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SPECIFIC ISOTYPE IMMUNE RESPONSE IN THE DIAGNOSIS OF HUMAN SCHISTOSOMIASIS PATHOLOGY?

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Abstract. Since the few indirect markers available for assessing the development and the stage of intestinal schistosomiasis morbidity are weakly specific, endoscopy is still the only method able to detect severe forms of pathology. Therefore, we evaluated the isotype antibody response to the current schistosome antigen preparation (soluble egg antigens [SEA]) in 142 Senegalese patients infected with *Schistosoma mansoni.* They were stratified into three different stages of pathology according to ultrasonographic, endoscopic, and clinical parameters (stage 1 = no detectable pathology; stage 2 = moderate morbidity; stage 3 = severe forms of pathology). Only median specific IgG4, IgE, and IgA responses changed according to the stage of pathology. The IgA level was significantly higher in stages 2 and 3 compared with stage 1, and the IgE level was higher in stage 3 compared with stage 1. A high specific IgG4 level was observed only in stage 3 and was significantly different compared with stage 2. We show an association between the variability of the specific response to SEA and the degree of morbidity, and demonstrate that IgA and IgG4 responses could be combined markers to easily discriminate the different stages of pathology due to infection with *S. mansoni*.

INTRODUCTION

Chronic infectious diseases are often easy to diagnose in regard to the detection of the infectious agent. In contrast, the evaluation of the intensity of the associated morbidity is difficult because of a lack of specific markers. For an efficient control of several chronic diseases, it seems appropriate not only to detect the presence of the infectious agent but also to diagnose the development of the related morbidity. Schistosomiasis is the second most common parasitic disease after malaria and it affects 200 million individuals.[1] Severe forms of its pathology could cause 200,000 deaths every year in sub Saharian Africa.[1] This chronic infection is a serious public health problem, particularly in Africa, where its recent expansion is mainly due to the consequences of human-made ecologic modifications.

Humoral immune responses directed to adult worm antigen soluble egg antigen (SEA) of schistosomes are well documented during human infection. Numerous immunoepidemiologic studies have demonstrated the potentially protective or harmful roles of the different isotype responses against infection with *Schistosoma mansoni* and *S. haematobium*. In particular, the antagonistic roles of specific IgE and IgG4 antibodies from epidemiologically different foci have been reported. Anti-schistosome IgE responses were closely correlated with resistance to reinfection, whereas high level of IgG4 were correlated with increased susceptibility to reinfection.[2–4] These results only concern the anti-schistosome humoral response during infection or reinfection after chemotherapy. In contrast, very few clinical data showing a relationship between specific antibody responses and morbidity to schistosomiasis have been presented.

The pathology of schistosomiasis is induced by fibrosis due to the deposition of eggs in tissues after laying by female parasites. In the case of *S. mansoni* infection, hepatic periportal fibrosis, hepatosplenomegaly, and portal hypertension with esophageal varices are the major causes of schistosomiasis morbidity and mortality.

Only a few indirect markers for assessing the development and the stage of schistosomiasis morbidity are available. The use of ultrasonography is a direct marker of morbidity, even for detecting the lowest form of pathology.[5] However, this method requires an experienced operator, and the results are often operator-dependent. Thus, the individual follow-up of morbidity, e.g., after chemotherapy, requiring the analysis of ultrasonography by the same operator or by two physicians, is now recommended.[6] For these reasons, it seems essential to develop complementary and effective methods to evaluate schistosomiasis morbidity and especially to diagnose the lowest clinical forms before the outcome of esophageal varices.

Therefore, the present study was undertaken to detect a possible variability of the specific humoral immune response in a stratified cohort of *S. mansoni*-infected patients with different pathologic stages. Our study was conducted in the Senegal River basin, a recent but dramatic focus of *S. mansoni* where the prevalence rates have reached 60–95% in many villages in a few years.[7]

METHODS

Study subjects. The studied population was drawn from the health center in Richard-Toll town, located near the Senegal River in northern Senegal. In this focus, no difference in prevalence or history of exposure were observed in relation to age or sex of the individuals and only *S. mansoni* infection is present.[7,8] The criteria of inclusion in this study were the presence of *S. mansoni* infection (evaluated by the presence of parasite eggs in feces) and/or the detection of schistosomiasis pathology. The intensity of infection was evaluated by the Kato-Katz method on two stool samples, and the results were expressed as the geometric mean number of eggs per gram of feces (EPG). Morbidity staging was estimated by clinical examination and abdominal ultrasonographic criteria

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according to a modified classification proposed by the World Health Organization.[6] The presence of esophageal varices was detected by endocospic examination.

Ultrasonographic patterns and endoscopic examination were used to evaluate schistosomiasis morbidity. As recently described,[9] patients with mild thickening (3–5 mm) of the portal vessel probably do not have portal hypertension and this was not discriminative for classification of patients. We classified the patients into three groups, independently of their portal vessel thickness (Table 1). Stage 1 included patients with *S. mansoni* eggs in stool samples and no ultrasonographic lesions and normal endoscopic results. Stage 3 included the patients with portal hypertension and esophageal varices found by endoscopic examination. Intermediate between these two stages, we identified patients with ultrasonographic lesions but normal endoscopic examination results (stage 2). This intermediate group represented the patients with evolving pathology but without severe forms of morbidity.

Informed consent was obtained from all participants in the study. The study protocol was reviewed and approved by the Senegalese Medical Authority of the region as required by the Ethical Committee of the Senegalese Ministry of Health. All individuals found to be positive for *S. mansoni* were treated with praziquantel (40 mg/kg of body weight) immediately after blood sampling. Pregnant or lactating women were excluded from the study in accordance with the recommendations of the World Health Organization on the use of praziquantel at the time the study was held.[10] These recommendations changed in 2003 and some effort should be made to ensure inclusion of this vulnerable group (pregnant and lactating women) in any national deworming efforts.

Human antibody levels. Specific antibody levels to SEA in individual sera were determined by an enzyme-linked immunosorbent assay (ELISA) as previously described.[11] Briefly, SEA (5 μg/mL) from *S. mansoni* was coated on 96-well plates (Nunc, Roskilde, Denmark) and individual sera were incubated at dilutions of 1:30,000 for IgG (heavy plus light chain), 1:10,000 for IgG1, 1:200 for IgG2 and IgG3, 1:1,500 for IgG4, 1:100 for IgA, and 1:50 for IgE. Corresponding biotinylated monoclonal antibodies to human immunoglobulin isotypes (Southern Biotechnology Associates, Inc., Birmingham, AL) were incubated at a dilution of 1:1,000 and peroxidase-conjugated streptavidin (diluted 1:1,000) was then added (Amersham, Les Ulis, France). Colorimetric development was carried out with ABTS (2,2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid); Sigma, St. Louis, MO) and absorbance (OD) was measured at 405 nm. Identical ELISAs were performed in parallel for 40 uninfected European individuals. Individual results were expressed as a ΔOD value[10] calculated for each isotype test according to the formula ΔOD = ODx – ODn, where ODx represents the individual OD value of an infected patient and ODn is the arithmetic mean of individual OD values for the 40 uninfected control individuals (ODn values = 0.17 for IgG, 0.13 for IgG1, 0.13 for IgG2, 0.34 for IgG3, 0.18 for IgG4, 0.24 for IgA, and 0.22 for IgE).

Statistical analysis. All data were analyzed with GraphPad Prism 3.0 software (GraphPad Software, Inc., San Diego, CA). After analysis, the nonparametric two-tailed Mann-Whitney U test was used to compare the specific isotype levels between two independent groups of patients. The correlation between antibody levels and age or EPG was analyzed using the nonparametric Spearman's correlation coefficient. Differences and correlations were considered significant at $P < 0.05$.

TABLE 1
Characteristics of the studied population

	Patients groups*		
	Stage 1	Stage 2	Stage 3
Number	93	28	21
Sex (M/F)	43/50	21/7	21/5
Age mean, years (range)	25 (12–80)	29 (11–70)	31 (15–57)
Intensity of infection† (range)	450 (1–12,520)	69 (0–6,680)	20 (0–4,990)
Pathology	None	Mild Ultrasonographic abnormalities	Severe Esophageal varices

* Patients were classified in three groups according to ultrasonographic, endoscopic, and clinical parameters: stage 1 = infected controls without morbidity; stage 2 = moderate morbidity (ultrasonographic lesions); stage 3 = severe forms of pathology (portal hypertension and esophageal varices).

† Intensity of infection was expressed by the geometric mean (range) of number of eggs per gram of feces.

RESULTS

The data concerning age and intensity of egg excretion (EPG) parameters according to the stages of pathology are shown in Table 1. A similar average age was observed between each patient group, indicating that the stage of pathology seemed to be independent of the age of individuals. In contrast, EPG count was significantly lower in the groups of patients in stage 2 and stage 3 compared with patients in stage 1 ($P = 0.015$ and $P = 0.001$, respectively). Similar intensity of infection was detected between stage 2 and stage 3 ($P = 0.17$). These results seem to indicate that intensity of infection was lower in patients presenting a pathology detected by ultrasonography compared with asymptomatic infected individuals.

The evaluation of individual specific isotypic responses to SEA was assessed and the results are shown in Table 2 according to the different stages of pathology. The specific IgG, IgG1, IgG2, and IgG3 responses were similar between patients groups. In contrast, IgG4, IgE, and IgA responses to SEA varied according to the stage of pathology. Indeed, IgA levels were significantly higher in stages 2 and 3 compared with stage 1. The IgA response was higher in stage 3 compared with stage 2, but no statistically significant difference was observed. A high specific IgG4 level was observed only in stage 3. Indeed, a similar IgG4 response was observed in stage 1 and stage 2 groups, and the increase in this isotype response in stage 3 compared with stage 2 was highly significant. These results indicate that only differences in specific IgA and IgG4 responses showed a correlation with the stage-by-stage evolution of pathology. A statistically significant difference was found for the IgE response between stage 1 and stage 3. This isotype level was higher with increasing morbidity (stage 1 to stage 2 and stage 2 to stage 3) but the difference was not statistically significant. No differences were observed considering age and sex in the three groups.

The correlation between these isotypic responses and intensity of infection was then analyzed using Spearman's correlation coefficient. For IgG4, IgE, and IgA levels, no significant correlation was observed with intensity of infection, suggesting that observed differences were only related to the

TABLE 2
Specific antibody immune responses to *Schistosoma* soluble egg antigens (SEA)

Antibody to SEA†	Patients groups*				
	Stage 1		Stage 2		Stage 3
IgG	1.55 (0.466–2.822)		0.658 (0.129–3.437)		1.47 (0.33–3.398)
P‡		0.122		0.094	
IgG1	0.677 (0.323–1.749)		0.574 (0.124–1.048)		1.015 (0.235–2.411)
P		0.252		0.200	
IgG2	0.446 (0.109–1.257)		0.128 (0.055–1.091)		0.435 (0.137–1.4)
P		0.081		0.115	
IgG3	0.154 (0.071–0.848)		0.121 (0.06–0.696)		0.18 (0.108–0.478)
P		0.799		0.578	
IgG4	1.522 (0.04–3.107)		1.051 (0.009–2.91)		3.147§ (2.736–3.387)
P		0.537		0.003	
IgE	0.071 (0.011–0.274)		0.114 (0.0475–0.375)		0.148§ (0.073–0.531)
P		0.147		0.425	
IgA	0.33 (0.083–0.721)		0.465 (0.239–1.326)		0.758§ (0.278–2.928)
P		0.02		0.486	

* Patients were classified into three groups (see Table 1).
† Values are median (25–75% percentile) individual optical density values determined by enzyme-linked immunosorbent assay as described in the Methods.
‡ The nonparametric two-tailed Mann-Whitney U test was used to calculate *P* values between patients groups (stage 1 vs stage 2 and stage 2 vs stage 3). Differences are considered significant at $P < 0.05$.
§ Significantly higher ($P < 0.05$) in stage 3 group compared with stage 1 ($P = 0.0008$ for IgG4, $P = 0.024$ for IgE, and $P = 0.011$ for IgA).

stage of pathology (IgG4: r = –0.0529, $P = 0.267$; IgE: r = –0.0623, $P = 0.232$; IgA: r = –0.0529, $P = 0.267$)

DISCUSSION

In contrast to the relationships between specific isotype responses and the intensity of (re)infection, the association of antibody responses to *Schistosoma* and disease pathology has not been investigated. Only one study has recently showed that levels of anti-SEA IgG4 were higher in patients presenting with initial fibrosis.[12] However, this study did not take into account a possible association between antibody levels to *Schistosoma* and the evolution of severity of the pathology. Such correlations between immune response and morbidity have also been demonstrated in other human diseases such as malaria[13] and in infection with *Trypanosoma cruzi*,[14] but they are not used routinely.

In our study, we have demonstrated that anti-SEA IgA and IgG4 responses showed specific variations according to the stage of schistosomiasis pathology. The IgA response was significantly higher in patients in stage 2 compared with stage 1, and progressively increased from stage 1 to stage 3. Specific IgA could be thus the first step in identifying patients with moderate (stage 2) and severe pathology (stage 3) compared with infected individuals. We have previously suggested that the determination of stage 2 by ultrasonography will be predictive of the evolution of the severe (stage 3) form of pathology.[9] In regard to our present study, the evaluation of a specific IgA isotype response could thus act as a predictive marker for identifying individuals with moderate pathology that could develop to severe forms several years later.

The IgG4 response was significantly higher in stage 3 compared with stages 1 and 2. In regard to the known role of this isotype during human schistosomiasis,[2–4] it is probably not surprisingly to observe a positive relationship between the anti-schistosome IgG4 response and the severe form of morbidity. Indeed, studies have previously demonstrated that the presence of a specific IgG4 response was associated with the susceptibility to (re)infection,[2–4] strengthening the relationship of this isotype with the susceptibility of individuals to schistosomiasis.

In regard to our results, the evaluation of the acquired anti-SEA isotype response could be a new approach in evaluating the different stages of schistosomiasis when the use of ultrasonography is not sufficiently effective (e.g., in the earlier stage of portal hypertension). Indeed, analysis of a specific IgA response could be done initially to identify patients with pathology (stages 2 or 3), and evaluation of IgG4 levels to SEA would then be used to discriminate the degree of morbidity, either moderate (stage 2) or severe (stage 3). Indeed, patients with a high specific IgG4 response could be classified as stage 3, whereas individuals with a low IgG 4 response would be classified as stage 2. The use of these immunologic markers could be helpful in identifying patients with earlier stages of portal hypertension so that they could be included in a medical monitoring program. Also, it could indicate when to use endoscopy to confirm and if necessary cure esophageal varices. Moreover, the evaluation of the antibody response to SEA could be a predictive marker in identifying the development of severe forms of schistosomiasis pathology. The evolution of these markers in a longitudinal follow-up is currently under investigation. For example, we did not determine a cut-off isotype value that would discriminate higher or lower responders. Several future studies with a large number of individuals would be necessary to determine a precise threshold of IgA and IgG4 isotype values between each stage.

Although the evaluation of antibody levels by an ELISA is a sensitive, specific, and simple method, it requires equipment in a laboratory that is frequently not available in regions endemic for schistosomiasis. The development of auto-reactive dipsticks pre-coated with SEA for evaluating specific IgA and IgG4 levels could be more appropriate in field conditions and for the rapid diagnosis of schistosomiasis pathology on a large scale. In addition, dipsticks can be semi-quantitative and only finger prick blood would be required.

In conclusion, our study shows an association between specific isotype antibody responses to schistosome antigens and

the degree of schistosomiasis morbidity. This association seems to be isotype and stage dependent. Since this immunologic marker of morbidity is specific for infection with *Schistosoma*, it could be a useful and simple method for application in the field.

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