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BIOCHEMICAL AND IMMUNOLOGIC PREDICTORS OF EFFICACY OF TREATMENT OR REINFECTION RISK FOR *SCHISTOSOMA MANSONI*

ELIANA A. G. REIS, MITERMAYER G. REIS, RITA DE CÁSSIA R. SILVA, THEOMIRA M. A. CARMO, ANA MARLÚCIA O. ASSIS, MAURÍCIO L. BARRETO, ISABEL M. PARRAGA, MÔNICA LEILA P. SANTANA, AND RONALD E. BLANTON*

Oswaldo Cruz Foundation, Fiocruz, Salvador, Bahia, Brazil; School of Nutrition, Federal University of Bahia, Salvador, Bahia, Brazil; Institute of Collective Health, Federal University of Bahia, Salvador, Bahia, Brazil; Department of Nutrition, Case Western Reserve University, Cleveland, Ohio; Center for Global Health and Diseases, Case Western Reserve University, Cleveland, Ohio

Abstract. Most *Schistosoma mansoni* infections are egg-negative after a single dose of oxamniquine. A cohort of 661 infected children was treated at 6-month intervals and assessed for nutritional and parasitological status. Initial biochemical and immunologic markers were measured in a subset of 84 children. All were treated at the start of therapy and at 6 months. Immunoglobulins only served as markers for active infection. No markers were predictive of cure or reinfection, except initial infection intensity and serum low-density lipoprotein. Ten percent were persistently infected and had no change in infection intensity at any time-point. Several factors suggest that this group was biologically different. In addition to failing to reduce their worm burden, they had significantly higher initial intensity of infection (100 versus 65 eggs/g, $P = 0.001$) and significantly lower initial serum low-density lipoprotein (72 versus 104 mg/dL, $P = 0.045$). The biologic plausibility of this observation is discussed.

INTRODUCTION

Control of schistosomiasis at present depends on repeated rounds of chemotherapy at intervals of 1 or 2 years. On a national scale, such as the programs in Brazil or Egypt, follow-up stool surveys at 6 weeks after treatment are costly and not usually performed. Therefore, in practical terms, the efficacy of annual or biennial chemotherapy for control of schistosomiasis is the combined effects of the drug, the intensity of transmission, and the rapidity of reinfection. The two drugs most recently used in the treatment of *Schistosoma mansoni*, oxamniquine and praziquantel, have similar profiles for efficacy as single-dose therapies.[1] A consistent finding for both is that 10–20% of those treated fail to clear their infection, although nearly 100% experience a reduction in the intensity of infection.[1,2] The factors associated with this fairly constant level of treatment "failure" have been primarily associated with very heavy infection and intense transmission,[3,4] as well as some level of noncompliance with taking these drugs. Because the drug does not affect immature stages of the parasite, drug efficacy may seem to be lower where many individuals have experienced a new infection within 4–6 weeks of treatment. Reinfection after treatment also has some well-described associations. In addition to the intensity of transmission, age < 14[5] and sexual immaturity in humans have been associated with increased reinfection with *S. mansoni.*[6–8] Male sex has also been associated with increased susceptibility and severity of schistosomiasis, both in laboratory animals,[9] and population studies conducted in endemic areas.[10] To study whether other host factors, such as immune responsiveness or nutritional status, might be related to treatment outcome, we conducted a prospective cohort study of 106 infected children. We analyzed initial parasite burden, socioeconomic status, age, sex, anthropometric indices, a panel of biochemical markers, and immunoglobulin isotypes and assessed their ability to predict parasitological outcome in the treated children at 6 months and 1 year.

* Address correspondence to Ronald Blanton, Center for Global Health and Disease, Case Western Reserve University, Cleveland, OH 44106. E-mail reb6@case.edu

MATERIALS AND METHODS

Study site and population. The study was performed in the Brazilian town of Jequié, which is situated in a semi-arid region of the State of Bahia. There are ~ 135,000 inhabitants some of whom rely on a major river that borders the town for washing, sanitation, fishing, and recreation. A total of 13,771 children 7.0–17.9 years of age were surveyed for infection by school-based and house-to-house examinations in neighborhoods previously identified as at risk for schistosomiasis by the Brazilian National Foundation of Health (Fundação Nacional de Saude [FNS]). Of the 1,766 children infected, we elected to include all of those from the most heavily infected neighborhoods (661) in a prospective cohort study of determinants of response to oxamniquine. Some 19.3% of children were excluded for stool egg counts > 400/g of stool (400 epg). Those included were further evaluated for anthropometrics, dietary intake, hemoglobin level, and socio-economic status. Serum was collected for measurement of biochemical markers of nutrition, tumor necrosis factor (TNF), and immunoglobulins. This number was determined by the resources available for this part of the study. Complete data were obtained on 84 of those selected for serologic and biochemical studies. Informed consent was obtained for all study subjects before clinical and parasitological study and blood collection. This study was approved by the Human Investigation Committees of University Hospitals and the Oswaldo Cruz Foundation, Salvador, Bahia, Brazil.

Parasitology and treatment. Stools were collected in prelabeled plastic containers delivered to the children's homes. Two stool samples obtained on different days were examined for each participant. For each stool sample, two slides were made and examined using the quantitative method of Katz and others.[11] The slides were prepared and read within the first 2 hours, and every 10th slide was re-examined by an independent technician for quality control. Even three consecutive Kato-Katz thick smears may fail to detect up to 7% of infections[12]; thus, those in whom no eggs were detected in feces will be referred to as "egg-negative" rather than "cured" or "uninfected."

Oxamniquine (20 mg/kg) was administered to all partici-

pants under direct observation at the start of the study and again 6 months later. Only those still found to have schistosome eggs in stool were treated at 1 year.

Anthropometric studies. All measurements were carried out using standard anthropometric methods as described previously.[13,14] For each child measurement of weight, height, skinfold thicknesses, and arm circumference were made. Indices were calculated and converted to Z-scores for analysis.

Socioeconomic survey. To control for the effects of other covariates such as environmental factors, an index was constructed from data collected by questionnaire during home visits. To construct the index for environmental conditions, all of the variables were dichotomized and coded as 1 (poor) or 0 (good). Variables were selected to make up the index according to their association with anemia by a logistic regression model, because this is a significant morbidity associated with helminthic infection.[15] Sex, age, and degree of education of the head of household were used as covariates to adjust the regression model. Variables that remained in the model were source of water, regularity of water availability, place where water is kept, care taken with drinking water, presence and location of toilet, frequency of garbage collection, presence of trash around the house, the presence of an open sewer, type of flooring in the home, and composition of the house. The included variables were summed and classified as adequate (0–6) or inadequate (7–13) based on Reichenheim and Harpham.[16]

Biochemical assays. Morning blood was collected in mineral-free tube from all participants by venipuncture. Hemoglobin concentration was measured with a portable hemoglobinometer (HemoCue, Lagoon Hills, CA) from a drop of finger stick blood. All other analyses were made in Pediatric Gastroenterology Laboratory of the Paulista School of Medicine, Federal University of São Paulo using commercial kits for clinical laboratories. Assays performed were serum iron, total iron binding capacity (TIBC), transferrin saturation, zinc, albumin, and total protein. Total plasma cholesterol and triglycerides were measured using standardized kits that use enzymatic techniques (Synermed, Quebec, Canada).[17,18] High-density lipoprotein (HDL)-cholesterol was similarly measured after heparin manganese precipitation of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)-cholesterol.[19] LDL-cholesterol was calculated using the formula of Friedewald and others.[20]

Measurement of TNFα and immunoglobulins. Free TNFα was determined in serum by enzyme-linked-immunosorbent assay (ELISA), according to the manufacturer's directions (R&D Systems, Minneapolis, MN). Specific levels of IgM, IgA, IgE, IgG1, IgG2, IgG3, and IgG4 against soluble egg antigen (SEA) from *S. mansoni* were analyzed by ELISA, as described[21] with minor modifications. Normal human serum was obtained from individuals from a non-endemic area of Brazil with no history of helminth infection. Cut-off values were calculated using the mean for the endemic normal controls plus 3 SD.

Analytic approach. Statistical tests for intensity of infection were performed on log-transformed egg counts. Demographic and socio-economic differences of the study population and subsample were compared by χ^2 tests. ANACOVA was used to test the association between treatment outcome and baseline values for nutritional, biochemical, and immunologic indicators. In the final model, these parameters were adjusted for the confounders sex, age, and socio-economic status. ANACOVA was selected for this analysis, because there are both continuous and categorical independent variables. Student's *t* test was used to compare intensities of infection at 6 and 12 months with baseline. A significance level of 0.05 was used throughout. Statistical calculations were performed with the program SPSS (version 10.0).

RESULTS

Study population. Within the city as a whole the prevalence of infection among school children was 18.9% and the geometric mean intensity of infection was 109.5 eggs/g of feces (epg). Because one of the criteria was an epg < 400, 19.3% of children were excluded from the study on this basis. There were a total of 661 children between the ages of 7.0 and 17.9 entered in the study. Of these, serum was collected from 106 for measurement of immunologic and biochemical indicators. Slightly less than one half of the children were male, their mean age was 11.7 ± 2.7, and one half were considered to live in inadequate economic and environmental conditions (Table 1). There were no significant differences in sex, age, or socio-economic status between the total study group and those for whom biochemical and immunologic studies were performed. In the first 6 months, 4.4% were lost to follow-up and an additional 16.2% were lost in the final 6 months of the study. Similar losses were observed in the subsample. In all, 79.4% (525) of the total and 77.4% (84) of the subsample had complete parasitological follow-up. The characteristics of those lost to follow-up for repeat anthropometrics, stool examination, and/or blood studies did not differ significantly from the remaining population (data not shown).

Reinfection rates and characteristics of children. Of the total number of children examined, the prevalence of *S. mansoni* infection was 18.9%. All children were infected with *S. mansoni* at the start of the study. Six months after treatment, 27.5% (172/625) were still positive for *S. mansoni* eggs in their stool (Table 2). All children were treated again at that time and at 12 months. The proportion of those who were again egg-positive at the end of the study decreased to 21.1% (113/525, $P < 0.187$ by χ^2 compared with 6 months). The reinfection rate between 6 and 12 months was 15.6%. This rate was not significantly different for those included in the subsample.

Pretreatment factors associated with resistance to reinfection. For the whole sample, 10% were egg positive at both the

TABLE 1
Demographic of study population and subsample

	Study		Subsample*		
	N	Percent	*N*	Percent	*P*
Sex					
Male	389	58.9	56	52.8	0.269
Female	272	41.1	50	47.2	
Age (years)					
7–10	152	23.0	25	23.6	0.852
10–14	388	58.7	64	60.4	
14–18	121	18.3	17	16.0	
Socio-economic conditions					
Adequate†	299	49.0	43	43.0	0.264
Inadequate	311	51.0	57	57.0	

* Blood collection group for biochemical and immunoglobulin studies.
† According to a socio-economic and environmental index (see Methods).

TABLE 2
Infection characteristics of study and subsample

Infection characteristics	Study (n = 512)		Subsample* (n = 84)	
	Stool egg +	Percent	Stool egg +	Percent
Positive at 6 months	172	27.5	27	26.5
Positive at 12 months	113	21.5	20	23.3
Reinfected at 12 months	57	15.6†	10	16.9†
Persistently infected‡	51	10.0	8	9.5

* Blood collection group for biochemical and immunoglobulin studies.
† Egg negative at 6 months and positive at 12 months.
‡ Egg positive at 6 and 12 months. Persistently egg negative at 6 and 12 months. Study group: 308 negative/512 total; subsample: 49 negative/84 total. Between-group comparison for all infection characteristics, $P > 0.05$.

6- and 12-month periods. The initial intensity of infection was a strong factor in treatment outcome. Those still infected at 6 months had significantly higher mean geometric egg counts than those who were egg negative (Figure 1). Furthermore, the initial egg counts of those who became egg-negative after the second treatment were significantly lower than those persistently positive (Figure 2), but no different than those who were negative at 6 months. In those persistently positive, the intensity of infection was not significantly different at any time period before or after the initial treatment. A relatively higher initial egg count (in this case > 10 SD), therefore, was predictive only of those who would not respond despite only moderate intensities of infection.

Before treatment we measured immunoglobulin class, isotype, HDL, triglycerides, VLDL, LDL, cholesterol, total protein, albumin, globin, and hemoglobin. We evaluated how each was associated with outcome of treatment at 12 months in the 84 children with complete data. Variables that most closely correlate with nutritional status, such as total protein, albumin, and hemoglobin, did not differ between groups (Table 3). However, baseline cholesterol and LDL were significantly associated with negative stool examinations for *S. mansoni* at the end of 1 year (P = 0.011 and P = 0.008, respectively). Higher cholesterol and LDL were associated

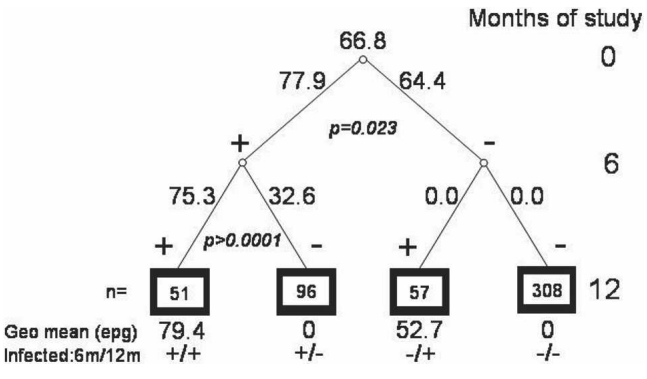

FIGURE 1. Informed consent was obtained for all study subjects before clinical and parasitological study and blood collection. This study was approved by the Human Investigation Committees of University Hospitals and the Oswaldo Cruz Foundation, Salvador, Bahia, Brazil. Geometric mean egg counts (epg) according to infection status at the 6- and 12-month interventions. The study population was retrospectively stratified according to their infection status at 6 and 12 months and the geometric mean epg of each group calculated. Thus, those infected still infected at 6 months started the study with a statistically higher epg (77.9) than those who were egg negative (64.4) at 6 months (P = 0.023).

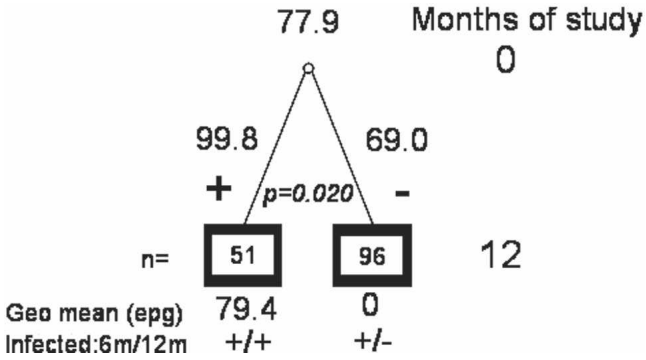

FIGURE 2. Geometric mean egg counts (epg) according to infection status comparing the 12-month intervention only. The study population was retrospectively stratified according to their infection status at 12 months and the geometric mean of each group calculated. Thus, the starting epg of those who would eventually be egg negative at the end of 12 months (99.8) were well separated from than those who were egg negative (69.0) at 12 months (P = 0.020).

with a better outcome at 1 year. Comparing the cholesterol and LDL levels of those with the best response (negative at 6 months and 1 year) with the worst (positive at 6 months and 1 year), the difference between the cholesterol and LDL measurements remained significant or borderline significant (cholesterol, 150.6 ± 34.9 versus 118.0 ± 118.00; P = 0.052; LDL: 104.0 ± 32.4 versus 72.0 ± 27.2, P = 0.045). There were no differences between those who were positive at only 6 months or only 12 months. By Pearson's correlation, there was no significant correlation between LDL and initial or 6-month intensity of infection, but LDL was significantly negatively correlated with intensity of infection at 12 months (r = –0.30, P = 0.029). No differences in socio-economic, anthropometrics, or lipid intake were noted between those persistently egg-positive and the other groups.

Changes in *S. mansoni*–specific immunoglobulins and resistance to reinfection. Most immunoglobulin isotypes show no pre-treatment differences between those who were egg-negative and those still positive at 6 or 12 months. The change in immunoglobulin class and IgG isotype between 0 and 12 months was consistent only with the presence of detectable eggs in the stool (Table 4). Parasite-specific IgG4, IgG3, and IgM at each time-point were elevated in those who were infected and not in those who were egg negative. A direct measurement of TNFα as well as VLDL concentration, an indirect measure of TNFα activity,[22,23] failed to show an association with cure at any time-point (Table 3).

DISCUSSION

In this study, ~ 10% of the cohort either failed treatment or were rapidly reinfected. Considering those that were negative at 6 months and became positive at 12 months, we know that the reinfection rate for some of the children is ~ 16% over a 6-month period. Because the percent of positives at 6 months was 27–26%, the drug "failure-to-cure" rate was ~ 10%. This is expected and only confirms that this group and this treatment were not extreme in any way. There were several findings, however, that suggest that the group that failed to cure despite two rounds of treatment were not merely unlucky, but differed significantly in their behavior or their biology. This

TABLE 3
Mean pre-treatment values for egg negative and egg positive individuals at 12 months

	Egg negative		Egg positive			
	Mean ± SD	*n*	Mean ± SD	*n*	*P**	*P*†
Total protein (g/dL)	7.39 ± 0.64	45†	7.38 ± 0.63	8	0.996	0.848
Albumin (g/dL)	4.38 ± 0.57	45	4.17 ± 0.48	8	0.348	0.295
Total cholesterol (mg/dL)	156.31 ± 41.50	49	123.90 ± 21.52	9	0.027	0.011
LDL (mg/dL)	108.05 ± 41.44	41	72.89 ± 23.68	9	0.018	0.008
VLDL (mg/dL)	17.55 ± 9.08	49	19.33 ± 12.81	9	0.614	0.374
TNFα§ (mg/dL)	8.34 ± 2.85	62	7.86 ± 2.60	16	0.546	0.763

* Unadjusted *P* values.
† *P* values adjusted for sex, age, and socio-economic status.
‡ Measurements of some variables was limited by quantities of serum available.
§ No differences were observed for immunoglobulin isotypes, globulin, hemoglobulin, HDL, or triglycerides, and anthropometric status.

group had a significantly higher intensity of infection from all other groups at the start of the study, at 6 months after one round of chemotherapy, and at 12 months after two rounds of chemotherapy. Whereas most infections, even when the mean intensity of infection is > 1,000 epg, will respond with nearly a 90% reduction in intensity,[24] there was no significant difference between the starting intensity and the final intensity for the persistently positive individuals.

In addition, while all nutritional parameters and markers including anthropometrics and measurement of macro- and micronutrients showed no differences between outcome groups, the lowest mean total cholesterol and LDL were associated with those who failed to clear their infection or even reduce its intensity at any time. This effect was essentially isolated to LDL and was not merely a marker for the adequacy of nutrition. Other and better markers of nutritional status showed no differences, and the concentrations of other types of cholesterol were similar for those egg-negative compared with those egg-positive at 1 year. Furthermore, LDL was not a marker for the initial intensity of infection, the other factor associated with drug efficacy, because there was no correlation between these variables. For the ages studied here, the normal range for LDL cholesterol is 50–170 mg/dL. No values were above the upper limit, and only two individuals were below the lower limit; therefore, the difference between the groups was not the result of pathologic lipid concentrations.

The trivial explanation for persistence in this group would be that these children consistently failed to take the oxamniquine. This is highly unlikely because the medicine was administered under direct observation as a suspension and not a pill. Resistance to oxamniquine might also explain persistence, but true resistance is not common and rarely clinically relevant at the scale of mass treatment or public health. In the Robert Toll outbreak in Senegal, "pseudo" resistance was observed because of the high level of transmission.[4] In this outbreak, many people probably presented with recent infections at the time of treatment. Because the parasite is only susceptible praziquantel at 4–6 weeks after infection, these individuals seemed not to respond. Furthermore, transmission was alarmingly intense when the mean infection intensity was near 1,000 epg and the prevalence was 91%. Thus, rapid reinfection was probably the rule. Neither of these factors would seem to have been operative in Jequié. First, the mean intensity of infection of all of those infected was relatively low as was the prevalence, which suggests a low rate of transmission. Second, oxamniquine in contrast to praziquantel seems to be able to affect immature stages of *S. mansoni.*[25]

Differences in host immune response or host behavior could also account for differences in apparent drug efficacy. The only immunologic markers tested (immunoglobulins and TNFα) did not show any differences between outcome groups. Although behavior cannot be entirely ruled out as a factor in the persistence of the infection, it should be noted that the prevalence and intensity of infection in this community suggests a relatively low level of transmission. True extremes in behavior such as occupational exposure to contaminated waters would be expected to observe such a constant high level of infection, and there is no direct evidence for such behavior among children attending school.

While multiple factors were tested, many of them were correlated or mutually dependent. The problem of multiple testing is usually addressed by correcting. While it is difficult to say exactly what value should be used for adjustment, we estimate that 12 factors were independent (height, weight, albumin/total protein, LDL, HDL, IgM, IgG1, IgG2, IgG3, IgG4/IgE, and IgA). Using the Bonferroni correction, $P < 0.004$ would be considered significant under these conditions. This correction is known to be very stringent and was taken as a guideline for significance. The *P* value obtained for differences in initial LDL concentration for those who remained negative compared with those who did not respond or became reinfected was highly significant before correction ($P = 0.009$), and there are multiple biologic mechanisms by which LDL could be associated with drug efficacy and/or resistance to reinfection.

Two potential mechanisms by which even normal LDL levels might affect drug efficacy are by LDL's influence on immune responses and by modification of drug metabolism and delivery. The immune response is an important component of the mechanism of action for praziquantel.[26–29] Immunosuppression is associated with decreased efficacy of oxamniquine

TABLE 4
Immunoglobulins at 12 months post-treatment

Immunoglobulin class/isotype	Egg negative ($n = 52$)	Egg positive ($n = 14$)	*P**	*P*†‡
IgM	0.45 ± 0.32	0.62 ± 0.25	0.070	0.013
IgA	0.06 ± 0.063	0.05 ± 0.03	0.416	0.532
IgE	0.010 ± 0.020	0.01 ± 0.02	0.862	0.935
IgG1	0.61 ± 0.61	0.88 ± 0.65	0.231	0.257
IgG2	0.01 ± 0.04	0.09 ± 0.01	0.423	0.386
IgG3	0.05 ± 0.01	0.05 ± 0.05	0.951	0.780
IgG4	0.07 ± 0.15	0.38 ± 0.35	< 0.001	< 0.001

Values are mean ± SD.
* Unadjusted *P* value.
† *P* value adjusted for sex, age, and socio-economic level.
‡ No differences were observed between the groups at the start of the study.

in experimental mice, but reconstitution of these animals with immune serum fully restores effectiveness. While much has been written about the deleterious effect of immune responses to LDL-cholesterol,[30] the pro-inflammatory properties of this form of lipid might be responsible for the effects we see here by altering the immune response to interluminal tissues including those of the parasite.

Because LDL is a major carrier of lipid-soluble or lipid-associated drugs, a second major possibility is that LDL levels correlate with the availability of this carrier in the blood stream. Oxamniquine is a weak amphipathic base that incorporates into liposomes with high efficiency (> 85%),[31] and the liposomal preparation of the drug has a higher experimental efficacy than the unmodified form. The incorporation of the drug into liposomes or its association with LDL may protect it from metabolism and earlier elimination leading to higher levels and or a longer half-life. Lipid-associated drug may also tend to be targeted to the liver or parasite membranes.

Because schistosomes are completely dependent on the host for cholesterol and fatty acids, they require a transport system for lipids. Multiple studies have shown the ability of parasite membranes to bind host LDL.[32,33] This is mediated at least in part by an abundant and efficient VLDL/LDL-binding protein.[34] The requirement for host lipids obligates transport of the drug as well. This may explain a mechanism by which the lipid status of the host influences this process. For trypanosomes, a lipid-associated protein, apolipoprotein-I, is responsible for parasite lysis.[35] It is possible that some component of the human lipid profile is also responsible for natural resistance to schistosomes or cooperates with oxamniquine in parasite killing. Such a component might be low, defective, or less efficient in those with lower LDL concentration.

Ancestry, diet, and metabolic diseases, such as diabetes mellitus, are the major determinants of lipid status in this population and this age group. Severely malnourished children were excluded from the study, and no differences in socio-economic status, anthropometrics, or lipid intake were noted between those persistently egg-positive and the other groups, so that diet is unlikely to explain differences in LDL. Diabetes in this age group in Brazil is very uncommon. The differences in LDL concentrations we observed here are most likely do to intrinsic biologic differences, perhaps genetics.

Several authors have correlated susceptibility to reinfection or response to chemotherapy to pre-treatment, parasite-specific antibody levels.[36] In this study, however, immunoglobulin levels were clearly correlated with actual infection status and were not predictive of response. Thus, IgM and IgG4 serve as markers of ongoing infection but do not participate in resistance or efficacy of drug treatment.

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Authors' addresses: Eliana A. G. Reis, E-mail: ereis@cpqgm.fiocruz.br. Mitermayer G. Reis, E-mail: miter@cpqgm.fiocruz.br. Rita de Cássia R. Silva, E-mail: rcsilva@ufba.br. Theomira M. A. Carmo, Oswaldo Cruz Foundation, Fiocruz, Rua Waldemar Falcão, 121 Brotas, Salvador, Bahia, Brazil, CEP–40296-710. Telephone: 71-3176-2205, Fax: 71-3176-2289. E-mail: theo@cpqgm.fiocruz.br. Ana Marlúcia O. Assis, E-mail: amos@ufba.br. Maurício L. Barreto, Institute of Collective Health, Federal University of Bahia, Rua Basilio da Gama s/N°, Canela, Salvador-Bahia, Brasil Salvador, Bahia, Brazil 40-110-170. Telephone: 71-3263-7445, Fax: 71-3263-7460, E-mail: mauricio@ufbar.br. Isabel M. Parraga, Department of Nutrition, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106-4906. Telephone: 216-368-6626, Fax: 216-368-6644, E-mail: isabel.parraga@case.edu. Mônica Leila P. Santana, School of Nutrition, Federal University of Bahia, Rua Padre Feijó, 29/4° andar, Canela, Salvador, Bahia, Brazip CEP–40.110-170. Telephone: 71-3245-0544, Fax: 71-3237-5856, E-mail: monicalp@ufba.br. Ronald E. Blanton, Center for Global Health and Diseases, Case Western Reserve University, 2103 Cornell Road, 4th floor, Wolstein Research Building, Cleveland, Ohio 44106-7286. Telephone: 216-368-4814, Fax: 216-368-4825, E-mail: reb6@case.edu.

REFERENCES

1. Gryseels B, Nkulikyinka L, Coosemans MH, 1987. Field trials of praziquantel and oxamniquine for the treatment of schistosomiasis mansoni in Burundi. *Trans R Soc Trop Med Hyg 81:* 641–644.
2. Katz N, Rocha RS, de Souza CP, Coura Filho P, Bruce JI, Coles GC, Kinoti GK, 1991. Efficacy of alternating therapy with oxamniquine and praziquantel to treat *Schistosoma mansoni* in children following failure of first treatment. *Am J Trop Med Hyg 44:* 509–512.
3. van Lieshout L, Stelma FF, Guisse F, Falcao Ferreira ST, Polman K, van Dam GJ, Diakhate M, Sow S, Deelder A, Gryseels B, 1999. The contribution of host-related factors to low cure rates of praziquantel for the treatment of *Schistosoma mansoni* in Senegal. *Am J Trop Med Hyg 61:* 760–765.
4. Gryseels B, Mbaye A, De Vlas SJ, Stelma FF, Guisse F, Van Lieshout L, Faye D, Diop M, Ly A, Tchuem-Tchuente LA, Engels D, Polman K, 2001. Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Trop Med Int Health 6:* 864–873.
5. Roberts M, Butterworth AE, Kimani G, Kamau T, Fulford AJ, Dunne DW, Ouma JH, Sturrock RF, 1993. Immunity after treatment of human schistosomiasis: Association between cellular responses and resistance to reinfection. *Infect Immun 61:* 4984–4993.
6. Naus CW, Kimani G, Ouma JH, Fulford AJ, Webster M, van Dam GJ, Deelder AM, Butterworth AE, Dunne DW, 1999. Development of antibody isotype responses to *Schistosoma mansoni* in an immunologically naive immigrant population: Influence of infection duration, infection intensity, and host age. *Infect Immun 67:* 3444–3451.
7. Abel L, Demenais F, Prata A, Souza AE, Dessein A, 1991. Evidence for the segregation of a major gene in human susceptibility/resistance to infection by *Schistosoma mansoni. Am J Hum Genet 48:* 959–970.
8. Butterworth AE, Capron M, Cordingley JS, Dalton PR, Dunne DW, Kariuki HC, Kimani G, Koech D, Mugambi M, Ouma JH, et al., 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.
9. Harrison RA, Bickle Q, Doenhoff MJ, 1982. Factors affecting the acquisition of resistance against *Schistosoma mansoni* in the mouse. Evidence that the mechanisms which mediate resistance during early patent infections may lack immunological specificity. *Parasitology 84:* 93–110.
10. Webster M, Correa-Oliveira R, Gazzinelli G, Viana IR, Fraga LA, Silveira AM, Dunne DW, 1997. Factors affecting high and low human IgE responses to schistosome worm antigens in an area of Brazil endemic for *Schistosoma mansoni* and hookworm. *Am J Trop Med Hyg 57:* 487–494.
11. 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.
12. Eberl M, al-Sherbiny M, Hagan P, Ljubojevic S, Thomas AW, Wilson RA, 2002. A novel and sensitive method to monitor helminth infections by faecal sampling. *Acta Trop 83:* 183–187.
13. Assis AM, Barreto ML, Prado MS, Reis MG, Parraga IM, Blan-

ton RE, 1998. *Schistosoma mansoni* infection and nutritional status in schoolchildren: A randomized, double-blind trial in northeastern Brazil. *Am J Clin Nutr 68:* 1247–1253.

14. Parraga IM, Assis AM, Prado MS, Barreto ML, Reis MG, King CH, Blanton RE, 1996. Gender differences in growth of school-aged children with schistosomiasis and geohelminth infection. *Am J Trop Med Hyg 55:* 150–156.
15. Brito LL, Barreto ML, Silva Rde C, Assis AM, Reis MG, Parraga I, Blanton RE, 2003. Risk factors for iron-deficiency anemia in children and adolescents with intestinal helminthic infections. *Rev Panam Salud Publica 14:* 422–431.
16. Reichenheim ME, Harpham T, 1990. An intra-community profile of nutritional deficiency: a study of children under 5 years of age in a low-income community in Rio de Janeiro (Brizal). *Rev Saude Publica 24:* 69–79.
17. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC, 1974. Enzymatic determination of total serum cholesterol. *Clin Chem 20:* 470–475.
18. Fossati E, Harashina T, Fujino T, 1982. The reapplication of microvascular clamps after venous anastomosis: an experimental study. *J Microsurg 3:* 239–241.
19. Burstein M, Scholnick HR, Morfin R, 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res 11:* 583–595.
20. Friedewald WT, Levy RI, Fredrickson DS, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem 18:* 499–502.
21. Li Z, King CL, Ogundipe JO, Licate LS, Blanton RE, 1995. Preferential recognition by human IgE and IgG4 of a species-specific *Schistosoma haematobium* serine protease inhibitor. *J Infect Dis 171:* 416–422.
22. Skoog T, Dichtl W, Boquist S, Skoglund-Andersson C, Karpe F, Tang R, Bond MG, de Faire U, Nilsson J, Eriksson P, Hamsten A, 2002. Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J 23:* 376–383.
23. Svenungsson E, Fei GZ, Jensen-Urstad K, de Faire U, Hamsten A, Frostegard J, 2003. TNF-alpha: A link between hypertriglyceridaemia and inflammation in SLE patients with cardiovascular disease. *Lupus 12:* 454–461.
24. Stelma FF, Talla I, Polman K, Niang M, Sturrock RF, Deelder AM, Gryseels B, 1993. Epidemiology of *Schistosoma mansoni* infection in a recently exposed community in northern Senegal. *Am J Trop Med Hyg 49:* 701–706.
25. Coelho PM, de Mello RT, Gerken SE, 1993. *Schistosoma mansoni*: Evaluation of the activity of oxamniquine on schistosomules, at 24 hours after infection. *Rev Inst Med Trop Sao Paulo 35:* 557–561.
26. Brindley PJ, Sher A, 1987. The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *J Immunol 139:* 215–220.
27. Silva LM, Andrade ZA, 1997. Immunostimulation as adjuvant for the chemotherapy of experimental schistosomiasis. *Rev Inst Med Trop Sao Paulo 39:* 11–14.
28. Fallon PG, Fookes RE, Wharton GA, 1996. Temporal differences in praziquantel- and oxamniquine-induced tegumental damage to adult *Schistosoma mansoni*: Implications for drug-antibody synergy. *Parasitology 112:* 47–58.
29. Lambertucci JR, Modha J, Doenhoff M, 1989. *Schistosoma mansoni*: The therapeutic efficacy of oxamniquine is enhanced by immune serum. *Trans R Soc Trop Med Hyg 83:* 362–363.
30. Hansson GK, 2001. Regulation of immune mechanisms in atherosclerosis. *Ann N Y Acad Sci 947:* 157–165.
31. Frezard F, de Melo AL, 1997. Evaluation of the schistosomicidal efficacy of liposome-entrapped oxamniquine. *Rev Inst Med Trop Sao Paulo 39:* 97–100.
32. Chiang CP, Caulfield JP, 1989. Human lipoprotein binding to schistosomula of *Schistosoma mansoni*. Displacement by polyanions, parasite antigen masking, and persistence in young larvae. *Am J Pathol 135:* 1015–1024.
33. Rogers MV, Henkle KJ, Fidge NH, Mitchell GF, 1989. Identification of a multispecific lipoprotein receptor in adult *Schistosoma japonicum* by ligand blotting analyses. *Mol Biochem Parasitol 35:* 79–88.
34. Fan J, Gan X, Yang W, Shen L, McManus DP, Brindley PJ, 2003. A *Schistosoma japonicum* very low-density lipoprotein-binding protein. *Int J Biochem Cell Biol 35:* 1436–1451.
35. Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, Nolan DP, Lins L, Van Den Abbeele J, Pays A, Tebabi P, Van Xong H, Jacquet A, Moguilevsky N, Dieu M, Kane JP, De Baetselier P, Brasseur R, Pays E, 2003. Apolipoprotein L-I is the trypanosome lytic factor of human serum. *Nature 422:* 83–87.
36. Caldas IR, Correa-Oliveira R, Colosimo E, Carvalho OS, Massara CL, Colley DG, Gazzinelli G, 2000. Susceptibility and resistance to *Schistosoma mansoni* reinfection: Parallel cellular and isotypic immunologic assessment. *Am J Trop Med Hyg 62:* 57–64.