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Brief Definitive Report

Adult worm-specific IgE/IgG4 balance is associated with low infection levels of *Schistosoma mansoni* in an endemic area

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SUMMARY

Field studies have suggested an immune-mediated mechanism associated with resistance to Schistosoma mansoni *infection. Overall, levels of specific IgE have been correlated with resistance to infection, whereas levels of IgG4 have been associated with susceptibility. This study aimed to evaluate serum levels of soluble adult worm antigen preparation (SWAP)-specific IgE and IgG4 in relation to current infection in a large casuistic of individuals living in an endemic area of schistosomiasis in Bahia, Brazil. The prevalence of* S. mansoni *infection was 37·7% and the mean parasite burden was 55·4 (0–2100) epg/faeces. There was no significant difference in the levels of SWAP-specific IgE in individuals with different parasite burden, whereas high producers of parasite-specific IgG4 presented higher parasite burden when compared to low IgG4 producers. Additionally,* S. mansoni *parasite load was positively correlated with the levels of specific IgG4 or total IgE. No significant correlation was observed between parasite burden and SWAP-specific IgE. Nevertheless, SWAP-specific IgE/IgG4 ratio was higher in uninfected or lightly infected individuals (1–99 epg/faeces) than in heavily infected ones (≥400 epg/feces). These findings highlight the important role of IgE/IgG4 ratio in the resistance to infection, which could be useful for further studies in schistosomiasis vaccine candidates.*

Keywords *helminth, IgE, IgG4,* Schistosoma mansoni

INTRODUCTION

Schistosomiasis remains a major public health concern, and according to recent estimates, approximately 200 million individuals are infected, with a further 700 million people living at risk of infection (1). Several factors have been related to susceptibility to infection, such as genetic predisposition, host immune response and environmental or behavioural factors (2,3). Control strategies focusing on mass chemotherapy with praziquantel (PZQ) have significantly reduced severe pathology. However, post-treatment reinfection and accompanying morbidities such as anaemia, malnutrition and cognitive impairment persist despite subsequent rounds of treatment (4). Although repeated drug administration is necessary, chemotherapy alone does not seem to be sufficient for long-term control (5). A vaccine that confers sustainable immunity against the infection might be the best alternative for controlling schistosomiasis.

Although human immune responses to *Schistosoma mansoni* have been extensively studied, mechanisms by which human hosts resist to schistosome infection are not well understood. Results from longitudinal field studies suggest that resistance to reinfection can be naturally developed (6) or induced after multiple rounds of exposure, treatment and reinfection (7). These observations have led to the conception of an age-dependent model of resistance, which has been the main accepted model over

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the last several decades (8). This theory states that children under 15 years of age are highly susceptible to post-treatment reinfection, with a gradual increase in resistance over time (8). Nevertheless, an important study suggested that resistance to reinfection may not be strictly age-dependent, but rather exposure-dependent (9). It is worth noting, however, that usually age and exposure are inversely and closely related.

Resistance to reinfection has been associated with a Th2 immunity and consequent high level of IgE, as well as recruitment and activation of eosinophils and mast cells (2,10,11). Some authors have reported correlations between levels of anti-schistosome IgE with protection and IgG4 with susceptibility against reinfection. High levels of IgE against soluble adult worm antigen preparation (SWAP) have been associated with resistance to reinfection (12–14), whereas high levels of IgG4 against adult worm and egg antigens have been associated with susceptibility to reinfection (10,15). The balance between levels of IgE and IgG4 tends towards the production of IgE in a Th2 milieu with low levels of IFN-γ and IL-10 (16). On the other hand, an environment rich in IL-10, as seen in *S. mansoni* infection, is associated with high levels of IgG4 (17).

Evidences suggest that the balance between IgE and IgG4 might determine resistance or susceptibility to *S. mansoni* infection (15,18,19) and that IgG4 may attenuate the protective effect of IgE (10). However, most of the previous studies evaluating resistance to *S. mansoni* infection have been developed in small cohorts. Therefore, we wondered whether anti-SWAP IgE and IgG4 production would be associated with *S. mansoni* parasite burden in an endemic area of Bahia, Brazil. To address this question, we conducted a large casuistic study, measuring levels of total IgE and SWAP-specific IgE and IgG4, and correlated levels of these isotypes with parasite burden. Moreover, we assessed the effect of age and gender on infection status and antibody production.

MATERIALS AND METHODS

Study population

This study was conducted in Conde, a city located 200 km north of Salvador, the capital of Bahia, Brazil. It includes four small villages (Sempre Viva, Jenipapo, Camarões and Buri), with approximately 800 inhabitants. They live in poor sanitary conditions, and fishing is the predominant occupation.

Pregnant women and individuals using immunosuppressive drugs or with evidence of hepatomegaly, identified during the clinical evaluation, were not included in this study. All adults provided written informed consent and consent for children's participation was obtained from a parent or guardian. The research protocol was approved by the Ethical Committee of Maternidade Climério de Oliveira at the Federal University of Bahia. All study participants received a mass treatment for schistosomiasis 3 years before and were treated with PZQ in a split dose of 50 mg/kg of body weight after the diagnosis of schistosomiasis in this study.

Water contact surveys

An interview-administered questionnaire, which was previously developed, was administered to each participant (3). This environmental questionnaire was modified based on the previous studies conducted in the same area, taking local water usage habits into consideration (20). For each of the seven activities observed (farming, bathing, washing clothes, washing hair, washing dishes, fishing and playing), each subject was asked to provide a local name for the water source along with the frequency and duration of each activity. Water source names were compiled from all questionnaires and reduced to a nonredundant list, and snails sampled at these sites were tested for cercariae. For water sources positive for cercariae, subjects were classified across all activities into four categories to reflect the level of exposure: no exposure, low exposure (1 h/week), medium exposure (1–3 h/week), or high exposure (1–3 h/day) (3,20).

Parasite exams

Stool samples from a total of 707 subjects were tested by using the Kato-Katz method to estimate the number of *S. mansoni* eggs per gram of faecal matter from three subsamples taken from two independent samples per individual, and the arithmetic mean was calculated for each subject. Parasite burden was expressed as average eggs per gram (epg) of faeces. The presence of *Ascaris lumbricoides, Trichuris trichiura* and hookworm eggs was also determined.

Antibody determination

Blood was collected through venipuncture using Vacutainer serum separation tubes (Becton Dickinson, Franklin Lanes, NJ, USA). Serum was obtained by centrifugation and stored at −20°C in single-use aliquots. Total serum IgE level was measured using Chemiluminescence (ADVIA Centaur; Bayer Corporation, Tarrytown, NY, USA) in Salvador, Brazil. Specific IgE antibodies against *S. mansoni* were investigated by indirect ELISA performed on

polystyrene microtiter plates coated with 10 μg/mL of SWAP, as previously described (21). Given that IgE-specific response is not always detected in *S. mansoni*-infected patients, IgG was removed by using RF absorbent (Behring Diagnostics, Marburg, Germany) (21). Immunoglobulin G4 against SWAP was measured in serum using a previously described ELISA method (12,22). Specific antibody response was expressed as optical density (OD), which was recorded into a data file in DeltaSOFT II (BioMetallics Inc, Princeton Junction, NJ, USA) microplate analysis software.

Statistical analysis

Data were entered into STATA statistical package version 11.0 (Stata Corporation, College Station, TX, USA) for statistical analysis, and GRAPHPAD Prism version 5.0 (GraphPad Software, San Diego, CA, USA) was used to build graphs.

The parasite load and immunological variables were expressed as the geometric mean and 95% of confidence interval of observed values, whereas others continuous variables were expressed as arithmetic mean and standard deviation (SD). The variable age was also categorized into seven clusters: 0–9, 10–19, 20–29, 30–39, 40–49, 50–59 and ≥60 years. *Schistosoma mansoni* parasite load was classified into light (1–99 epg/faeces), moderate (100–399 epg/faeces) or heavy intensity of infection (≥400 epg/faeces) (23). For statistical analysis, egg counts per gram of faeces were log-transformed ($\log_{10}$[epg + 1]) to approach the Normal distribution. Cut-off points for specific IgE and IgG4 were obtained by adding two SDs to the mean absorbance of results from 10 healthy individuals (IgG4 cut-off = 0·11 OD; IgE cut-off = 0·14 OD).

The d'Agostino-Pearson omnibus normality test was used to designate the most appropriate statistical test for each evaluation. The Mann–Whitney test was used to compare parasite load between male and female, whereas the Student's *t* test was used to compare IgE levels according to infection status. A nonparametric ANOVA (Kruskal–Wallis test) was used to compare continuous variables between three and more groups. The chi-square test was used to compare proportion of infected and uninfected subjects regarding gender and groups of age. The difference in parasite load between low and high IgG4 producers was assessed by logistic regression, whereas partial correlation coefficients were obtained for the association between the explanatory variable parasite load and levels of antibodies. Both logistic regression and correlation were adjusted for the categorical variables gender, age (0–9, 10–19, 20–29, 30–39, 40–49, 50–59 and ≥60 years) and level of exposure (no exposure, low, medium or high exposure). The alpha level for statistical significance was established as 0·05 for all analyses.

RESULTS

Baseline characteristics

A total of 707 individuals were included in the study. The mean age of the population was 28 ± 19 years, ranging from 6 to 85 years of age, and slightly more than half of studied individuals were female (56·6%). A high proportion of subjects had no exposure to infested water in the region (45·2%). Among those who reported water contact, 32·0% were exposed up to 1 h per week (low exposure), 26·8% were exposed to 1–3 h/week (medium exposure) and 41·2% were exposed to infested water daily (high exposure; Table 1).

The overall prevalence of *S. mansoni* infection was 37·7%, and the parasite load was 55·4 (46·8–65·7) epg/faeces, ranging from 0 to 2100 epg/faeces. Among

Table 1 Baseline characteristics of the population (n = 707)

Variables	Values
Age in years	
Mean (SD)	28 ± 19
Median (minimum-maximum)	23 (6–85)
Gender [*n* (%)]	
Male	307 (43·4)
Female	400 (56·6)
Water contact [*n* (%)]	
No reported exposure	296 (45·2)
Reported exposure	359 (54·8)
Low exposure (1 h/week)	115 (32·0)
Medium exposure (1–3 h/week)	96 (26·8)
High exposure (1–3 h/day)	148 (41·2)
Helminth infections [*n* (%)]	
Schistosoma mansoni	197 (37·7)
Epg/faeces (mean [95% CI])	55·4 [46·8–65·7]
Trichuris trichiura	347 (66·3)
Ascaris lumbricoides	280 (53·5)
Hookworm	150 (28·7)
S. mansoni parasite load categories [*n* (%)]	
Light-intensity infection (1–99 epg/faeces)	142 (72·1)
Moderate-intensity infection (100–399 epg/faeces)	42 (21·3)
Heavy-intensity infection (≥400 epg/faeces)	13 (6·6)
Coinfection[a] [*n* (%)]	172 (87·3)

[a]Coinfection defined as infection with *S. mansoni* plus one or more helminths.

those who were infected, 72·1% had a parasite burden up to 99 epg/faeces, 21·3% had moderate intensity of infection (100–399 epg/faeces), and only 6·6% were heavily infected, with a parasite load ≥400 epg/faeces (Table 1). The prevalence and intensity of *S. mansoni* infection was significantly higher in males (45·4% and 64·5 [50·3–82·7] epg/faeces, respectively) than females (31·6% and 46·8 [37·3–58·7] epg/faeces, respectively; $P < 0{\cdot}001$). When organizing individuals into age groups with intervals of 10 years, *S. mansoni* parasite load was 55·1 [38·7–78·4] epg/faeces in individuals up to 9 years old, 60·6 [44·9–81·9] epg/faeces in 10–19 years, 73·8 [45·3–120·3] epg/faeces in 20–29 years, 46·4 [27·9–77·3] epg/faeces in 30–39 years, 52·5 [32·3–85·5] epg/faeces in 40–49 years, 48·4 [25·0–93·7] epg/faeces in 50–59 years and 16·2 [7·3–35·7] epg/faeces in subjects over 59 years old ($P < 0{\cdot}001$). Besides the increased prevalence of infection and high parasite load, a great proportion (62·9%) of young individuals (0–9 years) was exposed to the infested water, whereas 55·6% of 50–59 years old individuals and only 23·8% of elderly individuals (≥60 years) were exposed. Approximately 87% of *S. mansoni*-infected patients were coinfected with one or more helminths, while among individuals negative for *S. mansoni* one or more helminths was found in 66%. The most common helminth found in the region was *T. trichiura* (66·3%), followed by *A. lumbricoides* (53·5%) and hookworm infection (28·7%; Table 1).

Total IgE and SWAP-specific IgE and IgG4 in infected subjects

The mean level of total IgE in the whole population was 1000 (914–1094) U/L, ranging from 1 to 9673 U/L. As expected, total IgE level was higher among those currently infected with *S. mansoni* (1335 [1151–1548] U/L) as compared to *S. mansoni* uninfected subjects (790 [686–909] U/L; $P < 0{\cdot}001$). Higher IgE levels were also observed in subjects infected with any helminth (1140 [1021–1272] U/L) compared to non-infected individuals (515 [397–667] U/L; $P < 0{\cdot}001$).

Independent of current *S. mansoni* infection status, SWAP-specific antibody isotypes were detected in a large proportion of individuals. Anti-SWAP IgG4 was positive in 91% of the tested samples, while 98% of the samples were positive for anti-SWAP IgE. The mean levels of SWAP-specific IgE and IgG4 in the overall population were 0·68 (0·64–0·72) and 0·67 (0·58–0·76) OD, respectively (Figure 1a). Regarding *S. mansoni* infection status, similar levels of SWAP-specific IgE were observed among infected (0·69 [0·61–0·78] OD) and uninfected individuals (0·68 [0·63–0·73] OD; $P > 0{\cdot}05$), whereas higher levels of SWAP-specific IgG4 was found in those infected (1·48 [1·22–1·79] OD) when compared to uninfected ones (0·38 [0·31–0·47] OD; $P < 0{\cdot}001$). Moreover, while levels of specific IgE were uniformly distributed around the mean, levels of IgG4 had a bimodal distribution, allocating subjects into phenotype of low (below 0·6 OD, $n = 241$) or high (above 3·0 OD, $n = 120$) IgG4 producer. When adjusted by age, gender and level of exposure to contaminated water, those subjects considered as high IgG4 producers presented a higher parasite burden (1·01 ± 0·98 $\log_{10}$epg) as compared to low IgG4 producers (0·27 ± 0·62 $\log_{10}$epg; $P < 0{\cdot}001$, Figure 1b).

Additionally, *S. mansoni* parasite load was positively correlated with levels of SWAP-specific IgG4 ($r = 0{\cdot}30$, $P < 0{\cdot}001$) or total IgE ($r = 0{\cdot}13$, $P < 0{\cdot}01$), whereas no significant correlation was observed between parasite

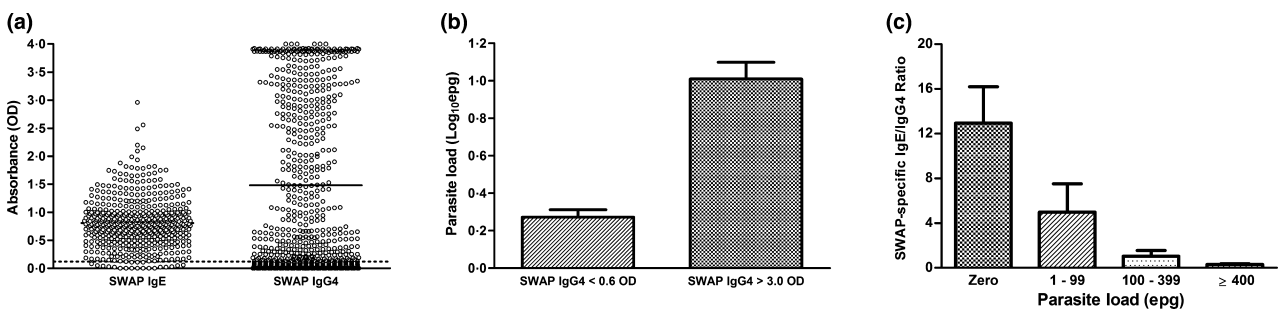

Figure 1 Soluble adult worm antigen preparation (SWAP)-specific IgE and IgG4 (a), parasite load according to categories of IgG4 production (b) and SWAP-specific IgE/IgG4 ratio according to intensity of infection (c) in subjects from an endemic area for schistosomiasis. (a) Each point represents data from one individual. Horizontal black lines indicate the mean, whereas discontinuous horizontal black lines represent the cut-off level for anti-SWAP IgE [0·14 optical density (OD)] and IgG4 (0·11 OD). (b) First bar shows *Schistosoma mansoni* parasite load of low anti-SWAP IgG4 producers; the second bar represents parasite load of high IgG4 producers. Horizontal black lines above each bar represent the standard error of the mean (SEM). Difference in parasite load between low and high IgG4-producers was statistically significant ($P < 0{\cdot}001$). (c) Horizontal black lines above each bar represent the standard error of the mean (SEM). Difference in SWAP-specific IgE/IgG4 ratio between groups of intensity of infection was statistically significant ($P < 0{\cdot}0001$).

burden and SWAP-specific IgE ($r = 0{\cdot}06$, $P > 0{\cdot}05$) when adjusted by age, gender and level of exposure. Despite this lack of association between specific IgE and intensity of infection, the SWAP-specific IgE/IgG4 ratio was higher in uninfected and light-intensity infected individuals (1–99 epg/faeces) compared to heavily infected ones (≥400 epg/faeces; $P < 0{\cdot}0001$; Figure 1c).

Levels of specific IgE were similar among individuals with different degrees of exposure to infested water (not shown). The levels of specific IgG4, however, were higher (1·88 [1·56–2·28] OD) in the highly exposed individuals when compared to the not exposed ones (0·41 [0·33–0·51] OD, $P < 0{\cdot}001$).

DISCUSSION

Prevalence of *S. mansoni* infection as well as parasite load is influenced by several factors within endemic communities, which includes exposure-related factors such as local environment and behaviour, and aspects associated with host susceptibility or resistance to infection, specifically intrinsic immune response and genetic predisposition (19). In the current study, we found that the levels of anti-adult worm IgG4 and IgE/IgG4 ratio is inversely associated with *S. mansoni* parasite burden in four villages situated in the north-east of Bahia, Brazil.

A positive correlation between *S. mansoni* parasite load and total IgE levels was also observed in this study. This has also been found by other researchers (24) and could result from the polyclonal activation seen in some parasite infections (21). Additionally, our research group also demonstrated, in a previous work in the same population enrolled in the current study, that polymorphisms in the IL-10 promoter were associated with high total IgE levels (24).

Despite the notable association between total IgE and intensity of infection, no correlation between SWAP-specific IgE and parasite load was found. We showed previously a high heritability to total IgE levels implying that there are genes controlling total IgE production regardless of the relative proportion of schistosome antigen-specific IgE (3). While total IgE seems not to be associated with resistance against helminth infections, high levels of total IgE production in detriment of schistosome-specific IgE may result in a less protective immune response. Moreover, suppression of basophil histamine release *in vitro* is observed when polyclonal to antigen-specific IgE ratios are >500 : 1, which rarely occurs during helminth infections (25). In schistosomiasis, however, we have found high levels of policlonal IgE, as showed here and elsewhere (26).

Levels of SWAP-specific IgG4, however, were positively associated with *S. mansoni* parasite load in this study, and this is in agreement with other studies (10,15,27). It is speculated that parasite-specific IgG4 can compete with specific IgE for the antigen binding, interfering in the IgE-mediated mast cell degranulation. Thus, IgG4 production would impair the protective role of specific IgE in the resistance to *S. mansoni* infection (10).

Despite the lack of correlation between SWAP-specific IgE and parasite load, specific IgE/IgG4 ratio was lower in infected individuals when compared to non-infected ones. High levels of specific IgE has been associated with resistance to infection (12,18,27); however, it seems that the expression of protective immunity does not depend exclusively on the levels of specific IgE or IgG4, but rather on the favourable balance toward IgE production (18,27). Elevated SWAP-specific IgG4/IgE balance was associated with increased rate of reinfection in a 2-year post-treatment follow-up study (15). Moreover, the influence of high specific IgE/IgG4 ratio in the resistance to *S. mansoni* (18) and in the intensity of *Schistosoma haematobium* infection (28) has been demonstrated by other authors.

In this study, higher intensity of infection was observed in males compared to females. Gender-related differences in infection prevalence or intensity of infection are frequently attributed to sociocultural or behavioural factors (29,30); however, experimental evidence shows that they may also be related to differences in susceptibility to infection (31). Consistent with other studies, the prevalence and parasite load were higher in the first three decades of life, suggesting an age-related resistance to infection in schistosomiasis endemic areas (3,32). The observation that antibodies, particularly IgE, may play a role in protection against schistosomiasis and tends to increase with age in endemic areas led to the hypothesis that age-related resistance to infection may be mediated by parasite-specific IgE (22). The prevalence of infection and infection status in this study was associated with exposure to the water, as expected. Higher levels of specific IgG4 was seen in the higher exposed individuals, while the levels of specific IgE were similar among the various levels of exposure.

We acknowledge, however, that the cross-sectional design and the use of questionnaires to access information on exposure to infested water were the major limitations of this study. It is possible therefore that misclassification of exposure status may have occurred in this study. Moreover, the study design did not allow us to make causal inference and confounding factors such as previous treatment and past infection may not be equally distributed between groups. To minimize bias, we performed statistical analysis taking into account the more known confounding factors in this subject.

It is hard to control all variables in field works. However, in agreement with previous studies, we were able to show, in a large casuistic study, that reduction in schistosome intensity of infection observed amongst subjects in endemic community may be mediated by acquired immunity. Specifically, the immune response associated with resistance to *S. mansoni* infection in this study was a positive balance of SWAP-specific IgE/IgG4 production. On the other hand, high levels of parasite-specific IgG4 were associated with high parasite burden. These findings, using a cohort of individuals living in an endemic area of schistosomiasis in Brazil, highlight the key role of parasite-specific IgE/IgG4 ratio in resistance to *S. mansoni* infection. Ongoing prospective studies are being conducted to determine whether the level of reinfection after treatment will be greater in those subjects with a low IgE/IgG4 ratio, who are likely more susceptible to infection.

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