

Human Immune Responses to *Schistosoma mansoni* Vaccine Candidate Antigens

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To determine the naturally occurring immunological responses to the *Schistosoma mansoni* antigens paramyosin, IrV-5, Sm-23 (MAP-3), and triose phosphate isomerase (MAP-4), a total of 119 subjects from an area of endemicity for schistosomiasis, including “resistant” subjects ($n = 17$) were evaluated. Specific immunoglobulin G1 (IgG1), IgG2, IgG3, IgG4, and IgA levels for each of the antigens and the cytokine profile in culture supernatants from antigen-stimulated peripheral blood mononuclear cells (PBMC) were determined. Although all the subjects had a high degree of contaminated water exposure, their infection levels were variable (0 to 1,128 eggs/g of stool). There were direct correlations between infection levels and levels of SWAP- and paramyosin-specific IgG1 and IgG4 ($P < 0.05$). However, an inverse correlation between infection levels and specific IgG2 to IrV-5 ($P < 0.01$) was observed. The evaluation of the cytokine profile (interleukin 5 [IL-5], IL-10, gamma interferon [IFN- γ], and tumor necrosis factor alpha) in response to these antigens showed inverse correlations between the degree of infection and IFN- γ levels in PBMC supernatants stimulated with paramyosin ($P < 0.05$) and IrV-5 ($P < 0.01$). Additionally, inverse correlations between the degree of infection and IL-5 levels in MAP-3- and MAP-4-stimulated PBMC supernatants ($P < 0.01$) were found. Logistic regression analysis was performed to adjust the results of cytokine profile by age. IL-5 production in MAP-3-stimulated PBMC supernatants was associated with lower infection levels (odds ratio = 11.2 [95% confidence interval, 2.7 to 45.8]).

Schistosomiasis is a chronic parasitic infection that affects 200 million people in Africa, South America, and Asia (35). Although treatment of infected people with schistosomicidal drugs has in part controlled the morbidity of the disease, transmission is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease arose from the fact that the parasite does not multiply in human beings and that reduction of infection levels by schistosomicidal drugs reduces the prevalence of severe forms of the disease. Moreover, in experimental models, partial immunity can be induced by vaccination with irradiated cercariae or specific antigens (2, 11, 14, 26, 36, 37, 58, 59). Immunological studies of subjects from areas of endemicity have demonstrated a naturally occurring resistance to reinfection (4, 19, 22–25, 27, 28). Both high levels of specific immunoglobulin E (IgE) in sera and gamma interferon (IFN- γ) in antigen-stimulated peripheral blood mononuclear cell (PBMC) cultures were associated with resistance to reinfection (1, 22, 24, 25, 27, 28, 56, 57). These data suggest the participation of immunological mechanisms in human resistance to *Schistosoma mansoni* infection, with mixed cellular and humoral responses.

Several *S. mansoni* antigens have been identified and tested in experimental models, with the induction of variable levels of protection against infection (11, 32, 49, 59, 61, 68–70, 74). The World Health Organization (WHO) has selected six of these antigens for further in vitro studies with PBMC from subjects

in areas of endemicity for schistosomiasis. The present study shows the immunological responses of subjects from an area where schistosomiasis is endemic to four of these antigens: paramyosin (49), irradiation-associated vaccine antigen (IrV-5) (68), triose phosphate isomerase (TPI) (32, 61), and *S. mansoni* 23-kDa antigen (Sm-23) (31). The immunological responses of subjects who, though exposed to contaminated water, appear on the basis of negative stool examinations to be uninfected were compared to those of infected patients with similar degrees of contaminated water exposure.

MATERIALS AND METHODS

Area of endemicity. Caatinga do Moura is a village of 3,913 inhabitants located on the banks of a river in Bahia, a northeastern state of Brazil. Agriculture is the main economic activity of the village, and irrigation is carried out by a primitive system of canals. The river and canals are populated by snails infected by *S. mansoni*. The population is infected during irrigation and through the domestic use of water. The prevalence of disease is about 40%, according to a parasitological evaluation performed in 1995. Active transmission of the parasite is still present in this area.

Study subjects. A sample of 1,064 subjects, residents of 208 houses located at about 500 meters from the river, have been monitored with parasitological examinations since 1992. In 1993 this population was treated with oxamniquine (25 to 30 mg/kg of body weight). In 1995, another parasitological evaluation was performed and water contact levels were determined by interview. The water contact levels were classified according to previous publications (19, 23), and a sample of those subjects with a high level of water contact (1 to 3 h per day) was selected to participate in the present study. This group's water contact was confirmed by a health post agent from the village through direct daily observations of activities in the river. The selection of subjects with similar water contact levels was done in an attempt to standardize both the opportunity to be infected and the natural exposure to parasite antigens. This study was approved by the Ethical Committee of the Hospital Universitário Prof. Edgard Santos. Informed consent, following the guidelines of the Brazilian Ministry of Health for research with human subjects, was obtained from all patients. The inclusion criteria were age between 5 and 60 years, high water contact levels (1 to 3 h/day), male and female representation, and at least two parasitological examinations performed

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on different days. The exclusion criteria were age less than 5 years and greater than 60 years, absence of water contact or doubt about water contact levels, pregnancy, and immunological disorders that may interfere with the results of immunological tests. A group ($n = 119$) was selected to participate in the present study and was divided into two groups. Group 1 was composed of subjects ($n = 17$) with negative examinations (three to six samples) in 1992 and 1995 but with high exposure to contaminated water. All but two members of this group, who were uncertain, were treated with schistosomicidal drugs more than 10 years ago. The mean age of this group was 43 ± 13 years (range, 13 to 60 years), with 9 males and 8 females. There was no difference in the conditions (type of activity and time of day) under which these subjects and the infected subjects were exposed to contaminated water. In addition to having no eggs in their stools, individuals in this group were all negative for serum schistosome circulating cathodic antigen (CCA) (data not shown). Group 2 was composed of subjects with positive parasitological examinations ($n = 102$) with different levels of infection, including 47 with more than 200 eggs/g of stool. The mean age of this group of patients was 23 ± 15 years (range, 5 to 50 years), and their infection levels ranged from ≥ 24 to 1,128 eggs/g of stool. They had intestinal or hepatointestinal forms of schistosomiasis but were otherwise apparently healthy without signs of malnutrition. In both groups there were subjects infected with other intestinal parasites, such as *Ascaris lumbricoides*, *Entamoeba histolytica*, and *Trichuris trichiura*, without significant differences between the groups. A control group was formed from students and hospital employees who were free of *S. mansoni* infection but who may have been also infected with other parasites such as *A. lumbricoides*, *E. histolytica*, and *T. trichiura*.

Parasitological methods. Parasitological examinations were performed periodically using Kato-Katz's method (44–46). Two to six stool samples were collected from each subject on different days. The results were shown as the arithmetic means of the numbers of eggs obtained at different days. A negative examination by Kato-Katz's method represents < 24 eggs/g of stool. Kato-Katz's method is the quantitative method of choice to measure infection level and has been used extensively in epidemiological studies due to its simplicity and reproducibility when three to five parasitological examinations are performed on different days during 2 to 3 weeks (44, 45, 47). Measurements of CCA levels were performed with serum samples from a group of 52 subjects, which includes the group with negative parasitological examinations and others with different infection levels, according to a previously described technique (18, 20, 21).

Antigens. *S. mansoni*-specific antigens were provided as part of a WHO project to evaluate in vitro immune responses to *S. mansoni*-specific antigens. The antigens used were soluble extract of whole adult *S. mansoni* (SWAP), purified native paramyosin, IrV-5, and multiple antigenic peptides containing T- and B-cell epitopes designed from the antigens Sm-23 (MAP-3) and TPI (MAP-4). Each antigen was tested for cytotoxicity by measuring the inhibition of the lymphoproliferative response of healthy controls to a suboptimal concentration of phytohemagglutinin mitogen. Nonspecific contaminants, such as lipopolysaccharide, were excluded because the antigens did not induce responses in healthy control subjects ($n = 10$).

Immunological procedures. The cellular immune response was evaluated from January to December 1996. Blood was heparinized, and plasma was separated and stored at -20°C for the evaluation of the humoral immune response. PBMCs were isolated from heparinized blood by density gradient centrifugation using Histopaque 1077 (Sigma Diagnostics, St. Louis, Mo.).

Humoral immune response. Analysis of Ig isotypes (IgG1, IgG2, IgG3, IgG4, IgE, and IgA) specific to SWAP and *S. mansoni*-specific antigens was performed by enzyme-linked immunosorbent assay (ELISA), using a modification of a previously described technique (48). Briefly, plates were coated with each antigen at previously established concentrations (SWAP and paramyosin at $10 \mu\text{g/ml}$ and IrV-5, MAP-3, and MAP-4 at $1 \mu\text{g/ml}$) and left overnight at 4°C . For specific IgE measurement, IgG antibodies were removed by RF absorbent (Behring Diagnostics Inc., Westwood, Mass.) following the manufacturer's instructions. The sera were diluted 1:2 in phosphate-buffered saline (PBS)–0.05% Tween (PBST) and incubated with the same volume of RF absorbent that had been resuspended in 1.5 ml of distilled water. After incubation at room temperature for 15 min, samples were centrifuged at $650 \times g$ for 5 min. The IgG-preabsorbed plasma samples were used at a final dilution of 1:4. The plates coated with the antigens were blocked with PBS–3% bovine serum albumin, and 100- μl plasma samples were incubated overnight at 4°C at dilutions previously tested for each of the isotypes for the different antigens. Mouse anti-human antibodies against each Ig isotype were added to the plates (1:500). After incubation for 2 h at 37°C and six washes with PBST, anti-mouse Ig coupled with peroxidase was added and the plates were incubated for 1 h at 37°C (1:1,000). These antibodies were kindly provided by Victor Tsang from the Centers for Disease Control and Prevention. After six more washes with PBST, the reaction was developed by the addition of TMB substrate (tetramethylbenzidine urea peroxide-stabilized chromogen; ICN Biomedicals Inc.) and stopped with H_2SO_4 . Plates were read in a spectrophotometer at 450 nm, and results were expressed as optical density (OD). Sera from control subjects, free of *S. mansoni* infection but contaminated with other intestinal parasitic infections, were used to calculate the cutoff for each of the specific isotypes (mean plus 2 standard deviations [SD] of the OD of the controls). Standardization of the serum dilutions, as well as the dilutions of the anti-isotype and the conjugate, were performed for each specific isotype using sera from two schistosomiasis subjects with high and low antibody titers to SWAP. To minimize

intra-test variation, the IgG isotype analyses for each patient were performed on the same plate. The brand of substrate solution TMB and the duration used for the color reaction were the same for all experiments.

Determination of cytokine profile from PBMC-stimulated cultures. Levels of the cytokines (interleukin 5 [IL-5], IL-10, IFN- γ , and tumor necrosis factor alpha) were measured in supernatants from PBMC cultures stimulated with the different antigens. Briefly, 3×10^6 cells in 1 ml of RPMI 1640 (GIBCO-BRL) supplemented with 10% AB Rh-positive sera were stimulated with the different antigens at optimal concentrations determined previously. The optimal antigen concentrations were the ones that induced significant responses in the schistosomiasis patient PBMCs but that did not induce a response in the control PBMCs. The concentrations of the antigens were as follows: SWAP, $10 \mu\text{g/ml}$; paramyosin, $60 \mu\text{g/ml}$; IrV-5, $2 \mu\text{g/ml}$; MAP-3, $100 \mu\text{g/ml}$; MAP-4, $100 \mu\text{g/ml}$. After a 72-h incubation, the supernatants were collected and stored at -20°C until cytokine measurement. Cytokine concentration was determined by a sandwich ELISA technique, and the results were expressed as picograms per milliliter based on a standard curve. Cytokine levels above 30 pg/ml were considered positive responses (value not seen in normal control subjects).

Statistical methods. The humoral and cellular immune responses to each of the antigens were analyzed as continuous variables and correlated with infection levels (number of eggs per gram of stool) by the Spearman correlation test, since they do not fit a Gaussian distribution. The design of the study, taking only subjects with similar water contact levels, eliminated any influence of this variable on the differences found in infection levels. The differences in immune responses between the group of subjects with negative examinations and the group with > 200 eggs/g were analyzed by the Mann-Whitney test. These statistical analyses were performed using the program InStat for Macintosh.

Because of the age dependence of the intensity of infection, logistic regression analysis was performed to adjust the immunological data by age. Cytokine data were categorized as positive ($\geq 30 \text{ pg/ml}$; levels not seen in control subjects) or negative, and infection levels were categorized as either less than or greater than or equal to 200 eggs/g of stool. Odds ratios (OR) and confidence intervals (CI) were calculated. The significance in terms of the logistic regression models was tested using χ^2 , the difference in deviance, which was assumed to follow a χ^2 distribution under the null hypothesis that the term was unimportant. The Hosmer and Lemeshow goodness-of-fit test was used in the final model. The logistic regression analysis was performed using the SAS system, version 6.12 (SAS Institute Inc., Cary, N.C.), for IBM.

RESULTS

Study subjects and infection levels. The mean age of the 119 studied patients was 23 ± 15 years, with 73 males and 46 females. In spite of having similar contaminated water contact levels, these subjects had a high variability in infection levels. The mean (\pm SD) number of eggs per gram of stool for these subjects was 246 ± 361 (range, 0 to 1,128 eggs/g of stool), including 17 with no detectable eggs, 42 with 1 to 100, 15 with 101 to 200, 18 with 201 to 400, and 27 with greater than 400 eggs/g of stool. An inverse correlation between age and infection levels from 1992 ($r_s = -0.24$; $P < 0.01$) and 1995 ($r_s = -0.22$; $P < 0.01$) was observed (Spearman's correlation test) (data not shown). Additionally, a direct correlation between infection levels in 1992 and reinfection levels in 1995 was observed ($r_s = 0.38$; $P < 0.01$; Spearman's correlation test) (data not shown). Levels of the *S. mansoni* CCA were below the cutoff (OD = 0.043) in all subjects with negative parasitological examinations. In subjects with positive parasitological examinations ($n = 80$), there was a direct correlation between the number of eggs per gram of stool and the CCA levels ($r_s = 0.40$, $P = 0.0002$; Spearman's correlation test).

Humoral immune responses to SWAP- and *S. mansoni*-specific antigens. Antibodies to SWAP were detected in a high percentage of subjects, with 95% responding with total IgG (mean of OD \pm standard error of the mean [SEM], 0.4 ± 0.02), with IgG1 (mean of OD \pm SEM, 1.4 ± 0.07), 75% with IgG4 (mean of OD \pm SEM, 0.4 ± 0.06), and 58% with IgA (mean of OD \pm SEM, 0.7 ± 0.04). Among the specific *S. mansoni* antigens, paramyosin-specific IgG1 was detected in 50% of the subjects (mean of OD \pm SEM, 1.35 ± 0.07) and IgG4 was detected in 48% (mean of OD \pm SEM, 0.23 ± 0.05). IrV-5-specific IgG4 was detected in 25% of subjects (mean of OD \pm SEM, 0.14 ± 0.05), and higher titers of IgG3 and IgG2

TABLE 1. Correlation between infection levels and antigen-specific isotype response in plasma and cytokine profile in antigen-stimulated PBMCs from subjects from an area where schistosomiasis is endemic

Variable correlated with infection levels ^c	Spearman's r_s
IgG1 specific to SWAP.....	0.31 ^a
IgG1 specific to paramyosin.....	0.31 ^a
IgG4 specific to SWAP.....	0.30 ^a
IgG4 specific to paramyosin.....	0.33 ^a
IgG2 specific to SWAP.....	-0.15 ^b
IgG2 specific to IrV-5.....	-0.27 ^a
IFN- γ to SWAP.....	-0.19 ^b
IFN- γ to paramyosin.....	-0.23 ^a
IFN- γ to IrV-5.....	-0.24 ^a
IL-5 to MAP-3.....	-0.32 ^a
IL-5 to MAP-4.....	-0.33 ^a

^a $P < 0.01$.

^b $P = 0.05$.

^c Numbers of eggs per gram of stool.

were found in 22 (mean of OD \pm SEM, 1.3 \pm 0.08) and 12% (mean of OD \pm SEM, 0.7 \pm 0.08) of the subjects, respectively. MAP-3-specific IgG3 was found in 53% of the subjects (mean of OD \pm SEM, 0.16 \pm 0.03), but higher levels of IgA were found in 21% of them (mean of OD \pm SEM, 1.3 \pm 0.07). Only 22% of the subjects responded to MAP-4 with specific IgG1 (mean of OD \pm SEM, 0.26 \pm 0.01). Table 1 shows the results of the correlation analysis. Direct correlations between infection levels (number of eggs per gram of stool) and levels of specific IgG1 (OD) and IgG4 to SWAP and paramyosin were observed ($P < 0.05$; Spearman's correlation), suggesting that these isotypes are markers for high levels of infection. However, inverse correlations between infection levels and the levels of specific IgG2 to SWAP ($P = 0.05$) and IrV-5 ($P < 0.05$) were found (Spearman's correlation test). This indicates that people with lower infection levels produced higher levels of IgG2 specific for these antigens. A direct correlation between IrV-5-specific IgG2 and IFN- γ levels in response to this same antigen ($P < 0.01$; Spearman's correlation test) (data not shown) was observed. No correlation between infection levels and levels of SWAP-specific IgE ($P > 0.05$; Spearman's correlation test) was observed. However, an inverse correlation between SWAP-specific IgE/IgG4 ratio and infection levels ($P < 0.05$; Spearman's correlation test) was found.

Cytokine profile of stimulated PBMC cultures from subjects from an area of endemicity for schistosomiasis. The percentages of patients who produced IL-5 and IFN- γ and the ranges and means \pm SEMs of cytokine concentrations in antigen-stimulated PBMC cultures from schistosomiasis patients are shown in Table 2. Paramyosin stimulated IL-5 production by PBMC from 53% of the studied subjects. Compared to other defined antigens, paramyosin induced the highest IL-5 production (1,013 \pm 170 pg/ml) but also induced IFN- γ production (126 \pm 25 pg/ml). In contrast, IrV-5 induced little IL-5 production but strongly promoted IFN- γ production (284 \pm 83 pg/ml) by PBMC from 61% of the subjects. MAP-4 induced more IL-5 than IFN- γ production in 40% of the subjects (67 \pm 20 pg/ml), and MAP-3 induced lower levels of both IFN- γ and IL-5 in a smaller proportion of the study group (26 and 3%, respectively). Figure 1 shows IFN- γ production in response to the different antigens in subjects classified according to their degrees of infection. A higher production of IFN- γ is observed in PBMC supernatants stimulated with IrV-5, SWAP, and paramyosin in subjects with negative stool examinations but exposed to contaminated water, and decreasing levels in in-

TABLE 2. IL-5 and IFN- γ responses from PBMCs stimulated with *S. mansoni* vaccine candidate antigens for subjects from an area where schistosomiasis is endemic

Antigen	Cytokine	No. of patients	% Responders ^a	Cytokine level (pg/ml)	
				Range	Mean \pm SEM
Paramyosin	IL-5	116	53	0-9,713	1,013 \pm 170
	IFN- γ	119	50	0-1,937	126 \pm 25
IrV-5	IL-5	116	29	0-2,388	57 \pm 82
	IFN- γ	119	61	0-9,027	284 \pm 83
MAP-3	IL-5	111	26	0-399	37 \pm 8
	IFN- γ	115	3	0-516	11 \pm 5
MAP-4	IL-5	116	40	0-2,208	67 \pm 20
	IFN- γ	118	14	0-629	24 \pm 8

^a Responder level, ≥ 30 pg/ml.

fect subjects are observed. There was an inverse correlation between IFN- γ in response to paramyosin and IrV-5 and infection levels ($P < 0.05$; Spearman's correlation test) (Table 1). Figure 2 shows IL-5 levels in PBMC supernatants stimulated with the different antigens from the study subjects classified according to their infection levels. Interestingly, higher levels of IL-5 were observed in subjects with 101 to 200 eggs/g of stool in response to the antigens SWAP, paramyosin, and

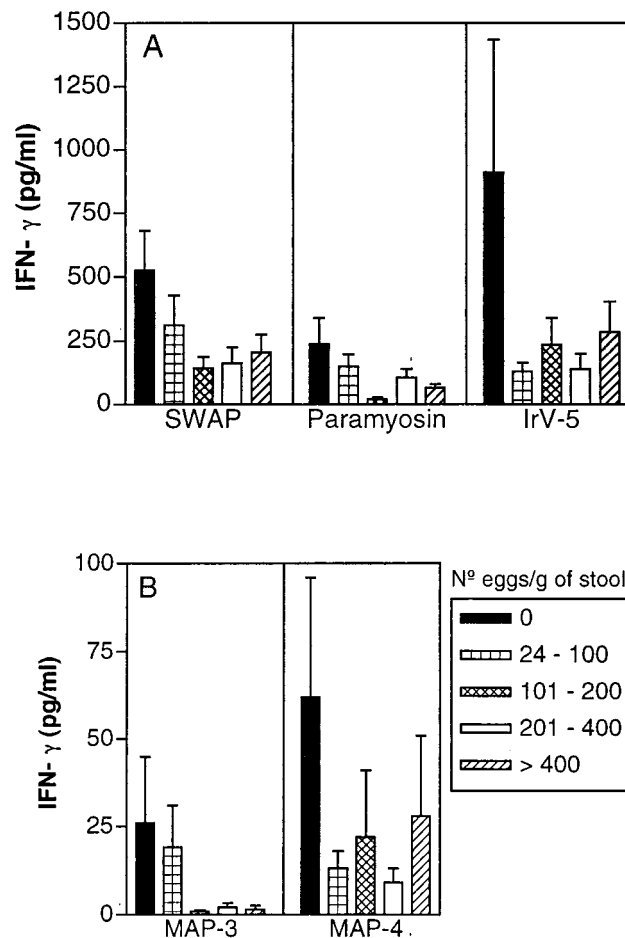


FIG. 1. IFN- γ levels (mean \pm standard error) in PBMC supernatants stimulated with SWAP, paramyosin, and IrV-5 (A) and MAP-3 and MAP-4 (B) from subjects from an area of endemicity for schistosomiasis with negative parasitological examinations or with different infection levels (numbers of eggs per gram of stool).

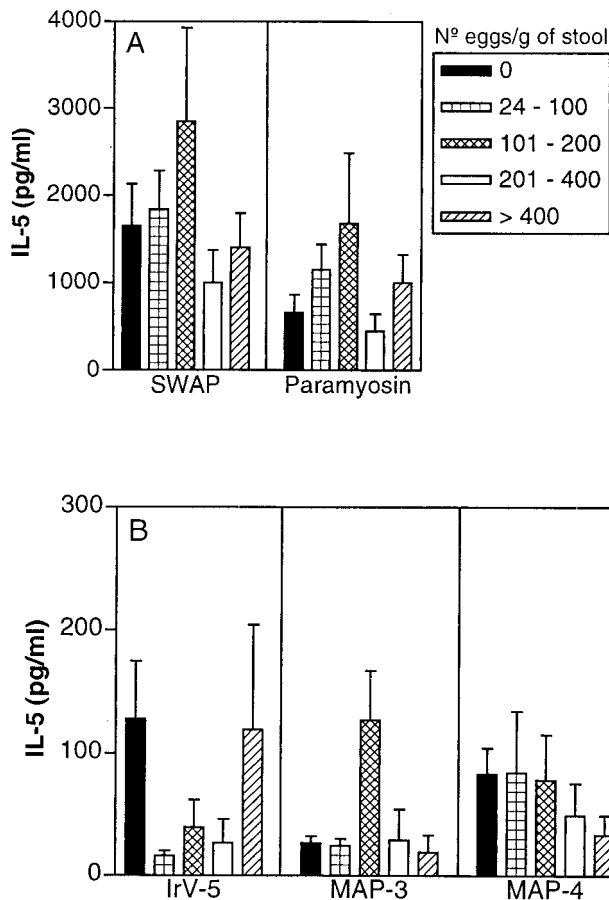


FIG. 2. IL-5 levels (mean \pm standard error) in PBMC supernatants stimulated with SWAP and paramyosin (A) and IrV-5, MAP-3, and MAP-4 (B) from subjects from an area of endemicity for schistosomiasis with negative parasitological examinations or with different infection levels (numbers of eggs per gram of stool).

MAP-3, corresponding to the subjects who produced lower levels of IFN- γ (Fig. 1). However, the subjects with negative stool samples also produced high levels of IL-5 in response to SWAP and paramyosin. Inverse correlations between IL-5 levels in PBMC supernatants stimulated with MAP-3 and MAP-4 and infection levels were also observed ($P < 0.01$; Spearman's correlation) (Table 1). The logistic regression analysis showed that age below 20 years was considered a risk for high infection levels (≥ 200 eggs/g of stool; OR, 12.5; 95% CI, 4.1 to 38.2; $P < 0.0001$). After adjustment by age, only the IL-5 response to MAP-3 was associated with lower infection levels (OR, 11.2; 95% CI, 2.8 to 45.8; $P < 0.0007$).

Comparison of humoral and cellular immune responses between subjects with "resistant" phenotype and subjects infected with ≥ 200 eggs/g of stool. Immunological responses of resistant subjects ($n = 17$) with negative examinations in 1992 and 1995 (3 to 6 samples) but highly exposed to contaminated water were compared with those of the infected subjects with more than 200 eggs/g of stool ($n = 47$). The mean age (\pm SD) of these resistant subjects (42 ± 13 years; range, 13 to 60 years) was significantly higher than that of the group with ≥ 200 eggs/g of stool (mean \pm SD, 16 ± 8 years; range, 8 to 40 years) ($P < 0.01$; Student's t test). Similar percentages of males and females were found in both groups.

The comparison of specific isotype levels between subjects with the resistant phenotype and the ones infected with ≥ 200 eggs/g confirmed the data shown in the correlation analysis.

SWAP-specific IgG1 and IgG4 levels were higher in the infected group ($P < 0.01$; Mann-Whitney test), as shown in Fig. 3A. Moreover levels of paramyosin-specific IgG1, IgG3, and IgG4 were also higher in the infected group ($P < 0.05$; Mann-Whitney test) (Fig. 3B). Although there were no differences in SWAP-specific IgE levels between the groups, the IgE-to-IgG4 ratio was higher in the group with the resistant phenotype ($P < 0.05$; Mann-Whitney test) (data not shown). Paramyosin-specific IgE was detected in only five people, one from the resistant group and four from the infected group (OD variation, 0.031 to 0.101). IrV-5-specific IgE was also detected in five people, two from the resistant group and three from the infected group (OD variation, 0.132 to 0.789). Specific IgE to MAP-3 and MAP-4 was not detected in any of the subjects tested. Additionally, levels of IgG2 specific to paramyosin and IrV-5 were higher in the resistant group ($P < 0.05$; Mann-Whitney test), as shown in Fig. 4. No differences in the other antigen-specific isotypes between these two groups of patients were found.

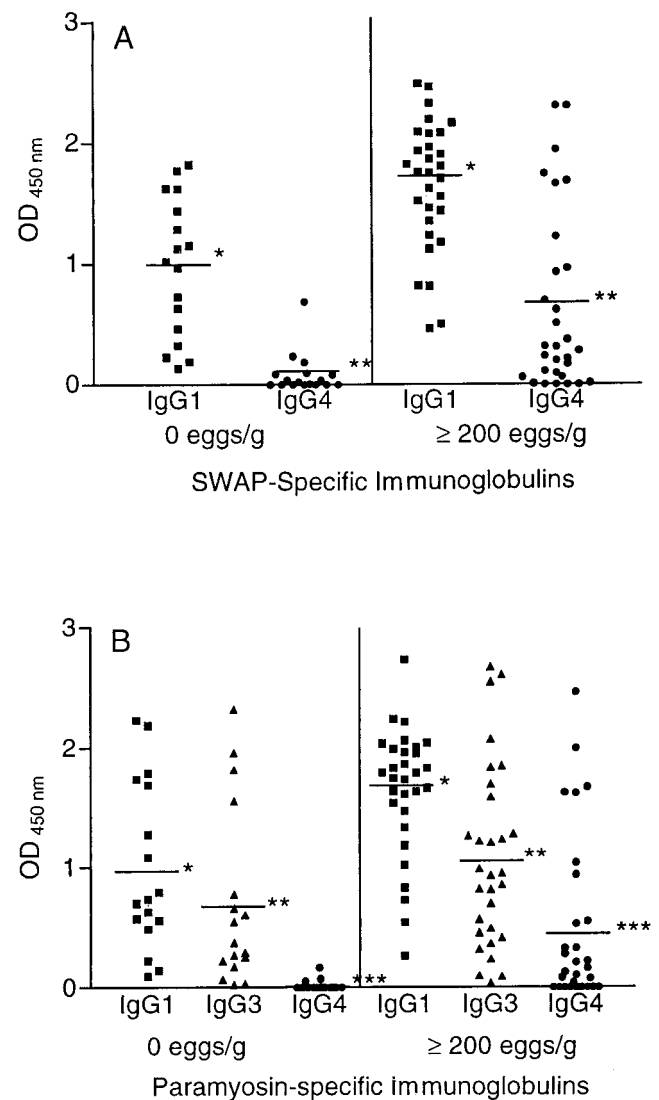


FIG. 3. Levels of SWAP-specific IgG1 and IgG4 (A) and paramyosin-specific IgG1, IgG3, and IgG4 (B) in subjects from an area where schistosomiasis is endemic with negative parasitological examinations and, in infected subjects with ≥ 200 eggs/g of stool. The Mann-Whitney test was used. * and ***, $P < 0.01$; **, $P < 0.05$.

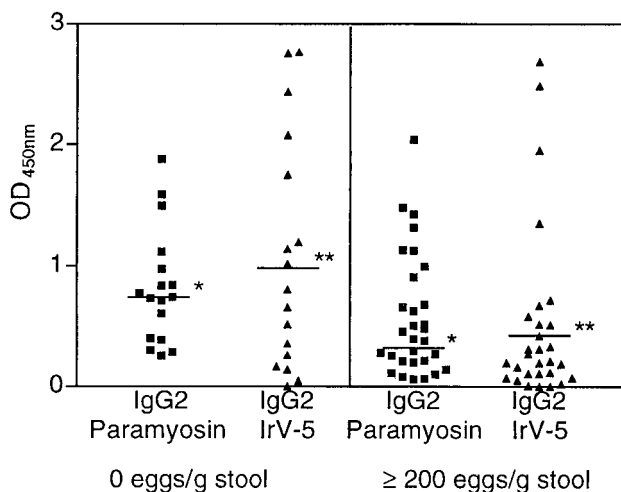


FIG. 4. Levels of paramyosin- and IrV-5-specific IgG2 in subjects from an area where schistosomiasis is endemic with negative parasitological examinations and in infected subjects with ≥ 200 eggs/g of stool. *, $P < 0.05$; **, $P < 0.01$ (Mann-Whitney test).

The levels of production of cytokines in supernatants of antigen-stimulated PBMCs in these groups of resistant and infected (≥ 200 eggs/g of stool) patients were compared. Higher levels of IFN- γ were seen in PBMCs from resistant subjects stimulated with SWAP, IrV-5, and MAP-3 (mean \pm SEM, 527 ± 154 , 911 ± 523 , and 25 ± 19 pg/ml, respectively) than in PBMCs from highly infected subjects (mean \pm SEM, 188 ± 46 , 217 ± 72 , and 2 ± 1 pg/ml, respectively; $P < 0.05$; Mann-Whitney test). IL-5 levels were higher in PBMCs from resistant subjects stimulated with IrV-5, MAP-3, and MAP-4 (mean \pm SEM, 128 ± 47 , 26 ± 5 , and 83 ± 21 pg/ml, respectively) than in PBMCs from highly infected subjects (mean \pm SEM, 85 ± 55 , 22 ± 13 , and 38 ± 14 pg/ml, respectively; $P < 0.05$; Mann-Whitney test). No differences in IL-10 and TNF- α levels between the groups ($P > 0.05$; Mann-Whitney test) were found (not shown).

DISCUSSION

Resistance to *S. mansoni* infection is well demonstrated in experimental models of schistosomiasis, either after natural infection or after immunization with irradiated cercariae or certain defined *S. mansoni* antigens (9, 11, 26, 29, 33, 36, 40, 50, 59, 71, 73, 74). The protective immune responses differ in these experimental models, being mainly humoral (IgE and IgG2a) in rats and mixed cellular and humoral in mice (13, 14, 34, 38, 39, 41, 42, 51, 52, 59). Although human immune responses to *S. mansoni* have been studied extensively, the mechanism by which humans resist schistosome infection are still unclear. Data from two different research groups in areas of endemicity in Kenya and Brazil have shown an association between resistance to reinfection and high levels of specific IgE and high IgE-to-IgG4 ratios (22, 24, 25, 28, 56, 57). Other studies in two areas of endemicity in Minas Gerais, Brazil, have described subjects who exhibit complete resistance to infection (16). These individuals showed higher levels of IFN- γ in PBMC supernatants stimulated with a schistosome membrane extract and IgG antibodies to paramyosin and higher levels of schistosome antigen-specific IgE than infected subjects (1, 72).

The defined *S. mansoni* antigens tested in the present study were selected as vaccine candidates by WHO because they were shown to be protective in vaccination experiments in

animals. Paramyosin was first identified in sera from mice immunized with an *S. mansoni* adult worm antigen in association with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG). Immunization promotes 39% protection in mice (50, 60), and this protein was recognized by sera from putative resistant subjects from areas where schistosomiasis is endemic (16). The gene encoding IrV-5 was cloned from a cDNA library using an antibody (IrV-3) from immunized mice that was not present in sera from infected animals. The IrV-5 recombinant protein induced 75% protection in mice and 25% protection in baboons (2, 65–68). TPI is an enzyme from the glycolytic pathway, identified by a monoclonal antibody that is capable of passively immunizing naive mice against infection. The enzyme itself induces 30 to 60% protection in mice (30, 53, 61). Sm-23 is an integral membrane protein, part of a superfamily of proteins which includes CD9 and TAPA-1, first described in hematopoietic cells. Sm-23 gives 40 to 50% protection in mice (2, 31). Because of the high homology of TPI and Sm-23 with mammalian proteins, epitope mapping of them was carried out, and multiple antigenic peptides containing T- and B-cell epitopes from the less-conserved regions were designed (54, 55). Aside from data for paramyosin, nothing is known about the immune responses to these antigens in *S. mansoni*-resistant subjects.

The present study shows the in vitro humoral and cellular immune responses of subjects from an area of endemicity with high exposure to infection but with variable degrees of infection, including complete resistance (negative parasitological examinations). An inverse correlation between levels of IFN- γ in response to paramyosin and IrV-5 and infection levels (number of eggs per gram of stool) was observed. Moreover, there is an inverse correlation also between levels of IL-5 production in response to MAP-3 and MAP-4 and infection levels. Considering that an inverse correlation between immunological parameters and infection levels is considered a sign that a given response is protective, an argument can be made that IFN- γ production in response to paramyosin and IrV-5 and IL-5 production in response to MAP-3 and MAP-4 are protective immune responses. Although the levels of IL-5 in PBMC supernatants stimulated with MAP-3 and MAP-4 were very low, these values correlate inversely with infection levels. As MAP-3 and MAP-4 are peptidic antigens, even low production of this cytokine may have biological relevance, as it might be expected that fewer specific lymphocytes are in the circulation at any given time. Moreover, the IL-5 response to MAP-3 was associated with resistance even after adjustment by age. These data suggest that both types of cellular immune responses, Th1 and Th2, are involved in the protective response to *S. mansoni*. Moreover, different specific antigens induce different types of protective immune responses, such that paramyosin and IrV-5 induce IFN- γ , which correlates inversely with infection levels, and MAP-3 and MAP-4 induce IL-5, which also inversely correlates with infection levels.

The present study also shows a direct correlation between the levels of IgG1 and IgG4 specific to SWAP and paramyosin and infection levels, confirming previously published data (22, 72). These data suggest that IgG1 and IgG4 are markers of higher infection levels. High levels of IgG2 specific for SWAP and IrV-5 were associated with lower infection levels, contradicting previously published experiments done with schistosome membrane extract (22). In mice, IgG2a antibodies are induced by IFN- γ . In the present study we showed a direct correlation between IFN- γ levels in PBMC culture supernatants stimulated with IrV-5 and levels of IgG2 specific for the same antigen. This finding suggests that IFN- γ in humans also stimulates the production of IgG2. It remains to be clarified

whether this isotype is involved in protective immunity or if it is only a consequence of IFN- γ production, with IFN- γ being the protective mediator.

The present study supports the idea that both cellular and humoral immune mechanisms may be important to control *S. mansoni* infection and that they may be active in different sites in humans. Specific IgE, interacting with eosinophils and phagocytic cells, has been demonstrated to be effective in schistosomula destruction *in vitro* (6–8, 10, 12, 13, 15) and may be an important immune defense mechanism in the skin, where these cell types are found at the time of cercarial penetration. The majority of studies done with subjects from areas of endemicity support a role for IgE in resistance to *S. mansoni* infection (22, 24, 25, 28, 56, 57). In the present study, the levels of SWAP-specific IgE were not correlated with infection levels. However, the IgE/IgG4 ratio was indeed correlated inversely with infection levels, supporting the idea that the presence of IgG4 might block a protective role of IgE in the most-infected subjects. In mice, IFN- γ -activated macrophages are important in parasite destruction in the lungs (17, 43, 62–64). The present study supports the role of IFN- γ production in the protective response in human beings by showing higher levels of this cytokine in partially or completely resistant subjects. The mechanisms involved in parasite destruction in humans are still unclear, as well as the site of parasite killing and other cytokines involved in this process.

The choice of an antigen to be further tested as a vaccine candidate for schistosomiasis is difficult. The present data evaluating the human immune response to four of these antigens suggest that all of them naturally induce an immune response that is correlated with protection against reinfection. The data also suggest that perhaps a vaccine that induces both Th1- and Th2-like responses would be necessary to achieve higher levels of protection. The choice will also depend on the facility to produce the antigen in large scale and on the possibility of achieving a good interaction between the antigens chosen, should a multicomponent vaccine be selected. An additional concern is that a vaccine should not lead to a potentiated immunopathologic response, since the granulomatous reaction and resulting hepatic fibrosis in schistosomiasis are also immunologically mediated. Fortunately, immunized animals do not present with increased hepatic fibrosis due to schistosome infection, indicating that it is possible to achieve a balanced immunological response that protects against reinfection without being deleterious to the host. Although naturally resistant subjects from areas of endemicity do not present with increased hepatic fibrosis due to natural *S. mansoni* infection, immunization, especially with adjuvants, has to be carefully evaluated.

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