

SUSCEPTIBILITY AND RESISTANCE TO *SCHISTOSOMA MANSONI* REINFECTION: PARALLEL CELLULAR AND ISOTYPIC IMMUNOLOGIC ASSESSMENT

IRAMAYA R. CALDAS, RODRIGO CORREA-OLIVEIRA, ENRICO COLOSIMO, OMAR S. CARVALHO, CRISTIANO L. MASSARA, DANIEL G. COLLEY, AND GIOVANNI GAZZINELLI

Centro de Pesquisas René Rachou-FIOCRUZ, Belo Horizonte, Brazil; Departamento de Estatística de Ciências Exatas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. Cellular and humoral immune responses to *Schistosoma mansoni* antigen preparations were evaluated in individuals presumed to be susceptible or resistant to reinfection after chemotherapeutic cure. A consistent proliferative increase in the response to soluble egg antigen (SEA) was observed post-treatment in both the susceptible and resistant groups. However, this change was not related to resistance. Isotype studies showed that IgM antibody levels to soluble worm antigen preparation (SWAP) and cercariae antigens were significantly higher in the resistant group than in the susceptible group. Post-treatment, an increase in IgE anti-SWAP and anti-schistosomular tegument (STEG) responses and a decrease in IgG4 anti-SEA and anti-STEG responses were observed in the resistant group. These findings are similar to those we have reported previously for a putative resistant group termed endemic normals, and are compatible with immunologic studies in different endemic areas. Together, these findings indicate that even on the population level, high IgE specificities coupled with low IgG4 specificities correlate well with documented resistance to reinfection.

Based on field studies following curative chemotherapy of either *Schistosoma mansoni*^{1–3} or *S. haematobium*,^{4,5} individuals are often categorized as being either susceptible or resistant to natural reinfection by schistosomes. These studies have shown that resistance to reinfection is an age-related phenomenon, with most people in endemic areas becoming resistant, or expressing their resistance during their second decade of life. When determined directly or by estimation, resistance appears to be unrelated to the degree of contact that the susceptible and resistant groups have with cercariae-containing water, and is usually attributed to immunity rather than physiologic or behavioral changes with age.^{4,6,7} These studies have cataloged a variety of humoral immune responses, and are in agreement with several of the correlations demonstrated between given immune responses and susceptible or resistant groups.^{8,9}

A number of studies have shown associations between resistance status and a balance between the level of effective anti-schistosomula antibodies and the presence of blocking antibodies.^{5,10–12} The latter are sometimes most easily demonstrated as antibodies to egg antigens that cross-react with epitopes present in the schistosomula tegument.^{3,10}

The most common features that show relationships to resistance to reinfection include high levels of IgE against adult worm or larval antigens, while high levels of IgG4 and IgM antibodies against egg antigens generally parallel susceptibility.^{5,9,11–15}

Recent studies have also reported correlations between resistance and elevated levels of IgA against a schistosome vaccine candidate (Sm 28 glutathione-S-transferase).^{16,17} Further studies with other antigens have demonstrated that higher levels of IgE against a 22-kD schistosomula moiety⁹ and higher levels of IgM against a 68-kD adult schistosome antigen¹⁸ also correlate with resistance to infection. Most of these studies have examined selected subpopulations of patients in depth for the immune correlations described, and in most cases have focused on individuals from endemic areas <20 years of age.

The current study was performed in a small village and

included the evaluation of all inhabitants who agreed to participate. We followed the proliferative response of patient's peripheral blood mononuclear cells (PBMCs), as well as their isotype levels of circulating antibodies to schistosome antigens. The patients were retrospectively identified as susceptible or resistant, and their immune responses were compared. Several significant immunologic differences were observed between the susceptible and resistant groups.

PATIENTS, MATERIALS, AND METHODS

Patients and study area. The study population is from the endemic area of Siqueira, a community of approximately 250 people located 25 km northwest of the metropolitan area of the Minas Gerais State Capital, Belo Horizonte in Brazil. One hundred sixty-two persons agreed to participate in this study and their feces were examined using the Kato-Katz method.¹⁹ Of these, 102 persons 3–88 years of age were positive for *S. mansoni*, with a mean infection intensity of 63 eggs per gram of feces. All of these patients were included in this reinfection study. The stool survey was repeated at 2, 9, and 21 months after treatment. Three stool samples were collected from each individual and were examined on duplicate slides to provide an estimate of the intensity of infection. Following each stool survey, infected individuals were treated with oxamniquine (15 mg/kg for adults and 20 mg/kg for children <14 years old). From this group of 102 individuals, 29 failed to provide post-treatment stool samples and in 11 the cure was not clearly demonstrated. Twenty-one months after treatment, the 62 remaining patients were retrospectively classified as susceptible (25 individuals) or resistant (37 individuals). The selection procedures used for the susceptible and resistant groups for in-depth immune response studies were 1) individuals were included in the susceptible group only when reinfection was observed after a clear demonstration that chemotherapeutic cure had occurred; and 2) those categorized as resistant did not have any egg-positive stool examination result during the entire 21 months following treatment, as shown in Table 1.

TABLE 1

Study protocol used for identification of susceptible (SUS) and resistant (RES) patients from the endemic area of Siqueira, Brazil (62 patients)*

Groups	Before treatment	After treatment (months)		
		2	9	21
SUS	+	-	-	+
RES	+	-	-	-

* Shown are the + (positive) or - (negative) results of stool examinations for *Schistosoma mansoni*.

All patients diagnosed with *S. mansoni* infection or other helminthic infections were treated independent of their participation in these studies. Written consent was obtained from all patients included in this study. Protocols involving human subjects were approved by the institutional review committees of the Fundação Oswaldo Cruz-FIOCRUZ and Vanderbilt University School of Medicine (Nashville, TN). Blood samples were collected immediately before and 21 months after treatment with oxamniquine, which was given in the standard Brazilian dose of 15 mg/kg for adults and 20 mg/kg for children.

The water contact index was determined using a questionnaire in accordance with Lima e Costa and others.²⁰ The water contact information was provided by each individual, or by the mother or another relative in the case of children <10 years of age. The degree of water contact was calculated using the formula $\Sigma (R \times F)$, where R is the score for the reason for the contact and F is the score for the frequency of contact. The following scores were given to the various reasons for water contact: 5 for bathing, swimming, or playing in the streams; 4 for doing laundry, watering agricultural fields, or sand extraction from streams; 3 for collecting water for the household or washing dishes; and 2 for fishing or crossing streams. The frequency of contacts was scored as 28 (at least 1 contact per day), 4 (at least 1 contact per week), 2 (at least 2 contacts per month), and 1 (less than 2 contacts per month). Indices of 2-99 were considered as a low risk of exposure to cercariae and those ≥ 100 were considered as a high risk.

Antigen preparations. *Schistosoma mansoni* antigens were prepared according to methods previously described.^{21,22} Briefly, adult worms and eggs were collected from outbred Swiss mice infected with the LE strain of *S. mansoni* maintained in our laboratory. Adult worms, eggs, or cercariae were resuspended in 1.7% saline and homogenized 3-5 times in a tissue grinder (30 sec per homogenization at 60-sec intervals). The homogenate was then centrifuged for 1 hr at $50,000 \times g$, the supernatant was collected, and its protein content was determined by the method of Lowry and others.²³ Cercariae were mechanically transformed *in vitro* into schistosomula as described by Ramalho-Pinto and others.²⁴ The schistosomular tegument (STEG) was prepared as previously described by extraction with 0.3M CaCl_2 .²⁵ Briefly, freshly transformed larvae were resuspended in 0.5ml of 0.3 M CaCl_2 and vortexed for 7 min. Schistosomula were then centrifuged at $240 \times g$ for 15 sec, resuspended in 0.25 ml of 0.3 M CaCl_2 , and vortexed again for 1 min. The membrane-containing supernatants were combined and centrifuged for 1 hr at $50,000 \times g$. The pellet

was collected, dialyzed against phosphate-buffered saline (PBS), and the protein content was determined by the method of Lowry and others.²³

Cellular proliferation. Peripheral blood mononuclear cells were prepared from heparinized (25 units/ml) blood and cultured as previously described.²² Briefly, whole blood was centrifuged ($400 \times g$ for 40 min at 20°C) over ficoll-diatrizoate (1.077 g/L, lymphocyte separation medium; Organon Teknika, Charleston, SC). The PBMC layer was collected, washed 3 times with minimal essential medium (Gibco, Grand Island, NY), and resuspended in RPMI 1640 medium (Gibco) at the concentration of 10×10^6 cells/ml. For antigen stimulation, 250,000 cells were added per well and for mitogen stimulation, 150,000 cells were added per well. Final concentrations of stimulants per milliliter of culture determined to be optimal were 25 μg of soluble egg antigen (SEA), 25 μg of soluble worm antigen preparation (SWAP), 30 μg of cercariae antigens (CERC), and 2.5 μg of phytohemagglutinin (PHA) (Difco Laboratories, Detroit, MI) in triplicate cultures in 200 μl containing 5% heat-inactivated normal AB + human serum. Tritiated thymidine (0.5 μCi /culture, specific activity = 6.7 Ci/mM) was added to cultures on the third day (mitogen stimulation) or sixth day (antigen stimulation) of culture. The cells were harvested 6 hr later and proliferation was estimated by liquid scintillation spectroscopy. The data are expressed as the mean counts per minute (cpm) of stimulated cultures (experimental) minus the mean cpm of the unstimulated culture (control).

Enzyme-linked immunosorbent assay. The ELISAs were performed using SEA (5 $\mu\text{g}/\text{ml}$), SWAP (50 $\mu\text{g}/\text{ml}$), CERC (25 $\mu\text{g}/\text{ml}$), or STEG (25 $\mu\text{g}/\text{ml}$) as antigens used to coat the surface of microtiterplate wells. For each antigen preparation, anti-human isotype-specific antibodies were used to quantitate the levels of IgG1, IgG2, IgG3, IgG4, IgM, IgA, and IgE antibodies present in the serum of each individual. The method used, with some modifications, was previously described.²⁶ Immulon II microtiter plates (Dynatech Laboratories, Inc., St. Louis, MO) were coated with antigens in 0.5 M carbonate-bicarbonate buffer, pH 9.6, at 4°C overnight, blocked for 90 min at 37°C with 2% bovine albumin (Biobrás, Montes Claros, Minas Gerais, Brazil) in PBS containing 0.05% Tween 20 (PBS-Tween), and washed 10 times with PBS-Tween. Serum at a dilution of 1:100 for IgA, IgE, IgG2, and IgG3 and 1:640 for IgM, IgG1, and IgG4 were added to each well. These dilutions were selected based on standard curves with a serum pool from all patients included in this study. After incubation for 1 hr at 37°C , the plates were washed 10 times with PBS-Tween and 0.1ml of a 1:10,000 dilution of biotin-conjugated goat anti-human IgA, IgM, and IgG1 or 0.1ml of a 1:2,000 dilution of biotin-conjugated monoclonal antibodies to human IgG2, IgG3, IgG4, and IgE (Southern Biotechnology Associates, Inc., Birmingham, AL) was added to each well and incubated for 1 hr at room temperature. The plates were then washed in PBS-Tween, and a 1:4,000 dilution of peroxidase-labeled avidin (Sigma Chemical Co., St. Louis, MO) was added. After incubation for 1 hr at room temperature, the plates were washed again and the substrate 2,2'-azino-bis-(3-ethylbenzthiazolinesulfonic acid) (ABTS) (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added. After appro-

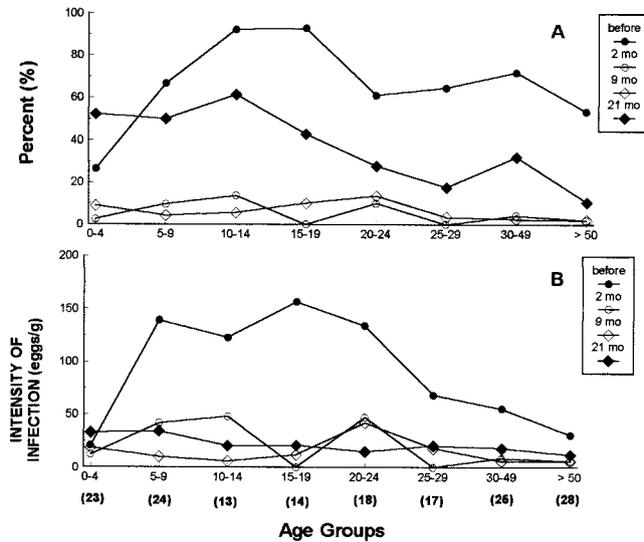


FIGURE 1. Prevalence (A) and intensity of infection with *Schistosoma mansoni* (B) in Siqueira, Brazil before and 2, 9, and 21 months (mo) after treatment. Values in parentheses are numbers of patients.

appropriate color development, the reaction was measured at 405 nm using an automated ELISA reader.

Statistical analysis. The lymphoproliferative data were expressed as the mean cpm of triplicate experimental cultures minus the mean cpm of triplicate control (unstimulated) cultures. Statistical differences were assessed by using a paired Student's *t*-test when comparing the group before and after treatment and an unpaired *t*-test when comparing the 2 groups. Analysis of variance was used with multiple measures. The isotype data were expressed as optical density and the Student's *t*-test was used to evaluate the statistical significance of differences between and or within groups. The effect of the co-variables age, sex, water contact, and other parasites in the cellular response to schistosome antigens were analyzed using regression analysis.

RESULTS

Figure 1 shows the age/prevalence and age/intensity curves for schistosome infections in Siqueira before and after treatment. As observed in most studies of endemic areas, before treatment the prevalence, as well as the intensity of infection, are highest in younger individuals. In the second and third stool surveys (2 and 9 months after treatment), the prevalence decreased to 10.6% and 12.0% (Figure 1A), respectively, and the intensity of infection decreased to 27.3 and 16.4 eggs per gram of feces, respectively (Figure 1B). The susceptible and resistant groups were retrospectively selected 21 months after treatment by using the criteria described in the Patients, Materials, and Methods. At this time, a reasonable increase in the mean prevalence data (36.2%) was observed, although the intensity of the infection after treatment did not increase during the period of observation. As expected, the percent of reinfection observed was particularly high in individuals less than 19 years of age (Figure 1A).

Table 2 shows data on the pretreatment ages, fecal eggs

TABLE 2

Characteristics of the patients included in the susceptible (SUS) and resistant (RES) groups*

Characteristics	Patients groups	
	SUS	RES
Mean age ± SD (years)	16.5 ± 9.8	39.2 ± 17.2
Percent male	68	57
Percent female	32	43
Eggs/gram of feces ± SD	222 ± 457	96 ± 173
Water contact index ± SD	175 ± 143	191 ± 143

* Bold numbers indicate pair differences that are statistically significant (*P* < 0.001).

count, sex distribution, and water contact of subjects within each study group. Analysis of susceptible and resistant groups showed a significant difference only in relation to age, with a higher mean age for the resistant group, while pretreatment differences in the intensity of infection observed in the 2 groups were not statistically different. Furthermore, no significant differences were observed between the groups in regard to either water contact or sex distribution (Table 2). In addition, both the susceptible and resistant groups had qualitatively similar prevalences of infection for other helminths. An inverse correlation between intensity of infection (eggs per gram of feces) and age was found for the susceptible (*r* = -0.087) and resistant (*r* = -0.408) groups, but was significant only for the resistant group (*P* < 0.05).

Schistosome antigenic preparations (SEA, SWAP, and CERC) and the mitogen PHA were used to study the lymphoproliferative responses of the patients. We confirmed (Figure 2) previous observations that pretreatment responses of PBMC from chronically infected patients are high for SWAP, low for SEA and intermediate for CERC.^{21,22} A consistent increase in SEA-stimulated proliferation is seen 21 months after treatment (*P* < 0.037) (Figure 2). When the proliferative responses to SEA were examined in the susceptible and resistant groups, patients in both groups contributed to the increase in the responsiveness seen 21 months after treatment (Figure 3). The PHA-induced responses in these patients remained high throughout the study with var-

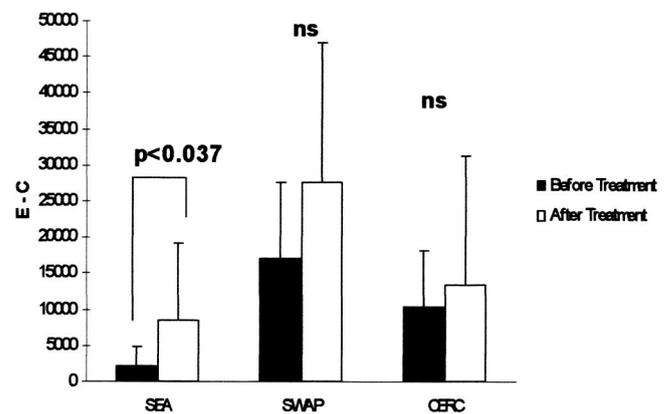


FIGURE 2. Peripheral blood mononuclear cell (PBMC) responses to *Schistosoma mansoni* antigens before and after treatment. Results are mean ± SD counts/minute. E-C = the mean cpm of triplicate experimental cultures minus the mean cpm of triplicate control (unstimulated) cultures; ns = not significant; SEA = soluble egg antigen; SWAP = soluble worm antigen preparation; CERC = cercariae antigens.

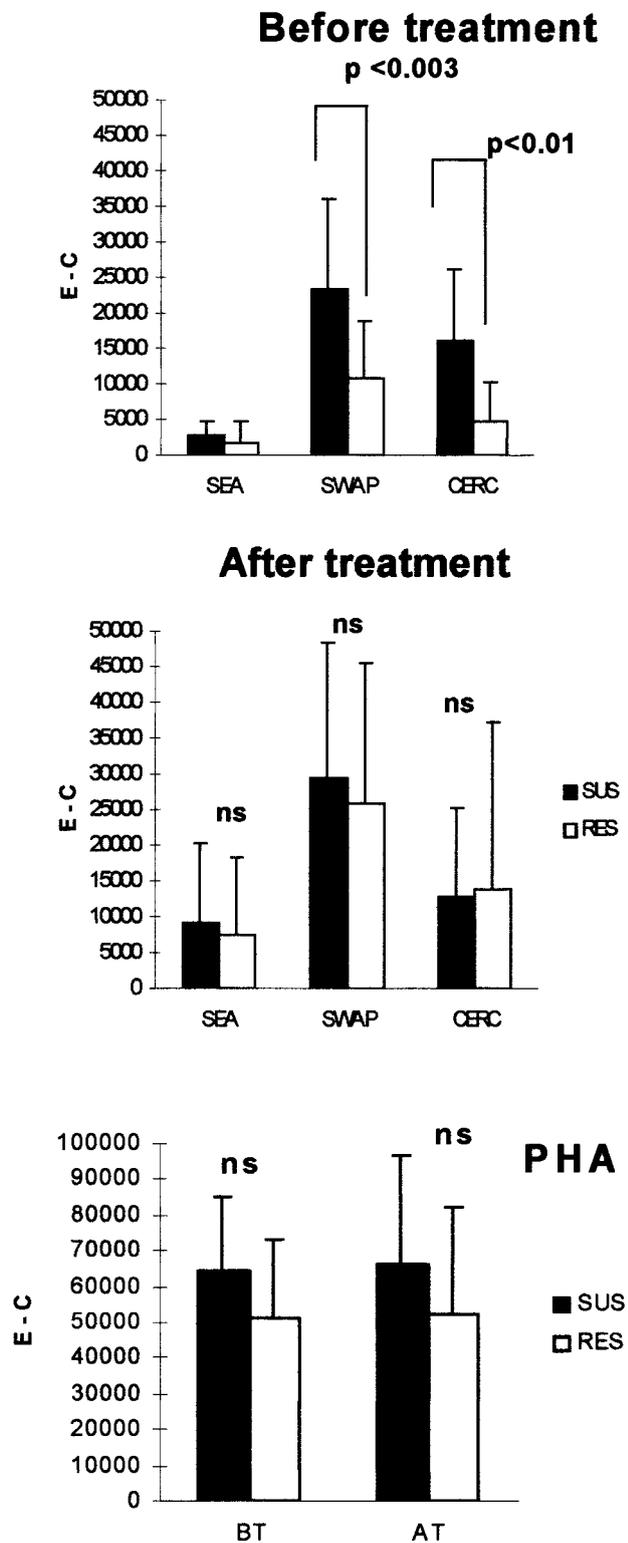


FIGURE 3. Peripheral blood mononuclear cell (PBMC) responses to *Schistosoma mansoni* antigens and the mitogen phytohemagglutinin (PHA) in the susceptible (SUS) and resistant (RES) groups before and after treatment. Results are the mean \pm SD counts/minute. BT = before treatment; AT = after treatment. For definitions of other abbreviations, see Figure 2.

iations that were not statistically significant ($P < 0.77$). Regression analysis did not show any effect of the co-variables age, sex, water contact, and other parasites in terms of the cellular proliferative responses to schistosome antigens.

The relative reactivity of the different antibody isotypes of all immunoglobulin classes and IgG subclasses against SEA, SWAP, and CERC was determined in serum from all individuals in the susceptible and resistant groups (Table 3). A comparison between both groups before treatment and after reinfection of the patients in the susceptible group showed significant differences in several isotypes. In addition, there were strong specific IgG1, IgG4, and IgM responses, particularly to SEA and CERC, in both groups. The reactivities of all isotypes were lower to SWAP. The anti-SWAP and anti-CERC IgM levels were significantly higher in resistant group compared with the susceptible group (SWAP; $P < 0.002$ and CERC; $P < 0.013$). This was true both before and after treatment for anti-SWAP and at 21 months after treatment for anti-CERC. An increase in anti-SWAP IgE and a decrease in anti-SEA IgG4 were observed in the resistant group after treatment (Table 3). Levels of IgE and IgG4 were further evaluated against antigens obtained from the tegument of the schistosomule (STEG), which may be a primary target of the protective immune response. Participation of these specific antibodies in resistance has been suggested by Rihet and others.¹¹ The level of antibodies to STEG in the susceptible and resistant groups did not differ before treatment. On the other hand, after treatment and reinfection, there was a significant increase in the level of IgE, which coincides with a decrease in the level of IgG4 (Figure 4). These findings are in agreement with previous observations of several laboratories using other antigens.^{5,9,11,12} Although the IgE response to CERC antigens was higher than those to the other antigens tested, there were no statistically significant differences between the susceptible and resistant groups.

DISCUSSION

It has been shown that persons living in areas endemic for *S. mansoni* express different levels of resistance to the parasite.^{1,2,4,10} For example, several studies of human schistosomiasis have demonstrated that children are generally more susceptible to reinfection than adults.^{1,4} However, the nature of this age-related resistance, although extensively studied, is still debated. It is possible that decreasing water contact with age could, in part, explain the age-intensity curve observed in some epidemiologic settings.^{27,28} In a study of immigrants that attempted to deal with the confounders age and exposure, it was observed that duration of infection may not contribute to these patterns, but that changes due to age, such as host physiology and immune system changes, may be most critical.²⁹ Nevertheless, in existing endemic settings, extant immunologic differences were also observed between these 2 age groups.^{1,2,4,9,11-13} Furthermore, in previous reports we have compared the immunologic profiles of adult individuals with patent infections with those of endemic normals; i.e., adults who live in endemic areas and have documented frequent water contact but do not become infected. These comparisons demonstrated

TABLE 3

Comparative analysis of anti-SEA, anti-SWAP and anti-CERC isotype reactivities of susceptible (SUS) and resistant (RES) groups before and 21 months after treatment*

Isotypes	Before treatment		21 months after treatment	
	SUS	RES	SUS	RES
Anti-SEA				
IgA	0.298 ± 0.205	0.225 ± 0.099	0.258 ± 0.091	0.334 ± 0.243
IgM	0.570 ± 0.339	0.541 ± 0.345	0.510 ± 0.206	0.495 ± 0.316
IgE	0.152 ± 0.051	0.151 ± 0.090	0.220 ± 0.068	0.169 ± 0.074
IgG1	0.518 ± 0.194	0.477 ± 0.163	0.388 ± 0.141	0.379 ± 0.125
IgG2	0.303 ± 0.325	0.284 ± 0.249	0.292 ± 0.277	0.281 ± 0.321
IgG3	0.137 ± 0.083	0.200 ± 0.111	0.192 ± 0.123	0.171 ± 0.063
IgG4	1.025 ± 0.359	0.845 ± 0.473	0.866 ± 0.342	0.568 ± 0.337
Anti-SWAP				
IgA	0.049 ± 0.041	0.036 ± 0.037	0.053 ± 0.053	0.081 ± 0.054
IgM	0.064 ± 0.087	0.212 ± 0.139	0.036 ± 0.046	0.268 ± 0.301
IgE	0.115 ± 0.053	0.140 ± 0.094	0.096 ± 0.065	0.159 ± 0.062
IgG1	0.379 ± 0.234	0.249 ± 0.137	0.253 ± 0.122	0.217 ± 0.087
IgG2	0.033 ± 0.028	0.065 ± 0.039	0.047 ± 0.037	0.005 ± 0.014
IgG3	0.106 ± 0.050	0.114 ± 0.073	0.051 ± 0.047	0.082 ± 0.057
IgG4	0.261 ± 0.249	0.192 ± 0.200	0.184 ± 0.185	0.109 ± 0.089
Anti-CERC				
IgA	0.414 ± 0.208	0.360 ± 0.146	0.366 ± 0.098	0.328 ± 0.110
IgM	0.454 ± 0.209	0.494 ± 0.316	0.387 ± 0.162	0.565 ± 0.238
IgE	0.590 ± 0.057	0.500 ± 0.170	0.585 ± 0.071	0.568 ± 0.088
IgG1	0.618 ± 0.122	0.501 ± 0.110	0.537 ± 0.056	0.365 ± 0.046
IgG2	0.526 ± 0.224	0.470 ± 0.126	0.409 ± 0.109	0.433 ± 0.187
IgG3	0.376 ± 0.072	0.453 ± 0.083	0.403 ± 0.106	0.424 ± 0.129
IgG4	0.378 ± 0.136	0.410 ± 0.170	0.361 ± 0.111	0.416 ± 0.078

* SEA = soluble egg antigen; SWAP = soluble worm antigen preparation; CERC = cercariae antigens. Data are the mean ± SD optical density. Bold numbers indicate differences that are statistically significant ($P < 0.05$) between groups before and after treatment. See details in Patients, Materials, and Methods.

distinctly different immunologic patterns between individuals with patent infections and endemic normals.^{30,31}

Some of the same parameters for the analysis of the immune response of endemic normals and infected individuals in this study were used to compare the immune responses of all participating susceptible and resistant individuals identified retrospectively 21 months after treatment in the endemic area of Siqueira. The resistant group in this study differed from those in other studies since we included only those individuals who did not become reinfected during the entire period of the observation. Also, the only selection bias for inclusion in the study and analysis was willingness to

participate throughout the study. It might be argued that partially resistant patients with very low worm burdens were included in the susceptible group and that this might change their immunologic pattern. However, it should be hypothesized that the presence of eggs in the host may also interfere with the development of resistance, resulting in immunologic patterns similar to those of infected individuals, as has been suggested in experimental *S. mansoni* infections.³²⁻³⁴ Therefore, our study using the criteria described above to identify the susceptible and resistant groups may provide additional information of considerable importance in the current understanding of acquired resistance after chemotherapy for human schistosomiasis. However, within the limits of 3 stool samples examined by double Kato-Katz smears, we can not distinguish between a very low infection and no infection.

In the present study, lymphoproliferative responses before treatment and 21 months after treatment were analyzed for SEA, SWAP, and CERC. As previously shown by Gazzinelli and others,²² Colley and others,³⁵ and Vieira and others,²⁵ responses of individual patients to the various schistosome antigens are highly variable. The common exception is the response to SEA, which in chronically infected patients before treatment is always low. An increase in the PBMC proliferative response to SWAP and CERC after treatment was not observed in this study, as has been previously reported.^{35,36} This apparent discrepancy may be explained by the difference in time of blood collection after treatment. However, our observation agrees with the report of Ribeiro and others,³⁷ in which an elevation in the cellular response to

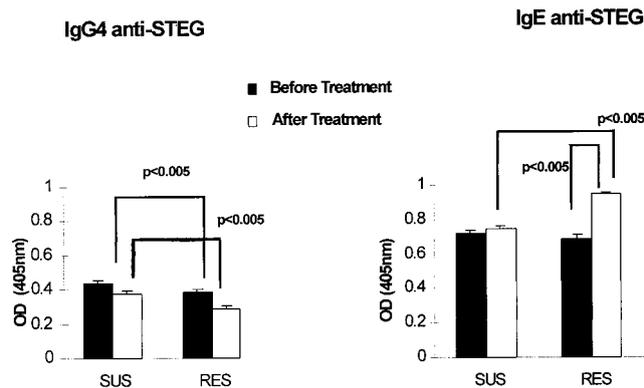


FIGURE 4. Levels of IgE and IgG4 antibodies to schistosomal tegument (STEG) in the susceptible (SUS) and resistant (RES) groups before and after treatment. Results are the mean ± SD optical density (OD) at 405 nm.

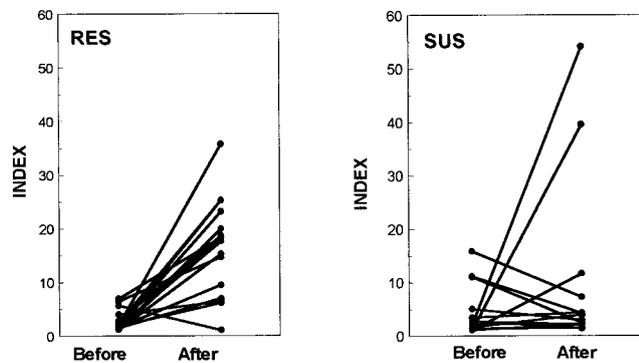


FIGURE 5. Peripheral blood mononuclear cell response, expressed as an index (counts/minute [cpm] of the stimulated culture divided by the cpm of the unstimulated culture), to soluble egg antigen in the susceptible (SUS) and resistant (RES) groups before and after treatment.

schistosomula and adult worm antigens after treatment in an endemic area in Brazil was not observed, except in some patients with very low responses to schistosome antigens prior to treatment. A significant elevation of the previously poor response to SEA after chemotherapy was observed in both the susceptible and resistant groups (Figure 2). However, when individual responses are compared, only 2 patients in the susceptible group showed an elevated response after treatment (Figure 5). These data indicate that only those responses that are strongly modulated during infection show increases after treatment and only in the group that was not reinfected. The blastogenesis response to PHA did not change during the period of observation either for the SUS or RES groups.

Several hypotheses have been proposed to explain the susceptibility of young individuals to reinfection. Although they are able to mount both cellular and humoral responses against the parasite antigens, as a group they are always more susceptible to reinfection after treatment. One hypothesis is that IgM and IgG2 antibodies elicited against carbohydrate epitopes expressed on the parasite eggs also react with epitopes present on the schistosomula surface, competing for the epitopes and therefore blocking the binding of protective antibodies.⁸ Other investigators have shown an association between an increased level of IgG4 and susceptibility to reinfection with a consequent inverse relationship between this isotype and IgE. These investigators suggested that IgG4 and IgE were blocking and effector (protective) antibodies, respectively.^{5,12,13}

In the current study, we observed a post-treatment reduction in the levels of IgG4 anti-SEA and anti-STEG in the resistant patients, which coincided with increases in the levels of IgE anti-STEG and anti-SWAP. These results were similar to our previous observations of pre-existent isotypes levels in endemic normals who exhibited high levels of anti-STEG IgE and low levels of anti-STEG IgG4. Together, these results from 2 different types of apparently protected groups of people support the hypothesis that an appropriate high IgE:IgG4 ratio is consistent with development of resistance to reinfection.^{5,9,11}

As previously reported,³¹ a relative increase in levels of IgM and IgG4, which are minor isotypes in non-infected

individuals, was observed in most of the patients in this study. The observation of certain antibody specificities and isotypes in comparisons of susceptible and resistant patients in relation to reinfection after chemotherapy has been reported by several investigators.^{2,5,8,9,11,12,14-16,38-41} Resistance in these studies was positively correlated with elevated IgE and IgA responses, and negatively correlated with IgG4, IgM, and IgG2 responses. In the present study, we observed higher levels of anti-SWAP and anti-CERC IgM in resistant patients when compared with the susceptible group. This was true both before and after treatment. The reactivity values for SEA did not differ significantly. Similar results were previously obtained when endemic normals (putatively resistant) were compared with individuals with patent infections.³⁰ This is an interesting finding because in spite of several studies regarding IgM reactivity reported earlier, it has also been reported that there is an increase in IgM anti-SmW68 with the age of patients and that high levels of this antibody have an inverse correlation with worm load.^{18,42}

We also observed higher anti-SEA (susceptible = 0.343, resistant = 0.468), anti-SWAP (susceptible = 0.470, resistant = 0.559), and anti-CERC (susceptible = 0.633, resistant = 0.943) IgG3:IgG1 ratios when the susceptible and resistant groups were compared. Similar findings have been observed in patients with onchocerciasis by Boyer and others, suggesting the possible involvement of IgG3 in acquired immunity to *Onchocerca volvulus*.⁴³ However, the role of the higher IgG3:IgG1 ratio in schistosomiasis still needs to be clarified.

In conclusion, we found several similarities between the immune responses expressed by persons categorized epidemiologically and parasitologically as resistant to schistosomiasis and as endemic normals. These include higher levels of IgE antibodies to *S. mansoni* STEG and SWAP antigens compared with levels of IgG4 antibodies to *S. mansoni* STEG and SEA antigens, and the relative increase in IgM levels in the resistant and putatively resistant groups compared with susceptible and patently infected individuals, respectively. These results suggest that these IgM antibodies do not function as blocking antibodies in our study group and that expression of immunity depends on balance between levels of IgE and IgG4 antibodies.

Financial support: This study was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, National Institutes of Health grant AI-26505-07, FINEP, CDC, CNPq (Brazil), and FAPEMIG (Minas Gerais, Brazil).

Authors' addresses: Iramaya R. Caldas and Rodrigo Correa-Oliveira, Laboratório de Imunologia Celular e Molecular do Centro de Pesquisas René Rachou-FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil. Enrico Colosimo, Departamento de Estatística de Ciências Exatas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. Omar S. Carvalho and Cristiano L. Massara, Laboratório de Helmintos Intestinais do Centro de Pesquisas René Rachou-FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil. Daniel G. Colley, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F22, 4770 Buford Highway, NE, Atlanta, GA 30341-3724. Giovanni Gazzinelli, Santa Casa Hospital and Centro de Pesquisas René Rachou-FIOCRUZ, Av. Augusto de Lima 1717, Barro Preto, CEP 30190-002 Belo Horizonte, Brazil.

Reprint requests: Giovanni Gazzinelli, Centro de Pesquisas René Rachou-FIOCRUZ, Av. Augusto de Lima 1715, Barro Preto, CEP 30190 002, Belo Horizonte, Minas Gerais, Brazil.

REFERENCES

- Butterworth AE, Capron M, Cordingley JS, Dalton PR, Dunne DW, Kariuki HC, Kimani G, Koech D, Muganbi M, Ouma JH, Prentice MA, Richardson BA, Arap-Siongok TK, Strurrock RF, Taylor DW, 1985. Immunity after treatment of human schistosomiasis mansoni II. Identification of resistant individuals and analysis of their immune responses. *Trans R Soc Trop Med Hyg* 79: 393–408.
- Dessein AJ, Begley M, Demeure C, Caillol D, Fueri J, Reis MG, Andrade Z, Prata A, Bina JC, 1988. Human resistance to *Schistosoma mansoni* is associated with IgG reactivity to a 37-kDa larval surface antigen. *J Immunol* 140: 2727–2736.
- Dunne DW, Bickle QD, 1987. Identification and characterization of a polysaccharide-containing antigen from *Schistosoma mansoni* eggs which cross-reacts with the surface of schistosomula. *Parasitology* 94: 255–268.
- Wilkins HA, Blumenthal VJ, Hagan P, Hayes RJ, Tulloch S, 1987. Resistance to reinfection after treatment of urinary schistosomiasis. *Trans R Soc Trop Med Hyg* 81: 29–35.
- Hagan P, Blumenthal UJ, Dunne DW, Simpson AJG, Wilkins HA, 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 349: 243–245.
- Sturrock RF, Butterworth AE, Houba V, 1976. *Schistosoma mansoni* in baboon (*Papio anubis*): parasitological responses of Kenyan baboons to different exposures of a local parasite strain. *Parasitology* 73: 239–246.
- Barbour AD, 1985. The importance of age and water contact patterns in relation to *Schistosoma haematobium* infection. *Trans R Soc Trop Med Hyg* 79: 151–157.
- Butterworth AE, Bensted-Smith R, Capron A, Dalton PR, Dunne DW, Grzych JM, Kariuki HC, Khalife J, Koech D, Muyambi M, Ouma JH, Arap Siongok JK, Strurrock RF, 1987. Immunity in human schistosomiasis mansoni: prevention by blocking antibodies of the expression of immunity in young children. *Parasitology* 94: 269–280.
- Dunne DW, Butterworth AE, Fulford AJC, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Strurrock RF, 1992. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 22: 1483–1494.
- Butterworth AE, Dunne DW, Fulford A, 1988. Immunity in human schistosomiasis mansoni: cross-reactive IgM and IgG2 antibodies block the expression of immunity. *Biochimie* 70: 1053–1063.
- Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ, 1991. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol* 21: 2679–2686.
- Demeure EC, Rihet P, Abel L, Ouattara M, Bourgois A, Dessein AJ, 1993. Resistance to *Schistosoma mansoni* in humans: influence of the IgE/IgG4 balance IgG2 in immunity to reinfection after chemotherapy. *J Infect Dis* 168: 1000–1009.
- Grogan JL, Kremsner PG, van Dam GJ, Metzger W, Mordmuller B, Deelder AM, Yazdanbakhsh M, 1996. Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. *J Infect Dis* 173: 1242–1247.
- Webster M, Fallon PG, Fulford AJC, Butterworth AE, Ouma HJ, Kimani G, Dunne DW, 1997. Effect of praziquantel and oxamniquine treatment on human isotype responses to *Schistosoma mansoni*: elevated IgE to adult worm. *Parasite Immunol* 19: 333–335.
- Zhaosong Z, Haiwei W, Suchen C, Lingshen H, Zhangwu X, Yingxi Q, Chuan S, Jian-Ping C, Yiqing W, Soaji Z, Gualing W, 1997. Association between IgE antibody against soluble egg antigen and resistance to reinfection with *Schistosoma japonicum*. *Trans R Soc Trop Med Hyg* 91: 606–608.
- Grzych JM, Grezel D, Xu BC, Neyrinck JL, Capron M, Ouma JH, Butterworth AE, Capron A, 1993. IgA antibodies to a protective antigen in human schistosomiasis mansoni. *J Immunol* 150: 527–535.
- Xu BC, Verwaerde C, Grzych JM, Fantaine J, Capron A, 1991. A monoclonal antibody blocking the *Schistosoma mansoni* 28 kDa glutathione-S-transferase activity reduces females worn fecundity and egg viability. *Eur J Immunol* 21: 1801–1807.
- King CH, Ibiary SE, Nawawi ME, Sawyer J, Griffin A, Hawey AE, Mahmoud AAF, 1989. Intensity of *Schistosoma mansoni* infection in a human population is inversely correlated with antibodies response to SmW68, a protective parasite antigen. *J Infect Dis* 160: 686–691.
- Katz N, Chaves A, Pellegrino J, 1972. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* 14: 397–400.
- Lima e Costa MFF, Magalhães MHA, Rocha RS, Antunes CMF, Katz N, 1987. Water-contact patterns and socio-economic variables in the epidemiology of schistosomiasis mansoni in an endemic area in Brazil. *Bull World Health Organ* 65: 57–66.
- Colley DG, Cook JA, Freeman GL, Bartholomew RK, Jordan P, 1977. Immune responses during human schistosomiasis mansoni I. *In vitro* lymphocyte blastogenic responses to heterogeneous antigenic preparations from schistosome eggs worms and cercariae. *Int Arch Allergy Appl Immunol* 53: 420–443.
- Gazzinelli G, Katz N, Rocha RS, Colley DG, 1983. Immune responses during human schistosomiasis mansoni X. Production and standardization of an antigen-induced mitogenic activity by peripheral blood mononuclear cells from treated but not active cases of schistosomiasis. *J Immunol* 130: 2891–2895.
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265–275.
- Ramalho-Pinto FJ, Gazzinelli G, Howells RE, Mota-Santos TA, Figueiredo EA, Pelegriño J, 1974. *Schistosoma mansoni*: defined system for stepwise transformation of cercariae to schistosomula *in vitro*. *Exp Parasitol* 36: 360–362.
- Vieira LQ, Colley DG, Souza CPS, Gazzinelli G, 1987. Stimulation of peripheral blood mononuclear cells from patients with schistosomiasis mansoni by living and fixed schistosomula and schistosomular membrane extracts and vesicles. *Trans R Soc Trop Med Hyg* 36: 83–91.
- Lunde MN, Ottesen EA, Cheever AW, 1979. Serological differences between acute and chronic schistosomiasis mansoni detected by enzyme-linked immunosorbent assay (ELISA). *Am J Trop Med Hyg* 28: 87–91.
- Butterworth AE, 1994. Human immunity to schistosomes: some questions. *Parasitol Today* 10: 378–380.
- Gryseels B, 1994. Human resistance to *Schistosoma* infections: age or experience? *Parasitol Today* 10: 380–384.
- Ouma JH, Fulford JC, Kariuki HC, Kimani G, Sturrock RF, Muchemi G, Butterworth AE, Dunne DW, 1998. The development of schistosomiasis mansoni in an immunologically naive immigrant population in Masongaleni, Kenya. *Parasitology* 117: 123–132.
- Viana IRC, Sher A, Carvalho OS, Massara CL, Eloi-Santos SM, Pearce EJ, Colley DG, Gazzinelli G, Correa-Oliveira R, 1994. Interferon- γ production mononuclear cells from residents of an area endemic for schistosomiasis mansoni. *Trans R Soc Trop Med Hyg* 88: 466–470.
- Viana IRC, Correa-Oliveira R, Carvalho OS, Massara CL, Colosimo E, Colley DG, Gazzinelli G, 1995. Comparisons of antibody isotype responses to *Schistosoma mansoni* antigens by infected and putative resistant individuals living in an endemic area. *Parasite Immunol* 17: 297–304.
- Pearce E, Caspar P, Grzych JM, Lewis FA, Sher A, 1991. Down-regulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth *Schistosoma mansoni*. *J Exp Med* 173: 159–166.
- Grzych JM, Pearce E, Cheever A, Caulada ZA, Gaspar P, Hieny F, Lewis F, Sher A, 1991. Egg deposition is the major stimulus for the production of TH2 cytokines in murine schistosomiasis mansoni. *J Immunol* 146: 1322–1327.
- Kullberg CM, Pearce EJ, Hieny SE, Sher A, Berzofsky JA, 1992. Infection with *Schistosoma mansoni* alters Th1/Th2 cytokine responses to a non-parasite antigen. *J Immunol* 148: 3264–3270.
- Colley DG, Barsoum IS, Dahawi SS, Gamil F, Habib M, El

- Alamy MA, 1986. Immune responses and immunoregulation in relation to human schistosomiasis in Egypt III. Immunity and longitudinal studies of *in vitro* responsiveness after treatment. *Trans R Soc Trop Med Hyg* 80: 952–957.
36. Roberts M, Butterworth AE, Kimani G, Kamau T, Fulford AJC, Dunne DW, Ouma JH, Strurrock RF, 1993. Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to reinfection. *Infect Immun* 61: 4984–4993.
37. Ribeiro AMJ, Almeida RP, Bacelar O, Araujo MI, Demure C, Bina JC, Dessein AJ, Carvalho EM, 1993. Correlation between cell-mediated immunity and degree of infection in subjects living in an endemic area of schistosomiasis. *Eur J Immunol* 23: 152–158.
38. Auriault C, Grass-Masse H, Pearce RJ, Butterworth A, Wolowczuk I, Capron M, Ouma JH, Balloul JM, Khalife J, Neyrinck JL, Tartar A, Koech D, Capron A, 1990. Antibody responses of *Schistosoma mansoni*-infected human subjects to the recombinant P28 glutathione-S-transferase and synthetic peptides. *J Clin Microbiol* 28: 1918–1924.
39. Iskander R, Das PK, Aalberse RC, 1981. IgG4 antibodies in Egyptian patients with schistosomiasis. *Int Arch Allergy Appl Immunol* 66: 200–207.
40. Khalife J, Capron M, Capron A, Grzych JM, Butterworth AE, Dunne DW, Ouma JH, 1986. Immunity in human schistosomiasis mansoni: regulation of protective immune mechanisms by IgM blocking antibodies. *J Exp Med* 164: 1626–1640.
41. Jassin RA, Hassan K, Catty D, 1987. Antibody isotypes in schistosomiasis mansoni. *Parasite Immunol* 9: 627–650.
42. King CH, Lett RR, Nanduri J, El Ibiary S, Peters PAS, Olds GR, Mahmoud AAF, 1987. Isolation and characterization of a protective antigen for adjuvant-free immunization against *Schistosoma mansoni*. *J Immunol* 139: 4218–4224.
43. Boyer AE, Tsang VCW, Eberhard ML, Zea-Flores G, Hightower A, Pilcher JB, Zea-Flores R, Zhou W, Reimer CB, 1991. Guatemalan human Onchocerciasis II. Evidence for IgG3 involvement in acquired immunity to *Onchocerca volvulus* and identification of possible immune-associated antigens. *J Immunol* 146: 4001–4010.