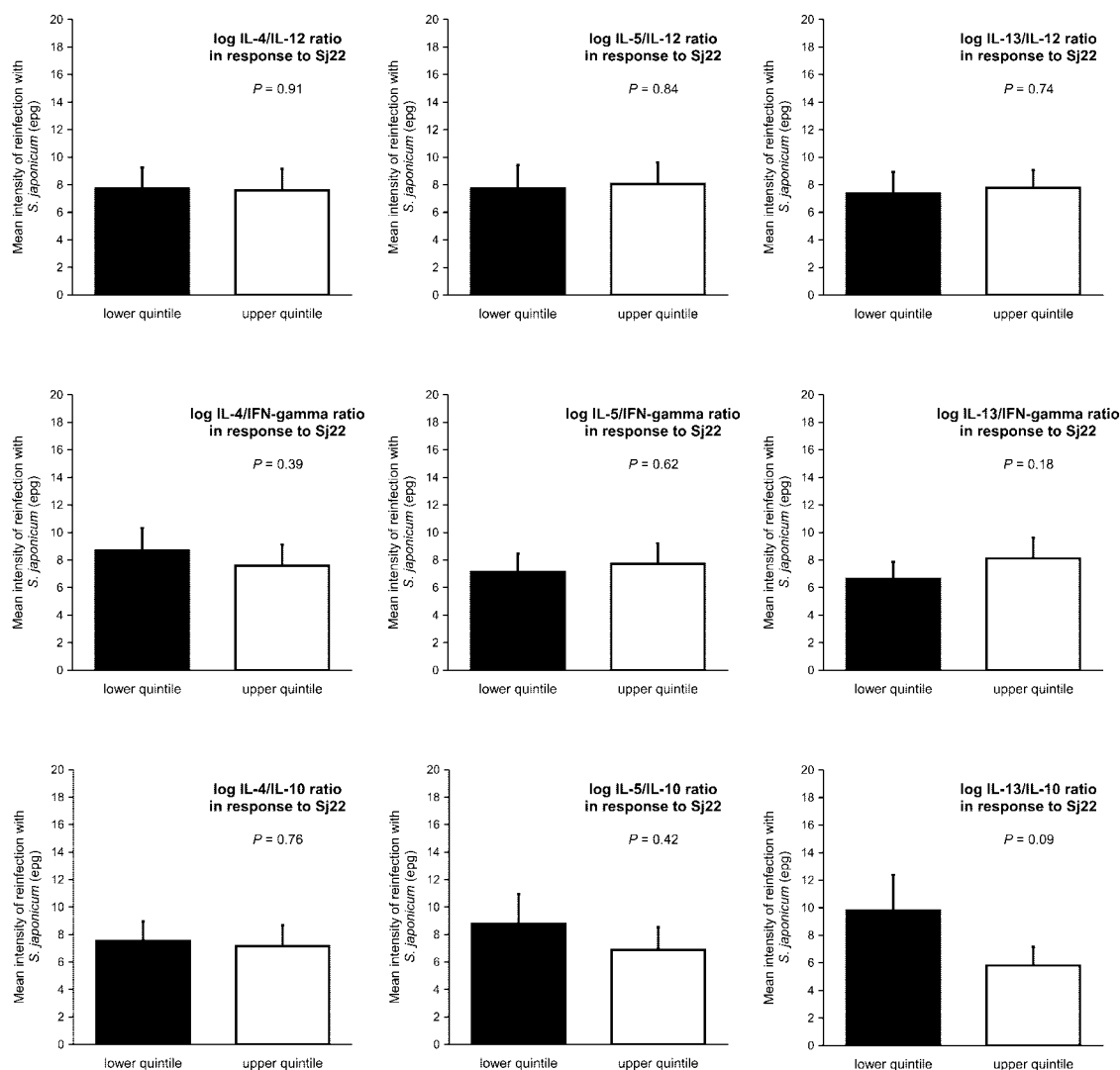


FIG. 7. Intensity of reinfection with *S. japonicum* 18 months after treatment with PZQ, as predicted by cytokine ratios in responses to Sj22.6 ( $n = 519$ ). White and black bars represent the back-transformed LS mean intensity of reinfection for the upper and lower quintile of each log cytokine-ratio distribution, respectively. Error bars represent standard errors. LS mean estimates, standard errors, and  $P$  values for differences in means between quintiles are adjusted for confounders and clustering (see Materials and Methods).

In survival and repeated-measures analyses, individual Th2-type cytokines made in response to SWAP and Th2/Th1 cytokine ratios made in response to SWAP, Sj97, and Sj22.6 predicted resistance to reinfection. Importantly, these associations remained after accounting for the potential confounding effects of age, sex, village, baseline intensity of infection, inter-current water contact, and clustering within households.

Current evidence suggests that the Th2/Th1 paradigm may oversimplify cellular immunologic phenomena, thus necessitating analysis of individual cytokines within the context of their potential antagonists (11, 15, 23). Because of potential antagonism between Th2 and Th1 cytokines, we hypothesized that the balance between individual cytokines made in response to *S. japonicum* vaccine candidates would more reliably predict potentially protective responses in vivo (11). Therefore, we evaluated the relationship between reinfection and individual cytokines as well as the ratio of potentially antagonistic cyto-

kines. Using this approach, we detected a remarkably consistent relationship between a high Th2/Th1 cytokine balance in response to SWAP and Sj97 and resistance to reinfection corresponding to a 1.4- to 2.9-months longer time to reinfection and a 27 to 55% decrease in intensity of reinfection. We also detected a protective relationship between the IL-5/IL-10 ratio in response to Sj22.6 and longer time to reinfection. These findings suggest that resistance to reinfection is related not simply to strong Th2 responses to these antigens but also to the combination of a strong Th2 and weak or absent Th1 response.

Our findings are broadly consistent with previous data identifying paramyosin (Sj97) and Sj22.6 as vaccine candidates (5). Paramyosin (Sm97) was originally identified by size fractionation of parasite extracts followed by murine vaccination trials in *S. mansoni* (42). Subsequent vaccination experiments with biochemically purified *S. japonicum* paramyosin and adoptive transfer of an anti-paramyosin IgE monoclonal antibody also

demonstrated significant protection (29). Using an immunology-based approach, Sm22.6 was identified as the target of IgE responses that were associated with resistance to reinfection with *S. mansoni* after PZQ treatment (19). The *S. japonicum* homologue Sj22.6 was identified by probing an *S. japonicum* cDNA library with sera obtained from infected individuals with high titers of anti-parasite IgE (40).

Current control strategies employing targeted or mass chemotherapy with PZQ have not reduced transmission and morbidity to acceptable levels, underscoring the urgent need for vaccines for schistosomiasis. Our current data, derived from a large-scale longitudinal treatment-reinfection study with simultaneous measurement of potential confounding variables, demonstrate that individuals who mount a polarized Th2 response to Sj97 under conditions of natural exposure are significantly protected from reinfection. These data strongly support the development of an Sj97-based vaccine formulated and adjuvanted to generate a polarized Th-2 response. Our data also suggest that a polarized Th2 response to Sj22.6 may contribute to resistance to schistosomiasis japonica in humans.

#### ACKNOWLEDGMENTS

This work was funded by National Institutes of Health grants R01AI48123 and K23AI52125.

We thank our field staff for their diligence and energy. We thank the study participants from Macanip, Buri, and Pitogo in Leyte, The Philippines.

#### REFERENCES

- Acosta, L. P., G. D. Aligui, W. U. Tiu, D. P. McManus, and R. M. Olveda. 2002. Immune correlate study on human *Schistosoma japonicum* in a well-defined population in Leyte, Philippines: I. Assessment of 'resistance' versus 'susceptibility' to *S. japonicum* infection. *Acta Trop.* **84**:127–136.
- Acosta, L. P., G. Waive, G. D. Aligui, W. U. Tiu, R. M. Olveda, and D. P. McManus. 2002. Immune correlate study on human *Schistosoma japonicum* in a well-defined population in Leyte, Philippines: II. Cellular immune responses to *S. japonicum* recombinant and native antigens. *Acta Trop.* **84**:137–149.
- Al-Sherbiny, M., A. Osman, R. Barakat, H. El Morshedy, R. Bergquist, and R. Olds. 2003. In vitro cellular and humoral responses to *Schistosoma mansoni* vaccine candidate antigens. *Acta Trop.* **88**:117–130.
- Allison, P. D. 1995. Survival analysis using the SAS System: a practical guide. SAS Institute, Inc., Cary, N.C.
- Bergquist, N. R. 1998. Schistosomiasis vaccine development: progress and prospects. *Mem. Inst. Oswaldo Cruz* **93**(Suppl. 1):95–101.
- Bergquist, N. R., L. R. Leonardo, and G. F. Mitchell. 2005. Vaccine-linked chemotherapy: can schistosomiasis control benefit from an integrated approach? *Trends Parasitol.* **21**:112–117.
- Booth, M., J. K. Mwatha, S. Joseph, F. M. Jones, H. Kadzo, E. Ireri, F. Kazibwe, J. Kemijumbi, C. Kariuki, G. Kimani, J. H. Ouma, N. B. Kabatereine, B. J. Vennervald, and D. W. Dunne. 2004. Periportal fibrosis in human *Schistosoma mansoni* infection is associated with low IL-10, low IFN- $\gamma$ , high TNF- $\alpha$ , or low RANTES, depending on age and gender. *J. Immunol.* **172**:1295–1303.
- Butterworth, A. E., E. L. Corbett, D. W. Dunne, A. J. C. Fulford, R. K. Gachuhi, R. Klumpp, G. Mbugua, J. H. Ouma, T. K. arap Singok, and R. F. Sturrock. 1989. Immunity and morbidity in human schistosomiasis, p. 193. In K. P. W. J. McAdam (ed.), *New strategies in parasitology*. Churchill Livingstone, Edinburgh, United Kingdom.
- Butterworth, A. E., P. R. Dalton, D. W. Dunne, M. Mugambi, J. H. Ouma, B. A. Richardson, T. K. Siongok, and R. F. Sturrock. 1984. Immunity after treatment of human schistosomiasis mansoni. I. Study design, pretreatment observations and the results of treatment. *Trans. R Soc. Trop. Med. Hyg.* **78**:108–123.
- Butterworth, A. E., A. J. Fulford, D. W. Dunne, J. H. Ouma, and R. F. Sturrock. 1988. Longitudinal studies on human schistosomiasis. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **321**:495–511.
- Cannon, J. G., J. L. Nerad, D. D. Poutsiaika, and C. A. Dinarello. 1993. Measuring circulating cytokines. *J. Appl. Physiol.* **75**:1897–1902.
- Chan, M. S., M. E. Woolhouse, and D. A. Bundy. 1997. Human schistosomiasis: potential long-term consequences of vaccination programmes. *Vaccine* **15**:1545–1550.
- Clarke, V. d. V. 1966. Evidence of the development in man of acquired resistance to infection of *Schistosoma* spp. *Cent. Afr. J. Med.* **12**:1–30.
- Coutinho, H. M., S. T. McGarvey, L. P. Acosta, D. L. Manalo, G. C. Langdon, T. Leenstra, H. K. Kanzaria, J. Solomon, H. Wu, R. M. Olveda, J. D. Kurtis, and J. F. Friedman. 2005. Nutritional status and serum cytokine profiles in children, adolescents, and young adults with *Schistosoma japonicum*-associated hepatic fibrosis, in Leyte, Philippines. *J. Infect. Dis.* **192**:528–536.
- Del Prete, G. 1998. The concept of type-1 and type-2 helper T cells and their cytokines in humans. *Int. Rev. Immunol.* **16**:427–455.
- Delgado, V., and D. J. McLaren. 1990. Evidence for enhancement of IgG1 subclass expression in mice polyvaccinated with radiation-attenuated cercariae of *Schistosoma mansoni* and the role of this isotype in serum-transferred immunity. *Parasite Immunol.* **12**:15–32.
- Demeure, C. E., P. Rihet, L. Abel, M. Ouattara, A. Bourgois, and A. J. Dessein. 1993. Resistance to *Schistosoma mansoni* in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. *J. Infect. Dis.* **168**:1000–1008.
- Dessein, A. J., M. Begley, C. Demeure, D. Caillol, J. Fueri, R. M. dos, Z. A. Andrade, A. Prata, and J. C. Bina. 1988. Human resistance to *Schistosoma mansoni* is associated with IgG reactivity to a 37-kDa larval surface antigen. *J. Immunol.* **140**:2727–2736.
- Dunne, D. W., A. E. Butterworth, A. J. Fulford, H. C. Kariuki, J. G. Langley, J. H. Ouma, A. Capron, R. J. Pierce, and R. F. Sturrock. 1992. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur. J. Immunol.* **22**:1483–1494.
- El Ridi, R., C. B. Shoemaker, F. Farouk, N. H. El Sherif, and A. Afifi. 2001. Human T- and B-cell responses to *Schistosoma mansoni* recombinant glyceraldehyde 3-phosphate dehydrogenase correlate with resistance to reinfection with *S. mansoni* or *Schistosoma haematobium* after chemotherapy. *Infect. Immun.* **69**:237–244.
- Fitzmaurice, G. M., N. M. Laird, and J. H. Ware. 2004. *Applied Longitudinal analysis*. John Wiley & Sons, Inc., Hoboken, N.J.
- Friedman, J. F., H. K. Kanzaria, L. P. Acosta, G. C. Langdon, D. L. Manalo, H. Wu, R. M. Olveda, S. T. McGarvey, and J. D. Kurtis. 2005. Relationship between *Schistosoma japonicum* and nutritional status among children and young adults in Leyte, the Philippines. *Am. J. Trop. Med. Hyg.* **72**:527–533.
- Gor, D. O., N. R. Rose, and N. S. Greenspan. 2003. TH1-TH2: a procrustean paradigm. *Nat. Immunol.* **4**:503–505.
- Hagan, P., H. A. Wilkins, U. J. Blumenthal, R. J. Hayes, and B. M. Greenwood. 1985. Eosinophilia and resistance to *Schistosoma haematobium* in man. *Parasite Immunol.* **7**:625–632.
- Hartel, C., G. Bein, M. Muller-Steinhardt, and H. Kluter. 2001. Ex vivo induction of cytokine mRNA expression in human blood samples. *J. Immunol. Methods* **249**:63–71.
- Hosmer, D. W., and S. Lemeshow. 1999. *Applied survival analysis: regression modelling of time to event data*. John Wiley & Sons, Inc., Hoboken, N.J.
- Joseph, S., F. M. Jones, K. Walter, A. J. Fulford, G. Kimani, J. K. Mwatha, T. Kamau, H. C. Kariuki, F. Kazibwe, E. Tukahebwa, N. B. Kabatereine, J. H. Ouma, B. J. Vennervald, and D. W. Dunne. 2004. Increases in human T helper 2 cytokine responses to *Schistosoma mansoni* worm and worm-tegument antigens are induced by treatment with praziquantel. *J. Infect. Dis.* **190**:835–842.
- Jwo, J., and P. T. LoVerde. 1989. The ability of fractionated sera from animals vaccinated with irradiated cercariae of *Schistosoma mansoni* to transfer immunity to mice. *J. Parasitol.* **75**:252–260.
- Kojima, S., M. Niimura, and T. Kanazawa. 1987. Production and properties of a mouse monoclonal IgE antibody to *Schistosoma japonicum*. *J. Immunol.* **139**:2044–2049.
- Mangold, B. L., and D. A. Dean. 1986. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against *Schistosoma mansoni* in mice. *J. Immunol.* **136**:2644–2648.
- Mascie-Taylor, C. G., and E. Karim. 2003. The burden of chronic disease. *Science* **302**:1921–1922.
- Medhat, A., M. Shehata, K. Bucci, S. Mohamed, A. D. Dief, S. Badary, H. Galal, M. Nafeh, and C. L. King. 1998. Increased interleukin-4 and interleukin-5 production in response to *Schistosoma haematobium* adult worm antigens correlates with lack of reinfection after treatment. *J. Infect. Dis.* **178**:512–519.
- Mountford, A. P., S. Anderson, and R. A. Wilson. 1996. Induction of Th1 cell-mediated protective immunity to *Schistosoma mansoni* by co-administration of larval antigens and IL-12 as an adjuvant. *J. Immunol.* **156**:4739–4745.
- Mountford, A. P., V. L. Shires, and S. Anderson. 1998. Interleukin-12 and protective immunity to schistosomes. *Braz. J. Med. Biol. Res.* **31**:163–169.
- Oswald, I. P., P. Caspar, T. A. Wynn, T. Scharton-Kersten, M. E. Williams, S. Hiery, A. Sher, and S. L. James. 1998. Failure of P strain mice to respond to vaccination against schistosomiasis correlates with impaired production of IL-12 and up-regulation of Th2 cytokines that inhibit macrophage activation. *Eur. J. Immunol.* **28**:1762–1772.

36. Ramirez, B. L., J. D. Kurtis, P. M. Wiest, P. Arias, F. Aligui, L. Acosta, P. Peters, and G. R. Olds. 1996. Paramyosin: a candidate vaccine antigen against *Schistosoma japonicum*. *Parasite Immunol.* **18**:49–52.
37. Ribeiro de Jesus, A., I. Araujo, O. Bacellar, A. Magalhaes, E. Pearce, D. Harn, M. Strand, and E. M. Carvalho. 2000. Human immune responses to *Schistosoma mansoni* vaccine candidate antigens. *Infect. Immun.* **68**:2797–2803.
38. Rihet, P., C. E. Demeure, A. Bourgois, A. Prata, and A. J. Dessein. 1991. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur. J. Immunol.* **21**:2679–2686.
39. Roberts, M., A. E. Butterworth, G. Kimani, T. Kamau, A. J. Fulford, D. W. Dunne, J. H. Ouma, and R. F. Sturrock. 1993. Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to reinfection. *Infect. Immun.* **61**:4984–4993.
40. Santiago, M. L., J. C. Hafalla, J. D. Kurtis, G. L. Aligui, P. M. Wiest, R. M. Olveda, G. R. Olds, D. W. Dunne, and B. L. Ramirez. 1998. Identification of the *Schistosoma japonicum* 22.6-kDa antigen as a major target of the human IgE response: similarity of IgE-binding epitopes to allergen peptides. *Int. Arch. Allergy Immunol.* **117**:94–104.
41. Shen, L., Z. S. Zhang, H. W. Wu, R. E. Weir, Z. W. Xie, L. S. Hu, S. Z. Chen, M. J. Ji, C. Su, Y. Zhang, Q. D. Bickle, S. N. Cousins, M. G. Taylor, and G. L. Wu. 2003. IFN-gamma is associated with risk of *Schistosoma japonicum* infection in China. *Parasite Immunol.* **25**:483–487.
42. Sher, A., E. Pearce, S. Hieny, and S. James. 1986. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine. IV. Fractionation and antigenic properties of a soluble adult worm immunoprophylactic activity. *J. Immunol.* **136**:3878–3883.
43. Singer, J. D. 1998. Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. *J. Educ. Behav. Stat.* **24**:323–355.
44. Solomon, J. S., C. P. Nixon, S. T. McGarvey, L. P. Acosta, D. Manalo, and J. D. Kurtis. 2004. Expression, purification, and human antibody response to a 67 kDa vaccine candidate for schistosomiasis japonica. *Protein Expr. Purif.* **36**:226–231.
45. Wilkins, H. A., U. J. Blumenthal, P. A. Hagan, R. J. Hayes, and S. Tulloch. 1987. Resistance to reinfection after treatment of urinary schistosomiasis. *Trans. R Soc. Trop. Med. Hyg.* **81**:29–35.
46. Wynn, T. A., A. Reynolds, S. James, A. W. Cheever, P. Caspar, S. Hieny, D. Jankovic, M. Strand, and A. Sher. 1996. IL-12 enhances vaccine-induced immunity to schistosomes by augmenting both humoral and cell-mediated immune responses against the parasite. *J. Immunol.* **157**:4068–4078.

Editor: W. A. Petri, Jr.