Human Immune Responses to *Schistosoma mansoni* Vaccine Candidate Antigens

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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 is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease is largely unaltered (3, 5, 24).

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 IL-5 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 is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease is largely unaltered (3, 5, 24). The possibility of a schistosomiasis vaccine as an additional measure to control the disease is largely unaltered (3, 5, 24).
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) of students and hospital employees who were free of S. mansoni infection and who had also been infected with other parasites such as A. lumbricoides, E. histolytica, and T. 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 each day, on different days. The results were shown as arithmetic means of the number 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 derived 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.). PBMCs were isolated from heparinized blood by density gradient centrifugation and stored at −20°C. Nonspecific contaminants, such as lipopolysaccharide, were excluded because the antigens did not induce responses in healthy control subjects (n = 10).

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 antigens at previously established concentrations (SWAP and paramyosin at 10 μg/ml and IrV-5, MAP-3, and MAP-4 at 1 μg/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 500 × g for 5 min. The IgG-precipitated 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 H2SO4. Plates were read in a spectrophotometer at 450 nm. The results were expressed as OD and as the arithmetic mean of the numbers of eggs obtained at different days. The results were shown as the arithmetic mean of the numbers of eggs obtained at different days. The results were shown as the arithmetic mean of the numbers of eggs obtained at different days. The differences in immune response between the groups of subjects with negative parasitological examinations and the group with >200 eggs/g of stool were categorized as positive (≥50 pg/ml; levels not seen in control subjects) or negative. The differences in immune response between the groups with different levels of infection were categorized as less 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 χ2, the difference in deviance, which was assumed to follow a χ2 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 (± 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 less than 100 eggs/g of stool, 42 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 = −0.24; P < 0.01) and 1995 (r = −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 = 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 = 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 95% of the subjects, with 95% responding with total IgG (mean of OD ± standard error of the mean [SEM], 0.4 ± 0.02), 98% with IgG1 (mean of OD ± SEM, 1.4 ± 0.07), 75% with IgG4 (mean of OD ± SEM, 0.4 ± 0.06), and 58% with IgA (mean of OD ± SEM, 0.7 ± 0.04). Among the specific S. mansoni antigens, paramyosin-specific IgG1 was detected in 50% of the subjects (mean of OD ± SEM, 1.35 ± 0.07) and IgG4 was detected in 48% (mean of OD ± SEM, 0.23 ± 0.05). IgE-5-specific IgG4 was detected in 35% of subjects (mean of OD ± SEM, 0.14 ± 0.05), and higher titers of IgG3 and IgG2
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VOL. 68, 2000 IMMUNE RESPONSE TO (mean of OD 6 6 found in 21% of them (mean of OD 22% of the subjects responded to MAP-4 with specific IgG1 6 6 of OD 6 MAP-3-specific IgG3 was found in 53% of the subjects (mean 

FIG. 1. IFN-γ levels (mean ± 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).
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 ($200 \text{ eggs/g of stool}$; 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 $\geq 200 \text{ eggs/g of stool}$. 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 ($\pm \text{SD}$) of these resistant subjects (42 ± 13 years; range, 13 to 60 years) was significantly higher than that of the group with $\geq 200 \text{ eggs/g of stool}$ (mean ± 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 $\geq 200 \text{ eggs/g}$ 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.
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 ± SEM, 527 ± 154, 911 ± 523, and 25 ± 19 pg/ml, respectively) than in PBMCs from highly infected subjects (mean ± 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 ± SEM, 128 ± 47, 26 ± 5, and 83 ± 21 pg/ml, respectively) than in PBMCs from highly infected subjects (mean ± 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 schistosomula 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 schistosomula 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 the 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 cyto-

The choice of an antigen to be further tested as a vaccine candidate for schistosomiasis is difficult. The present data evaluating that it is possible to achieve a balanced immunological response that protects against reinfection without being dele-

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Immune response to S. mansoni vaccine candidates


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