Am. J. Trop. Med. Hyg., 86(2), 2012, pp. 296–305
doi:10.4269/ajtmh.2012.11-0204

Factors Associated with Resistance to *Schistosoma mansoni* Infection in an Endemic Area of Bahia, Brazil

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Abstract. Detailed knowledge of factors associated with resistance to *Schistosoma mansoni* infection in endemic areas might facilitate more effective schistosomiasis control. We conducted a cross-sectional study of persons resistant to schistosomiasis and found no association between socioeconomic status and resistance to infection. Mononuclear cells of resistant subjects produced higher levels of interleukin-5 (IL-5), IL-13 and interferon-γ upon stimulation with soluble egg antigen (SEA) compared with infected persons. When stimulated with Sm21.6 or Sm22.6, levels of IL-10 were higher in cell culture of resistant persons. Levels of IgE against soluble adult worm antigen (SWAP) and against interleukin-4–inducing principle from *S. mansoni* eggs (IPSE) and levels of IgG4 against SWAP, SEA, and Sm22.6 were lower in the resistant group compared with the susceptible group. Our data suggest that socioeconomic status could not fully explain resistance to *S. mansoni* infection observed in the studied area. However, a mixture of Th1 and Th2 immune responses and low levels of specific IgG4 against parasite antigens could be mediating resistance to infection.

INTRODUCTION

Schistosomiasis is a tropical disease caused mainly by the blood flukes *Schistosoma mansoni*, *S. haematobium,* and *S. japonicum*. It is estimated that the disease affects 200 million persons worldwide, and is endemic to 74 countries in tropical regions of Africa, Asia, and South America.[1] The species that causes most infections in Brazil is *S. mansoni*. Soon after infection and before the egg-laying phase, the immune response is predominantly the T helper 1 (Th1) inflammatory immune response, with high levels of tumor necrosis factor-α, interleukin-1 (IL-1), IL-6 and interferon-γ (IFN-γ) characterizing the acute phase of schistosomiasis.[2] The natural progress of the disease leads to liver and intestinal injury caused mainly by the immune response against *S. mansoni* eggs deposited in these sites. In the chronic phase of the disease, there is a predominance of the Th2 immune response to egg antigens, with low production of IFN-γ, and high levels of IL-4, IL-5, IL-13, and IL-10.[3–5]

Current strategies of schistosomiasis control programs include use of molluscicides to eliminate the intermediate host, education of the population with respect to improvement in sanitation, and mass treatment programs.[6] The development of a vaccine is an important alternative strategy for the control of schistosomiasis. However, no suitable vaccine has yet been developed that confers more than partial protection against infection.[7] Membrane proteins including receptors, ion-binding proteins, immunomodulatory molecules, and enzymes accessible to the human immune system likely represent suitable vaccine targets.[8] Recently investigators have demonstrated that some recombinant *Schistosoma* spp. antigens, such as Sm22.6, Sm29, Sm21.6, and Sm14, are associated with resistance to infection and/or reduction of morbidity.[9–12] These particular proteins are found mainly in the tegument of *S. mansoni*, and induce high levels of IL-4, IL-10, and IFN-γ production that exert partial protection against experimental *S. mansoni* infection.[13] Additionally, a fraction of *S. mansoni* soluble adult worm antigen (SWAP) known as PIII is able to induce protection against a challenge infection after immunization in a murine model of schistosomiasis.[14] Egg antigens are also able to induce a Th1 or Th2 immune response in murine models.[15] The most abundant proteins in the egg, the interleukin-4–inducing principle from *S. mansoni* eggs (IPSE), by inducing IL-4 production is associated with a shift to a Th2 immune response.[16,17]

In schistosomiasis-endemic areas, resistance to reinfection after specific chemotherapy has been correlated with a Th2 immune response, with the presence of specific IgE[18–20] and production of IL-4 and IL-5.[21] Conversely, susceptibility to infection has been correlated with high levels of specific IgG4.[22,23] An appropriate high IgE:IgG4 ratio against egg or adult worm antigens is associated with development of resistance to reinfection.[18,24] This type of resistance to reinfection has been strongly correlated with the immune response as opposed to physiologic or behavioral alterations observed at different ages or with the level of exposure to infested water.[25,26]

Correa-Oliveira and others reported a group of persons living in schistosomiasis-endemic areas who had multiple egg-negative stools despite exposure to infested water; this group was called endemic normal (EN).[27,28] The immune response of these persons differs from that of infected persons and those resistant to reinfection after specific chemotherapy. They produce higher levels of IgE against schistosomula tegument and IgG against paramyosin than infected persons.[29] Moreover, when stimulated with *Schistosoma* antigens, peripheral blood mononuclear cells (PBMC) of EN persons proliferate vigorously and produce higher levels of IFN-γ than those of persons currently infected and those resistant to reinfection.[28,30,31] On the basis of this evidence, it appears that Th1 and Th2 immune response are involved in protection against the infection in this naturally resistant group of persons.

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In this cross-sectional study, we used previously collected data from persons residing in the schistosomiasis-endemic area of Conde-BA to identify schistosomiasis-resistant persons. These persons had multiple egg-negative stools in all parasitologic surveys performed, despite exposure to infested water. We assessed epidemiologic data and collected blood samples for identifying possible risk factors for infection, and to evaluate the immune response of schistosomiasis-resistant persons living in an endemic area in Bahia, Brazil.

MATERIALS AND METHODS

Schistosomiasis-endemic area and selection of resistant and susceptible persons. This study was carried out in Conde, a city 200 km north of Salvador, the capital of Bahia, Brazil. It includes four small villages named Sempre Viva, Jenipapo, Camarões, and Buri, which have approximately 800 inhabitants. These inhabitants live in poor sanitary conditions, and fishing is the predominant occupation.

Cross-sectional parasitologic surveys were conducted in 2001 and 2004, and stool specimens were examined for *S. mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs. Also, the intensity of exposure to *S. mansoni* infection was assessed by a previously developed questionnaire, which provides four categories of level of exposure to infested water: no exposure, low exposure (< 1 hour/week), medium exposure (1–3 hours/week), or high exposure (1–3 hours/day).[32,33]

The Immunology Service of the Federal University of Bahia has been studying these villages irrespective of the prevalence of schistosomiasis since 2001. At that time, the prevalence of *S. mansoni* infection was approximately 90%.[33] A schistosomiasis mass treatment program was implemented and the population was followed-up until 2004, when another parasitologic survey was conducted. This second survey showed an overall schistosomiasis prevalence of 44%, which preceded the second treatment of this population,[32] following the World Health Organization recommendations for areas with high or moderate schistosomiasis prevalence.[34]

The subject selection strategy was performed in two steps (Figure 1). First, 2001 and 2004 databases were checked to select exposed persons from those 10–60 years of age whose previous parasitologic results were all negative for *Schistosoma*. In the second part of the selection procedure, which was conducted in 2009, pre-selected persons were asked to provide two stool samples and answer a questionnaire, which included information about exposure to fresh water. Each person who was still negative for schistosomiasis in 2009 and who had a history of exposure to the infection was invited to be included

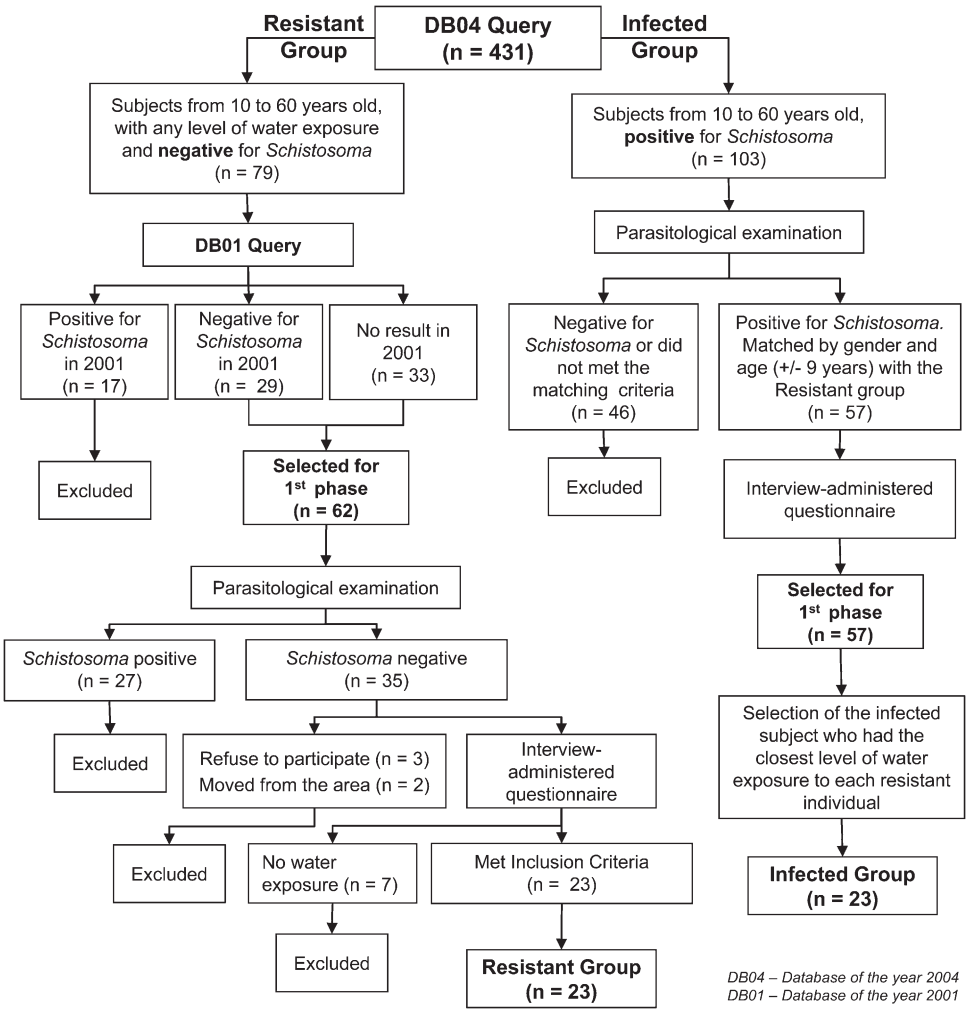

FIGURE 1. Flowchart of study participant selection strategy, Bahia, Brazil.

in a group of putative resistant persons. The infected group was composed of persons matched to the resistant persons by age (±9 years), sex, and level of exposure to infested water. To compose the infected group, the 2004 database was queried for persons from those 10–60 years of age who were positive for *S. mansoni*. These participants were asked to provide one stool sample. Four infected persons who met the matching criteria were then selected per resistant subject. All four potential controls per persons were asked to answer the same exposure questionnaire. Afterward, the infected person who had the closest level of water exposure to each resistant person was invited to participate in this study. An additional group of 10 persons with no helminth infections and who were living outside the schistosomiasis-endemic area was invited to be included in a healthy group to provide baseline information on cytokine and antibody levels.

Ethics. All adults provided written informed consent. Children gave written assent, and written consent for children's participation was obtained from a parent or guardian. The research protocol was approved by Institutional Review Boards at Weill Cornell Medical College and at the Federal University of Bahia.

Socioeconomic and water contact surveys. An interview-administered questionnaire was developed for the purpose of this study and administered to each participant. This questionnaire was divided into 3 sections: A) schistosomiasis-related questions, which included the water contact information and questions regarding the last schistosomiasis treatment; B) demographic and socioeconomic information, which included age, sex, housing conditions, sanitation, occupation, and income; and C) health-related questions, which included information about other related diseases, the CAGE questionnaire, and questions 1 and 7–9 of the Household Food Insecurity Access Scale. This scale is used to assess food insecurity information and was developed by the Food and Nutrition Technical Assistance project on the basis of validation studies.[35] The CAGE questionnaire is a four-item alcohol-screening instrument, an acronym indicating cut down on drinking; annoyed by complaints about drinking; guilty about drinking; had an eye-opener first thing in the morning.[36]

The water contact questionnaire obtained information about the reasons for and frequency of each contact. This questionnaire was modified on the basis of previous studies conducted in the same area and took into consideration the population habits. The reasons (R) and frequency (F) of contact were scored on the basis of previous work to obtain the Water Contact Index (WCI), a numeric coefficient that reflects the level of water exposure.[37] The reason for contact was scored from 2 to 5 as follows: 2 = crossing the streams; 3 = collecting water for the household and dish-washing; 4 = laundering and watering agricultural fields; and 5 = bathing, swimming, and playing in the streams and fishing. The frequency of contact was scored as follows: 1 = < 2 contacts/month; 2 = ≥ 2 contacts/month; 4 = ≥ 1 contact/week; and 28 = ≥ 1 contact/day. The WCI was obtained by the sum of each reason score multiplied by the respective frequency score according to the equation $WCI = \Sigma (R \times F)$.

Fecal examinations for parasites. To evaluate the intensity of *S. mansoni* infection and the presence or absence of *A. lumbricoides*, *T. trichiura*, and hookworm eggs, each person received one plastic container for fecal samples at two time points 2–30 days apart. Participants were instructed to deposit the stool sample and return the container immediately to the collection point, where the samples were stored at 4°C. Each stool sample had two slides prepared and tested by using the Kato-Katz method to estimate the number of *S. mansoni* eggs per gram of feces.[38]

Cell culture and laboratory investigations. Blood was collected by venipuncture. Serum was separated and stored at –20°C for evaluation of specific IgE and IgG4. The PBMCs were obtained for cell cultures and cytokine measurements.

The PBMCs were isolated from venous blood collected into heparin-treated tubes by Ficoll-Hypaque gradient sedimentation and adjusted to a concentration of 3×10^6 cells/mL in RPMI 1640 medium containing 10% normal human serum (AB positive and heat-inactivated), 100 U/mL of penicillin, 100 mg/mL of streptomycin, 2 mmol/L of L-glutamine, and 30 mmol/L of HEPES (all from Life Technologies GIBCO BRL, Gaithersburg, MS). We added 10 μg/mL of SWAP, SEA, Sm21.6, Sm22.6, PIII, IPSE, or phytohemagglutinin (PHA) to each well and incubated the plates at 37°C in an atmosphere containing 5% CO_2. Supernatants were removed after 72 hours of incubation and maintained at –20°C for measurement of cytokines by enzyme-linked immunosorbent assay. Levels of IFN-γ, IL-5, IL-13, IL-10, and IL-21 were determined by using Duoset (R&D Systems, Inc., Minneapolis, MN), and results were expressed as picograms per milliliter on the basis of a standard curve.

Recombinant proteins Sm21.6, Sm22.6, and IPSE were produced in *Escherichia coli* and tested for lipopolysaccharide (LPS) by using a commercially available chromogenic LAL end-point assay kit (Cambrex, Charles City, IA). Levels of LPS in Sm21.6, Sm22.6, and IPSE were less than 1.2 endotoxin units/mg of protein. To neutralize potential effects of the LPS present in low levels on the *S. mansoni* recombinant antigens, polymyxin B was added to cell cultures every 12 hours according to an established protocol.[39]

IgG4 specific for SWAP, SEA, IPSE, and Sm22.6 was measured by using an indirect enzyme-linked immunosorbent assay as described.[40,41] Briefly, microtiter plates (Immulon 2; Dynatech, Geneva, Switzerland) were coated overnight with the antigens at a concentration of 5 μg/mL for SEA, IPSE, and Sm22.6 and 10 μg/mL for SWAP in carbonate-bicarbonate buffer, pH 9.6. Plates were washed with phosphate-buffered-saline, 0.05% Tween 20 (Sigma, St. Louis, MO) and non-specific attachment sites were blocked with phosphate-buffered saline, 0.1% Tween 20 and incubated at 37°C for 2 hours. Samples were diluted 1:4 and distributed into the wells of the plates. Biotin-conjugated anti-human IgG4 and streptavidin horseradish peroxidase (BD Bioscience, San Jose, CA) were used to amplify the signal. Specific antibody responses were expressed as the mean optical density (OD). Conditions for the specific IgE measurement were the same as those described for IgG4 except for the plates used (Maxisorb; Nunc, Roskilde, Denmark), the specific antibodies, and the sample dilution (1:32). Given that the IgE-specific response is not always detected in *S. mansoni*-infected patients, total IgG was removed by using rheumatoid factor absorbent (Behring Diagnostics Inc., Westwood, MA).[41,42]

Data analysis. Statistical analysis was performed by using the STATA statistical package version 10.0 (Stata Corp., College Station, TX). GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) was used to construct graphs. Continuous variables, such as WCI, age, and immunologic

variables, were expressed as median and interquartile range (IQR) of observed values. A random effects logistic regression model was used to test for association between *S. mansoni* infection and covariates. Linear regression was used to compare cytokine and antibody levels between groups. All analyses took into account the matched design. Given that the water exposure was not well-matched within groups, statistical analyses were adjusted for WCI. The alpha level for statistical significance was established as 0.05 for all analyses.

For the random effects model, the villages of Sempre Viva and Jenipapo were analyzed as only one region (region A), and Buri and Camarões were analyzed as Region B. This procedure was conducted because the grouped villages share geographic proximity and similar population habits.

The CAGE questionnaire was composed of four questions, each of which yielded an answer in the form of binary responses as yes or no. Each yes answer accounted for one point and each no answer accounted for no points. The CAGE score was the resulting total point and varied from 0 to 4. For this study, the CAGE score was grouped in two categories: 1) score 0 or 1, which has no clinical significance; and 2) score ≥ 2, which indicates alcoholism or problem drinking.[43,44]

RESULTS

Study compliance and baseline characteristics. A total of 431 persons were evaluated for resistance to *Schistosoma* infection. The strategy to identify possible resistant persons living in Conde-BA was used initially with 62 persons who were exposed to but negative for *Schistosoma* in 2004, of whom 29 (46.8%) were also *Schistosoma* negative in 2001; 33 (53.2%) had no parasitologic results in 2001. In the parasitologic survey that was performed for this specific study, we found that 27 (43.5%) of 62 persons were currently *Schistosoma* positive, and 35 (56.5%) were still negative for *S. mansoni* infection. The questionnaire was then administered to 30 of these putative resistant persons; 2 of them had moved from the area and 3 did not consent to participate. Of these 30 persons, 7 were no longer exposed to the infection and were not included in the study. Therefore, the estimated prevalence of putative resistant persons living in the area was 5.3% (23 of 431 screened persons; 95% confidence interval = 3.4–7.9%). Age and sex did not differ between included and excluded persons.

Approximately 50% of the resistant persons had at least one stool sample analyzed in 2001 or 2004. They had ≥ 6 stool samples analyzed since the first survey and most (65%) provided 5 or 6 stool samples in all parasitologic surveys. In 2004, 57% of resistant persons had an intermediate (1–3 hours/week) or high (1–3 hours/day) level of exposure to infested water. Only 2 (9%) of the resistant persons were not treated in 2004 during the schistosomiasis mass treatment.

TABLE 1
Baseline characteristics of study participants, Bahia, Brazil

Characteristics	Resistant (n = 23)	Infected (n = 23)
Age, median (IQR)	24 (26)	24 (24)
Male sex (%)	26.1	26.1
Water Contact Index, median (IQR)*	48 (61)	86 (188)
No. stool samples provided, median (IQR)*	5 (2)	3 (1)
Schistosoma mansoni parasite load (EGF), median (IQR)	0	48 (96)

*$P < 0.05$, by linear regression.
IQR = interquartile range; EGF = eggs per gram of feces.

The infected group was composed of 23 persons of similar ages and sex (Table 1). However, exposure to infection estimated by the WCI was slightly higher in the infected group than in the resistant group ($P = 0.025$). Despite this significant difference in the WCI, 12 of 23 pairs were well matched regarding the WCI, with a difference in WCI between matched pairs $\leq 15\%$. We also included a group of healthy persons who lived outside the schistosomiasis-endemic area. The median age of these persons (26.5 years) was similar to what was observed in the resistant and infected groups. The healthy control group had a higher proportion of men (60%).

Factors associated with *S. mansoni* infection. As shown in Table 2, none of the demographic or other covariates assessed by the questionnaire were statistically associated with infection status in this population. Although persons from region B (75%) appeared more likely to be infected than persons living in region A (41.2%), this association was not statistically significant by matched analysis. Moreover, all participants reported an average income $\leq$ \$250.00 per month, which could be explained by the fact that most of them were students or unemployed. Nine persons were fisherman, mostly (88.9%) from the infected group. However when we adjusted for WCI in the random effects model, we found that there was no statistically significant association between this occupation and infection status.

The prevalence of other helminth infections among persons in the resistant and infected groups is summarized in Table 3. The frequency of *A. lumbricoides* and/or *T. trichiura* infection was significantly lower in resistant persons (47.6% and 52.4%, respectively) than in infected persons (73.9%; $P = 0.047$ and 95.6%; $P = 0.008$, respectively). This trend was not observed for hookworm infection, which was slightly less frequent in the resistant group than in the infected group (28.6% and 43.5%, respectively). However, this small difference was not statistically significant. When the presence of any helminth infection (*A. lumbricoides*, *T. trichiura*, or hookworm) was tested for an association with *S. mansoni* infection, we found that the *Schistosoma*-resistant persons were less likely to have any other helminth infection than persons from the *Schistosoma*-infected group (66.7% and 95.6%, respectively; $P = 0.046$).

Cytokine profile and humoral immune response. Levels of IL-5, IL-13, IL-10, IL-21, and IFN-γ measured in supernatant of PBMCs cultures unstimulated or stimulated with SWAP, SEA, Sm21.6, Sm22.6, PIII, IPSE, or PHA are shown in Figure 2. Levels of IL-5 and IL-13 were similar in both resistant and infected groups when cells were stimulated with all tested antigens, except for SEA. After stimulation with SEA, cells of resistant subjects produced significantly higher levels of IL-5 and IL-13 (median = 1,342 [IQR = 1,832] and median = 94 [IQR = 132] pg/mL) than cells of infected persons (median = 266 [IQR = 556], $P = 0.007$; and median = 94 [IQR = 0] pg/mL, $P = 0.022$). We also demonstrated that the recombinant antigens Sm21.6, Sm22.6, and IPSE were unable to stimulate the production of IL-5 or IL-13 because their levels were similar to those found in unstimulated cultures in both groups tested.

After antigen stimulation, levels of IL-10 increased in cultures of cells of all tested persons independent of the specific antigen or infection status. However, when compared with

TABLE 2

Random-effects model adjusted by Water Contact Index for the association between *Schistosoma mansoni* infection and covariates, Bahia, Brazil

Characteristic	Resistant, no. (%) (n = 23)	Infected, no. (%) (n = 23)	OR (95% CI)	*P*
Place of residence				
Region A	20 (87.0)	14 (60.9)	1.00	
Region B	3 (13.0)	9 (39.1)	4.45 (0.42–47.53)	0.217
Sanitation at home				
None	5 (21.7)	10 (43.5)	1.00	
Outhouse	18 (78.3)	13 (56.5)	0.40 (0.11–1.50)	0.175
Frequency of piped water				
Not daily	2 (8.7)	7 (30.4)	1.00	
Daily	21 (91.3)	16 (69.6)	0.30 (0.05–2.00)	0.214
Water intake treatment				
None	9 (39.1)	10 (43.5)	1.00	
Any	14 (60.9)	13 (56.5)	0.96 (0.28–3.24)	0.946
Education				
None	2 (8.7)	4 (17.4)	1.00	
Some	21 (91.3)	19 (82.6)	0.63 (0.09–4.26)	0.635
No. of persons per room				
1	5 (21.7)	2 (8.7)	1.00	
2	13 (56.5)	13 (56.5)	2.45 (0.37–16.20)	0.354
3	3 (13.1)	5 (21.7)	4.30 (0.45–41.00)	0.205
≥ 4	2 (8.7)	3 (13.1)	4.06 (0.34–49.20)	0.271
Occupation				
Student/none	17 (73.9)	15 (65.2)	1.00	
Fisherman	1 (4.4)	8 (34.8)	6.91 (0.73–65.65)	0.092
Agriculture	3 (13.0)	0	–	0.998
All others	2 (8.7)	0	–	0.999
Drinks alcoholic beverages				
No	19 (86.4)	16 (69.6)	1.00	
Yes	3 (13.6)	7 (30.4)	2.86 (0.62–13.20)	0.178
CAGE score				
0 or 1	21 (95.4)	20 (87.0)	1.00	
≥ 2	1 (4.6)	3 (13.0)	3.28 (0.31–34.71)	0.323
Past smoker				
No	18 (81.8)	18 (78.3)	1.00	
Yes	4 (18.2)	5 (21.7)	0.98 (0.21–4.64)	0.982
Food insecurity*				
No	20 (90.9)	22 (95.6)	1.00	
Yes	2 (9.1)	1 (4.4)	0.55 (0.05–6.65)	0.638

*Question no. 9 of the Household Food Insecurity Access Scale (Did you or any household member go a whole day and night without eating anything because there was not enough food?).
OR = odds ratio; CI = confidence interval; CAGE = Cut down, annoyed, guilty, eye opener.

levels of IL-10 produced by cells of infected or resistant persons, we found that IL-10 was significantly higher in Sm21.6- or Sm22.6-stimulated culture of cells from resistant persons (median = 2,126 [IQR = 405] and median = 2,103 [IQR = 412] pg/mL) than in cells from infected persons (median = 1,514 [IQR = 875] pg/mL, $P = 0.002$, and median = 1,490 [IQR = 940] pg/mL, $P = 0.001$). Conversely, levels of IL-10 after stimulation with PIII were lower in the resistant group than in the infected group (median = 593 [IQR = 476] versus median = 886 [IQR = 488] pg/mL, $P = 0.017$). Another cytokine with regulatory properties that was tested (IL-21) was produced in low levels by cells of persons from the schistosomiasis-endemic area.

Levels of IFN-γ were similar in both resistant and infected groups when cells were stimulated with the different antigens, except for SEA. After stimulation with SEA, the concentration of IFN-γ was higher in cultures of cells from resistant

TABLE 3

Random-effects model adjusted by Water Contact Index for the association between *Schistosoma mansoni* infection and other helminth infections, Bahia, Brazil

Organism	Resistant,* no. (%) (n = 21)	Infected, no. (%) (n = 23)	OR (95% CI)	*P*
Ascaris lumbricoides				
Negative	11 (52.4)	6 (26.1)	1.00	
Positive	10 (47.6)	17 (73.9)	4.03 (1.02–15.99)	0.047
Trichuris trichiura				
Negative	10 (47.6)	1 (4.4)	1.00	
Positive	11 (52.4)	22 (95.6)	19.95 (2.16–184.46)	0.008
Hookworm				
Negative	15 (71.4)	13 (56.5)	1.00	
Positive	6 (28.6)	10 (43.5)	1.96 (0.55–7.08)	0.302
Any helminth				
Negative	7 (33.3)	1 (4.4)	1.00	
Positive	14 (66.7)	22 (95.6)	9.57 (1.04–87.85)	0.046

*Data were not available for two resistant persons.
OR = odds ratio; CI = confidence interval.

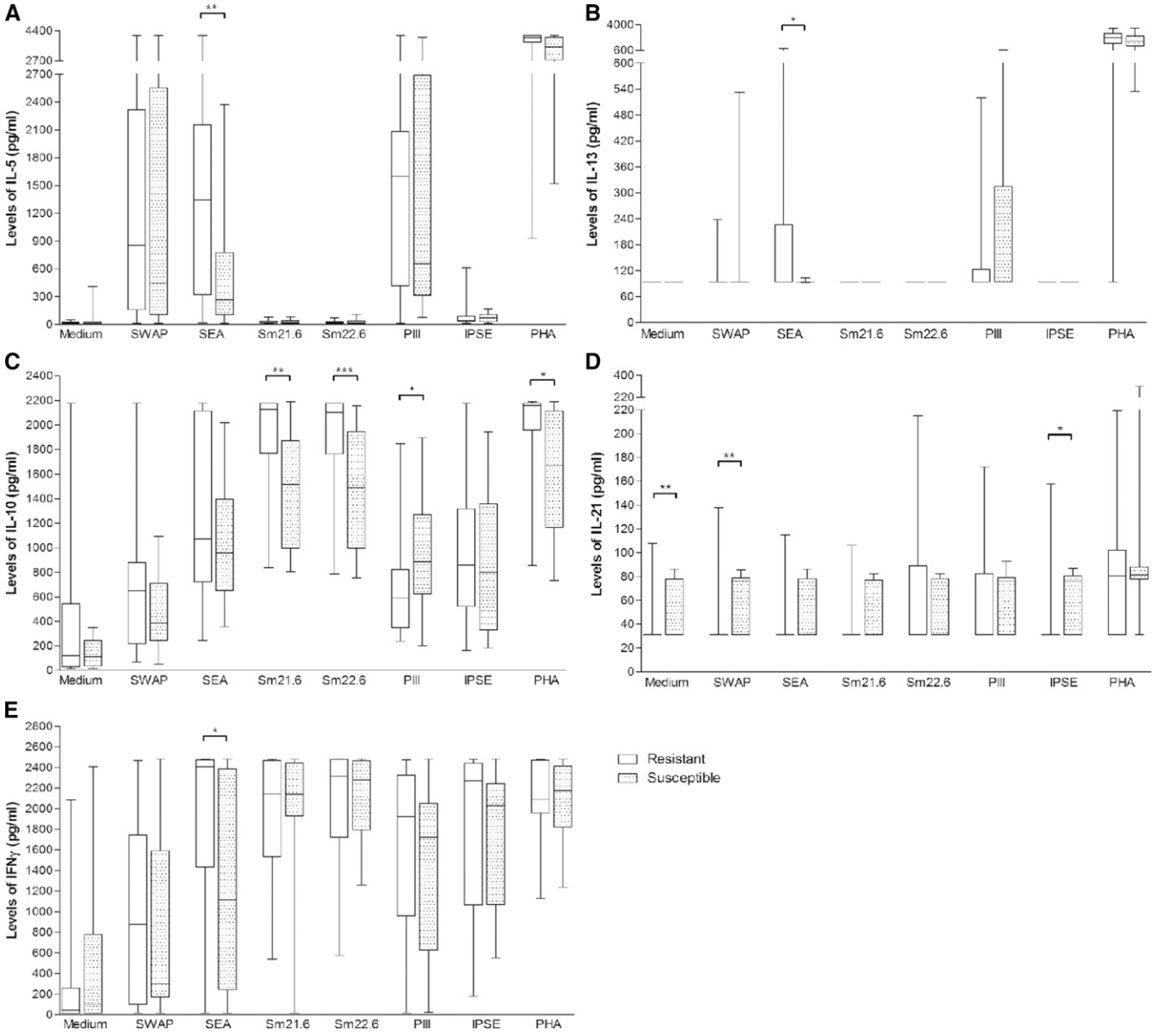

FIGURE 2. Levels of **A**, interleukin-5 (IL-5); **B**, IL-13; **C**, IL-10; **D**, IL-21; and **E**, interferon-γ (IFN-γ) produced by peripheral blood mononuclear cells of schisosomiasis-resistant and -infected persons, Bahia, Brazil. Minimum and maximum values, and 25th, 50th, and 75th percentiles are indicated by horizontal lines. Statistical difference in levels of cytokines between infected and resistant persons are indicated by asterisks (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

persons (median = 2,407 [IQR = 715] pg/mL) than in cells from infected persons (median = 1,117 [IQR = 2,128] pg/mL, $P = 0.034$).

Regarding the cytokine response in the health control group, we found that levels of IFN-γ, IL-5, IL-13, and IL-21 were below the detection limits and were detected in response to PHA (median = 1,461 [IQR = 1,135], 3,274 [814], 978 [1,749], and 31 [50] pg/mL, respectively). However, IL-10 was produced in response to *S. mansoni* antigens Sm21.6, Sm22.6, IPSE and to PHA (median = 1,140 [IQR = 485], 1,481 [849], 1,193 [835], and 1,792 [455] pg/mL, respectively).

Levels of specific IgE and IgG4 against SWAP, Sm22.6, SEA and IPSE are shown in Figure 3. IgE against SWAP and IPSE were lower in the resistant group (median OD units = 0.64 [IQR = 0.25] and median OD units = 1.33 [IQR = 1.36]) than in the infected group (median OD units = 0.99 [IQR = 0.50], $P = 0.013$; and median OD units = 1.82 [IQR = 0.66], $P = 0.048$). Conversely, there was no significant difference in the levels of IgE against Sm22.6 and SEA between groups. As expected, levels of specific IgE in both groups were higher than the median levels of healthy persons, except for IgE against Sm22.6, in which approximately half of the persons from the schistosomiasis-endemic area had lower or similar levels compared with healthy persons.

Regarding specific IgG4 levels, we demonstrated that infected persons had higher levels of IgG4 against SWAP (median 3.17 OD units [IQR = 2.11]), Sm22.6 (median = 0.06 OD units [IQR = 0.13]), and SEA (median = 3.09 OD units [IQR = 0.53]) than resistant persons (median = 0.89 OD units [IQR = 2.10], $P = 0.013$; median = 0.04 OD units [IQR = 0.11],

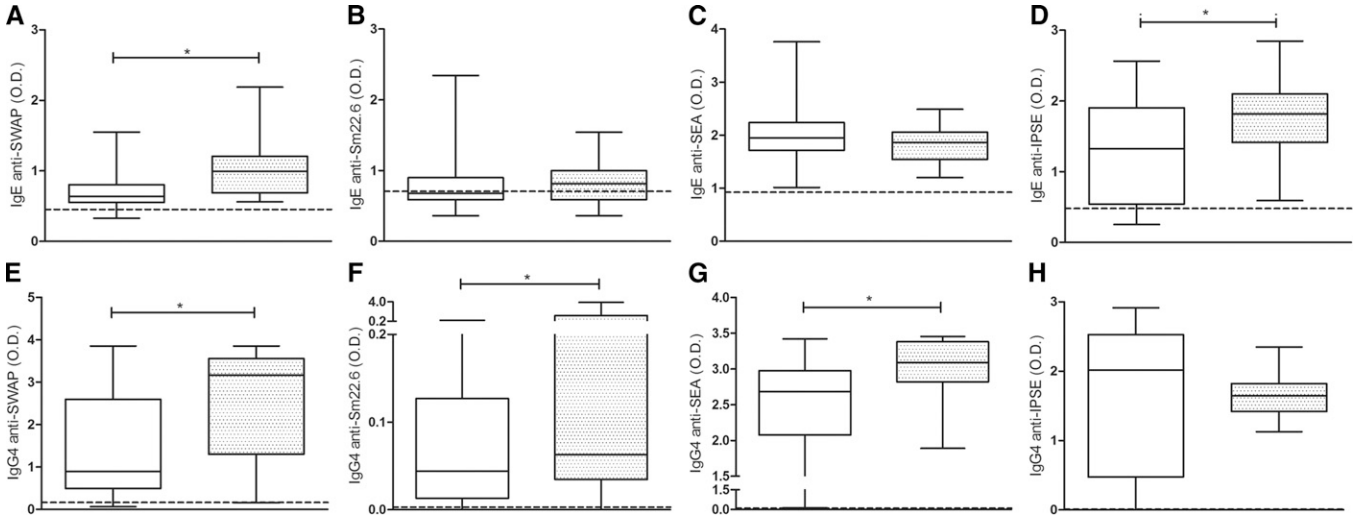

FIGURE 3. Levels of specific IgE (**A–D**) and IgG4 (**E–H**) in the study groups, Bahia, Brazil. Minimum and maximum values, and 25th, 50th, and 75th percentiles are indicated by horizontal lines. Discontinuous horizontal lines indicate median antibody levels of healthy persons. O.D. = optical density. Statistical differences between resistant (□) and infected (▨) persons are highlighted by asterisks (*$P < 0.05$).

$P = 0.047$; and median = 2.68 OD units [IQR = 0.90], $P = 0.044$), and levels of IgG4 against IPSE were similar in both groups.

DISCUSSION

The present study describes factors associated with resistance to *S. mansoni* infection in an endemic area of Bahia, Brazil. None of the environmental aspects evaluated, such as place of residence, sanitation at home, occupation, income, and education, was associated with resistance to infection. However, we observed a significantly higher Th1 and Th2 immune response when PBMCs of resistant persons were stimulated with *S. mansoni* antigens such as SEA. Unexpectedly, we found that IgE against SWAP and IPSE was lower in the resistant group than in the infected group. Conversely, levels of IgG4 against SWAP, Sm22.6, and SEA were higher in susceptible persons.

The risk of acquiring schistosomiasis has been directly associated with exposure to infested water, and direct observation and survey administration are the most efficient methods for estimating the level of exposure.[45] The WCI was obtained for all participants in this study by an interviewer-administered questionnaire, which was modified on the basis of observation of the main activities of the population in previous studies.[32,33] Although in the study design resistant persons were matched to infected persons by age, sex, and WCI, it was not always possible to match closely by WCI. However, ≥ 12 pairs of persons were well matched and showed a WCI difference $\leq 15\%$, which was reflected in similar WCI ranges observed in both groups. Furthermore, to reduce the potential effect of confounding by differences in exposure to water, all analyses performed were adjusted for WCI.

Our results showed no association between infection and housing parameters, such as number of persons per room or sanitation, in contrast to what has been reported by other authors.[46–49] Differences in study design may explain these discrepant findings because in this study we selected persons who were persistently negative for schistosomiasis over time and compared them with matched infected controls. Most other studies have considered resistant persons to be those who had negative parasite examination results in a small number of surveys over a short period of follow-up.

We found no association between resistance to infection and education status or income. This finding is consistent with other studies that have demonstrated that income was not an independent factor associated with infection, which suggests that improving income alone may not reduce the rate of infection.[50,51] Other studies have shown an inverse correlation between education or income with presence of infection,[46,47,49,52] but our ability to detect such an association was limited because levels of education and income were relatively homogeneous in our overall population.

Consistent with our data, studies of host genetics have demonstrated a low contribution from environmental factors to the variance of parasite burden.[53] Moreover, $\leq 66\%$ of the variation in *S. mansoni* worm burden is caused by a few host genes.[54] Therefore, our findings of stronger associations between resistance to infection and the immune response against *Schistosoma* antigens rather than environmental factors are not unexpected.

Interestingly, the *S. mansoni*-infected group had a higher prevalence of other helminth infections, which could not be explained by their behavior or socioeconomic status. These results are consistent with what was found in another study,[51] which suggested that resistance to *Schistosoma* infection could be conferring cross-resistance against other helminth infections, or that there is a genetic factor associated with resistance to helminth infections in general. However, we cannot rule out that the lack of association between epidemiologic data and resistance to *Schistosoma* infection could be caused by the small number of resistant persons found in the schistosomiasis-endemic area or by geographic factors.

Others factors related to resistance to reinfection in schistosomiasis-endemic areas are age and prior treatment for schistosomiasis. It is hypothesized that drug-dependent killing of worms *in situ* might induce protective immunity against reinfection by immunizing with newly exposed tegument antigens released from dying worms.[55,56] The mean life span of

S. mansoni worms is estimated to be 6–10 years.[57] Thus, exposure to antigens from naturally dying worms in untreated populations could explain the age-dependent resistance to reinfection. Age was unlikely to be a confounder in this study because the age of the resistant persons ranged from 10 to 60 years, and infected persons were matched by age. Some of the tegument antigens that are released during parasite death, such as Sm21.6 and Sm22.6, were tested in this study for a correlation with resistance to infection. Additionally, SEA and IPSE that are obtained from the *Schistosoma* eggs were also tested.

The PBMCs of resistant persons produced higher levels of IL-5, IL-13, and IFN-γ after stimulation with SEA than those of infected persons, as has been found in naturally resistant persons.[27,28] Although the resistant persons studied had no prior history of schistosomiasis and were negative by multiple screenings, 21 of 23 persons previously received schistosomiasis treatment. This treatment was given in a mass schistosomiasis treatment program conducted because of the high prevalence of infection detected in our previous surveys. Thus, it is plausible that some of these *Schistosoma*-negative persons had a low intensity infection by the time they were treated with praziquantel. Another possible explanation of this intense immune response detected in the resistant persons compared with infected persons would be the absence of immune suppression that is commonly observed in patients chronically infected with helminths.[33,58] However, it seems not to be the case in this study because cells from resistant persons were able to produce similar or higher levels of the regulatory cytokine IL-10 than cells from infected individuals.

A positive correlation has been described between resistance to reinfection and a high IgE:IgG4 ratio, suggesting that resistant persons have higher levels of IgE and lower levels of IgG4 than infected persons.[18–20,41,59] Although levels of specific IgE have been correlated with resistance to infection in putatively resistant persons,[29] in the present study, levels of IgG4 were more likely to be correlated with susceptibility to infection than IgE with resistance to infection, given that levels of specific IgG4 were higher in the infected group and levels of specific IgE were similar or lower in the resistant group. Because this was not a prospective study, we cannot rule out the possibility of IgG4 levels were higher in infected persons exclusively because of their infection status, as suggested in epidemiologic studies.[20,23] However, it is reasonable to believe that the presence of specific IgG4 might block a protective role of IgE in infected persons, which causes them to be more susceptible to infection than persons with lower levels of IgG4.

Overall, our data suggest that resistance to *Schistosoma* infection in the studied area is associated with a mixture of Th1 and Th2 cytokine responses and low levels of IgG4 against *S. mansoni* tegument and egg antigens. This finding is consistent with available data, which gives support to the idea that the antigens tested for a schistosomiasis vaccine should be able to induce Th1 and Th2 responses and some level of regulatory molecules to reduce morbidity.

Received April 6, 2011. Accepted for publication November 1, 2011.

Acknowledgments: We thank the persons from schistosomiasis-endemic areas in Conde (Buri, Camarao, Jenipapo and Sempre Viva) for participating in the study; the health agents (Adaliudes Conceição, Luciana Quintela, Ivanice Santos, and Analú Lima) for collaborating in the field work; and Dr. Audrey V. Grant, Dr. Mary E. Charlson, and Dr. Carol A. Mancuso for support.

Financial support: This study was supported by the Brazilian National Research Council (CNPq) Universal (482064/2010-2). Ricardo R. Oliveira and Marshall J. Glesby were supported in part by the Fogarty/Ellison International Clinical Research Scholars Program and National Institutes of Health/National Institutes of Allergy and Infectious Diseases grant K24Al078884, respectively. Kathleen C. Barnes was supported in part by the Mary Beryl Patch Turnbull Scholar Program. Maria Ilma Araújo and Edgar M. Carvalho are investigators supported by CNPq.

Disclaimer: The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

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