Serological Responses among Individuals in Areas Where Both Schistosomiasis and Malaria Are Endemic: Cross-Reactivity between *Schistosoma mansoni* and *Plasmodium falciparum*

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We examined specific immunoglobulin G1 (IgG1) and IgG3 responses to *Plasmodium falciparum* schizont and *Schistosoma mansoni* egg and worm antigens in individuals from Kenya, Uganda, and the Sudan who had been exposed to malaria and schistosomiasis. A strong correlation between malaria- and schistosome-specific IgG3 responses was observed. This association appears to result from the presence of cross-reactive components of the 2 parasites that bind IgG3 antibodies, rather than to be mediated by immunological cross-regulation or specific regulatory mechanisms induced by either parasite. Cross-reactivity of IgG3 antibodies was confirmed in a Brazilian cohort of individuals living in an area where schistosomiasis is endemic but no malaria occurs and in a Pakistani cohort from an area where malaria is endemic but no schistosomiasis occurs. An IgG3 interaction with antigens from both parasites was observed in individuals from both cohorts, but not in uninfected European control subjects. The immunological and biological implications of this observation require further exploration.

Malaria and schistosomiasis are 2 of the most important parasitic diseases in humans, both in terms of socioeconomic impact and from a public health perspective. Malaria often takes the form of acute disease, although chronic infections are not uncommon, whereas schistosomiasis is a chronic infection in which the life span of individual adult worms is measured in years. Infection is common in both diseases. Current estimates from the World Health Organization indicate that 300 million individuals contract acute malaria annually, whereas 200 million people are chronically infected with schistosomiasis. Most cases of both infections occur in sub-Saharan Africa, and malaria is a major public health problem.

Informed consent was obtained from all adults or from the parents or legal guardians of children who participated in this study, in accordance with the guidelines of the Kenyan Medical Research Institute and the Ugandan Ministry of Health, whose ethical review committees approved all protocols used, and the US Department of Health and Human Services.

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health problem in ~75% of countries where schistosomiasis is endemic. In areas where these diseases are coendemic and the transmission rates for both parasites are high, coinfection is very common; this may influence morbidity, immunology, distribution, and immunodiagnosis of both infections. For example, determining the etiology of splenomegaly and hepatomegaly in areas where these diseases are coendemic, particularly in children, is complicated by the effects of both infections on the spleen and liver [1]. Interactions between malaria and schistosomiasis are suggested by coinfection studies in animal models [2–4].

Schistosomiasis, like many helminth infections, induces Th2-like host responses, and, in murine models, Schistosoma mansoni can skew the host immune response to nonchistosomal “bystander” antigens from Th1-like to Th2-like [5]. It is likely that immunological interactions occur between these infections in humans; both schistosomiasis and malaria have been implicated in the modulation of human immunological responses. Thus, the prevalence of positive skin test reactions to house dust mite antigens is lower among children who have been highly exposed to malaria [6] and children infected with S. haematobium [7] than among children with less exposure to malaria or who do not have schistosomiasis, perhaps because of the effects of infection-induced interleukin-10 [7, 8]. Plasmodium falciparum–infected erythrocytes adhere to dendritic cells (DCs), inhibit their maturation, and subsequently reduce their capacity to stimulate T cells [8]. The peripheral blood DCs of children with acute malaria have lower expression of HLA-DR than do the DCs of healthy children [9]. There also is evidence that responses to vaccination with tetanus toxoid may be skewed by infection with S. mansoni [6] and P. falciparum [10]. It has been suggested that a human locus controlling P. falciparum blood parasitemia levels [5] could be located in the same genetic region as a locus controlling the level of S. mansoni infection [11].

In the present study, we looked for interactions between the humoral immune responses to P. falciparum and S. mansoni in individuals who were concurrently exposed to infection with both parasites and in populations with single-species infections. Malaria infections in humans typically induce predominantly IgG1 and IgG3 antibody responses [12, 13], and these isotypes appear to be protective [14, 15]. On the other hand, schistosome-specific IgG1, IgG4, and IgE, but not IgG3, predominate in S. mansoni–infected individuals, and no distinctive relationship between IgG3 response and either intensity of infection or host age has been seen in human populations in areas where schistosomiasis is endemic [16–18]. However, we have found that mean levels of anti-worm and anti-egg IgG3 were higher among Kenyan immigrants who were newly exposed to S. mansoni than in a resident population with long-term exposure to the infection [16]. These results suggested that there is an association between antischistosomal IgG3 responses and duration of exposure to S. mansoni. However, it was noted that, in the same relocation that led to the new exposure of these Kenyan immigrants to schistosomiasis, the population moved from an area that was free of malaria into an area where malaria was endemic. It is possible, therefore, that concurrent exposure to malaria and schistosomiasis in a population that previously had been exposed to neither might lead to modulation of the typical antibody responses to one or both parasites.

We retrospectively measured antimalarial antibody responses in 4 cohorts of individuals from areas of Kenya, Uganda, and the Sudan where S. mansoni and P. falciparum are coendemic, who were originally selected for study of immune responses to schistosomiasis. The Kenyan substudy focused on immigrants who previously had not been exposed to malaria or schistosomiasis and who were moving into an area where S. mansoni and P. falciparum were coendemic [19, 20]. The presence of a resident population in the area where these infections were coendemic made it possible to compare the newly exposed immigrants and the established residents of the area [16]. We also examined a cohort from Booma, Uganda. Blood samples were obtained from individuals in the Ugandan cohort before and 7 weeks after treatment for schistosomiasis. A third substudy examined Sudanese patients with schistosomiasis who also suffered from onchocerciasis. We examined antimalarial responses in Brazilian patients with schistosomiasis who lived in a malaria-free area and anti–S. mansoni responses in patients with malaria from Pakistan, which is free of schistosomiasis, as controls. Correlations between IgG subclass responses to S. mansoni and P. falciparum were examined, and apparent cross-reactivity between antigens from the 2 parasites was investigated by means of antigen-specific cross-blocking experiments and Western blot analysis.

SUBJECTS AND METHODS

Study populations. Kenyan subjects were from the Masongaleni area, an area with low-intensity endemicity for S. mansoni that has been described elsewhere [16, 20]. In March 1992, an area adjacent to an established Masongaleni settlement was allocated to several thousand displaced immigrants who were members of the same Kamba ethnic group as the residents of the area. The immigrants had previously inhabited the Chuylu Hills, an area that is free not only of schistosomiasis but also of malaria [19]. Random, age-stratified cohorts from both this immigrant community and the established resident community were selected for the study. Blood samples were obtained from the immigrant cohort (n = 184; age range, 5–59 years; 93 female subjects and 91 male subjects) within 1 year of their first arrival and again ~2 years later. Blood samples were obtained once from subjects from the established resident community.
Onchocerca volvulus, on the basis of active infection with west of Khartoum. Members of the cohort, originally selected from a displaced persons center at Dar Es Salam, 22 km north-range, 14–75 years; 24 male subjects and 22 female subjects.

O. volvulus infection was low, and no malaria transmission occurs in the area.

The Sudanese study cohort was from the fishing village of Booma, in the Butiaba area on the shore of Lake Albert. We have described this area and a very similar neighboring village elsewhere [21]. The cohort consisted of 277 individuals (age range, 7–50 years; 129 female subjects and 148 male subjects).

Blood samples were obtained from 219 subjects before schistosomiasis treatment (2 doses of 40 mg/kg praziquantel given 2 weeks apart) was administered and from 215 subjects 7 weeks after the first dose of praziquantel. Intensities of S. mansoni infection, as estimated by the number of parasite eggs in stool samples, were high. The pretreatment prevalences of malaria (determined by blood-smear examination) and S. mansoni (determined by measurement of circulating anodic antigen levels) in different age groups were as follows: prevalences among subjects 7–9 years of age (n = 34) were 50% and 81%, respectively; 10–12 years of age (n = 38), 46% and 100%; 13–16 years of age (n = 35), 42% and 100%; 17–22 years of age (n = 32), 38% and 92%; 23–30 years of age (n = 47), 29% and 85%; 31–38 years of age (n = 45), 25% and 88%; and 39–50 years of age (n = 46), 18% and 94%.

The Sudanese study cohort consisted of 46 individuals (age range, 14–75 years; 24 male subjects and 22 female subjects) from a displaced persons center at Dar Es Salam; 22 km northwest of Khartoum. Members of the cohort, originally selected on the basis of active infection with Onchocerca volvulus, originated from areas in Southern Sudan that are endemic for O. volvulus, S. mansoni, and P. falciparum. The prevalence of malaria infection in the area around Khartoum has been reported to be as high as 84.9% during peak transmission periods [22].

The Brazilian study cohort was from Corrego do Bernardo in Minas Gerais State. A total of 102 individuals (age range, 5–59 years; 44 female subjects and 58 male subjects) were randomly selected from a larger cohort described elsewhere [23]. The intensity of S. mansoni infection was low, and no malaria transmission occurs in the area.

The Pakistani cohort consisted of 50 patients from Afghan refugee camps on the Afghanistan/Pakistan border with acute P. falciparum infections (age range, 3–55 years; 28 female subjects and 22 male subjects). We divided subjects into those with IgG3 responses (“responders”) and those without responses (“nonresponders”) to S. mansoni soluble worm antigen (SWA) and P. falciparum schizont antigen (Pfs). The cutoff point separating responders from nonresponders was determined by calculating the mean optical density (OD) + 4 SD of the responses of 20 healthy European control subjects.

For all substudies, meetings were held to explain the proposed projects, and the communities involved expressed their willingness to participate. Treatment for schistosomiasis and/or malaria was offered as required to all individuals in study communities, regardless of their participation in the study.

Antigen preparations. Saline soluble antigen extracts from whole homogenized S. mansoni eggs (soluble egg antigen; SEA) and adult worms (SWA) have been described elsewhere [16]. For the preparation of Pfs, strain 3D7 parasites were grown in A+ human erythrocytes in RPMI 1640 medium (Gibco BRL) with 25 mM HEPES (Sigma), 1 mg/L hypoxanthine (Gibco BRL), 28 mM sodium bicarbonate, and 10% normal AB human serum (pH 7.3). Flasks were exposed to 3% O2/4% CO2/93% N2 and incubated at 37°C; parasite development was monitored by examination of Giemsa-stained thin-film blood smears. Cultures were routinely screened for mycoplasma contamination by polymerase chain reaction (BioWhittaker) and were found to be mycoplasma free. Mature schizonts were harvested from cultures with 6%–7% parasitemia by centrifugation at 10,000 g for 5 min through 60% Percoll (Sigma), resuspended in culture medium at a concentration of 10⁷ infected red erythrocytes/mL, and freeze-thawed 3 times in liquid nitrogen. The lysate was stored at −70°C until use. Freeze-thawed uninfected erythrocytes were used as a control antigen. Full-length recombinant merozoite surface protein (MSP)–2 from strains 3D7 and FC27 was kindly provided by Dr. Robin Anders (La Trobe University, Bundoora, Australia) and has been fully described by Riley et al. [24]. The preparation of O. volvulus worm antigen has been described elsewhere [25].

Determination of specific antibody levels. Specific IgG1 and IgG3 responses to Pfs, MSP-2 3D7, and MSP-2 FC27 were measured by ELISA, using methods similar to those described elsewhere [16]. All assays testing for a particular isotype response were carried out at the same time. Briefly, Immulon IV plates (Dynex Technologies) were coated with the malarial antigen at a dilution of 1:1000 (Pfs) or 1 µg/mL (MSP-2 antigens) and incubated overnight. The plates were washed 6 times between each incubation step. After the blocking step (1 h of incubation with 1% Marvel [dried skimmed milk powder, Premier Brands UK] at room temperature [RT]), serum samples, diluted to 1:400 (with PBS, 0.05% Tween 20, and 1% Marvel) and held for 1 h at RT (IgG1) or overnight at 4°C (IgG3), were randomly distributed into the wells of flat-bottomed microtiter plates (Dynex Technologies). These serum samples, which were tested in duplicate on different plates, had been treated against viral contamination [26]. Mouse anti–human IgG1 (added at
a dilution of 1:1000; plates held for 1 h at RT) or mouse anti-human IgG3 (added at a dilution of 1:2000; plates held overnight at 4°C) (each from Unipath) was used as a detecting antibody. Finally, biotinylated anti-mouse immunoglobulin (added at a dilution of 1:1000; plates held for 1 h at RT) and then streptavidin–biotinylated horseradish peroxidase complex (added at a dilution of 1:1000; plates held for 1 h at RT) (both from Amersham) were used to amplify the signal. Specific antibody responses were detected by o-phenylenediamine and expressed as the mean OD of duplicate samples. Specific IgG1 and IgG3 responses to SWA, SEA, and Pfs were measured in triplicate by ELISA, as described elsewhere [16].

**Antigen-blocking experiment.** Serum samples from Kenyan subjects that were known to have IgG1 and IgG3 reactions to SWA, SEA, and Pfs (n = 7) were diluted (1:100), and each sample was aliquoted (100 μL) into 6 Eppendorf tubes. In 4 tubes, 300 μg of SWA, 11 μg of SEA, or 160 μg of Pfs, each in 50 μL of PBS (pH 7.2), was added. The 3 remaining tubes served as controls. The concentration of each antigen used was predetermined in preliminary antigen titration experiments that identified the amount of each antigen that was sufficient to significantly block ELISA-detectable antibody activity in pooled serum samples from infected individuals against the same antigen. All antigen and control tubes were incubated overnight at 4°C. The samples were centrifuged for 15 min (13,000 g) in a Microcentrifuge centrifuge (Sanyo Gallenkamp) before being assayed for specific IgG1 and IgG3 responses to SWA, SEA, and Pfs, using the ELISA method described elsewhere [16]. All samples were tested against the 3 antigen preparations in duplicate on the same plate, and responses in the antigen-blocked samples were compared with those in the unblocked controls.

**Western blots.** For Western blot analysis, SWA and Pfs were separated in 12% SDS-PAGE minigels and then electro-transferred onto nitrocellulose sheets (Biotrace). After blocking for 1 h with PBS containing 0.5% Tween and 1% Marvel, the sheets were cut into vertical strips, which were incubated with serum overnight (diluted 1:100 in blocking buffer). Mouse anti-human IgG3 (Unipath; added at a dilution of 1:2000; held for 4 h at RT) was used as a detecting antibody. The signal was amplified using biotinylated anti-mouse immunoglobulin (added at a dilution of 1:1000; held for 1 h at RT), followed by streptavidin–biotinylated horseradish peroxidase complex (added at a dilution of 1:1000; held for 1 h at RT) (both from Amersham), and the strips were developed using 3,3′-diaminobenzidine (Sigma).

**Statistical analysis.** Associations between *P. falciparum* and *S. mansoni*-specific antibody responses were expressed as Spearman’s rank correlation coefficients. The data were log-transformed using SPSS software (log [OD + 0.1]), and Student’s t test for independent samples was applied to determine differences in specific antibody responses between subjects with IgG3 responses to SWA or Pfs and nonresponders.

**RESULTS**

**Specific antibody responses to *P. falciparum* in Kenyan, Ugandan, and Sudanese cohorts.** Specific IgG1 and IgG3 responses to Pfs were measured in all cohorts from areas where schistosomiasis and malaria are coendemic (Kenyan, Ugandan, and Sudanese cohorts), whereas IgG1 and IgG3 responses to recombinant proteins representing 2 prototypic alleles of *P. falciparum* MSP-2 were measured in the Kenyan cohorts only. The age distribution of the antimalarial response in the Kenyan cohorts is shown in figure 1. IgG1 and IgG3 responses to Pfs increased with age among residents, as well as among newly exposed immigrants. IgG3 responses to MSP-2 3D7 increased with age in both Kenyan cohorts, whereas IgG3 responses to MSP-2 FC27 increased with age in the resident cohort only. IgG1 responses to both MSP-2 antigens, as well as IgG3 responses to MSP-2 FC27, were similar in all age groups. Interestingly, all antimalarial responses were stronger in newly exposed immigrants, not only within the first year of exposure but also 2 years later. We observed in another study that IgG3 responses to SWA and SEA also were stronger among immigrants than among residents [16]. Because of the striking similarity between IgG3 responses to *S. mansoni* and *P. falciparum* in immigrants and residents, we considered the possibility that these responses were associated.

Correlations (Spearman’s rank correlation coefficients) between IgG1 and IgG3 responses to malarial antigens, SWA, and SEA in the Kenyan cohorts are shown in table 1. IgG3 responses to Pfs were significantly correlated with IgG3 responses to SWA and SEA in all cohorts. IgG1 responses to Pfs were significantly associated only with anti-SWA IgG1 in the first Kenyan immigrant survey and anti-SEA IgG1 in the second Kenyan immigrant survey. IgG3 responses to both types of MSP-2 were significantly associated with anti-SWA IgG3, and associations between anti-MSP-2 and anti-SEA IgG3 responses were only significant in the resident cohort and in the second Kenyan immigrant survey, and in the latter case for MSP-2 3D7 only. The results obtained in the Kenyan substudy were confirmed by analysis of specific responses to SWA, SEA, and Pfs in Ugandan and Sudanese cohorts in which similar associations were found (table 1).

In addition, because positivity for onchocerciasis was a selection criterion for Sudanese subjects, we measured IgG3 responses in this group to *O. volvulus* adult worm antigen. The Onchocerca-speciﬁc IgG3 response was signiﬁcantly correlated with IgG3 responses to SWA (R = 0.370; P < .05) and to Pfs (R = 0.533; P < .01).

**Specific antibody responses to malaria and schistosomiasis**
in the Brazilian and Pakistani cohorts. To examine whether the associations between schistosome-and malaria-specific IgG3 were dependent on coinfection with the 2 parasites, which would suggest that coregulation may play a role in response to infection, we measured these responses in 2 cohorts from areas where one, but not the other, parasite was endemic. We compared IgG1 and IgG3 responses to SEA and Pfs in responders and nonresponders to SWA and the responses to SEA and SWA in responders and nonresponders to Pfs (table 2). The percentage of samples that contained IgG3 to SWA was 36% among Brazilian subjects (where schistosomiasis is endemic) and 18% among subjects from Pakistan (where schistosomiasis is not endemic). IgG3 responses to SEA and Pfs were significantly stronger in both Brazilian and Pakistani individuals with responses to SWA than in nonresponders. In the Brazilian cohort only, IgG1 responses to S. mansoni antigens also were significantly stronger in subjects with responses to Pfs. Similar associations were observed when subjects with IgG3 responses to Pfs were compared with nonresponders: the numbers of positive serum samples were small, but IgG3 responses to SEA were significantly stronger in the 24% of Pakistani subjects with IgG3 responses than in nonresponders, which indicates that anti-Pfs IgG3 may cross-react with SEA. In the Brazilian cohort, IgG3 responses to both SWA and SEA were stronger in subjects who had IgG3 responses to Pfs than in nonresponders; again, this difference was highly significant, despite the small number of subjects with IgG3 responses to Pfs (9%). Spearman’s rank correlation coefficients describing associations between the strength of the IgG3 response to Pfs and the strength of the IgG3 response to SWA were 0.305 for Brazil (P < .01) and 0.602 for Pakistan (P < .001). The associations between the strength of the IgG3 response to Pfs and the strength of the IgG3 response to SEA were 0.233 for Brazil (P < .05) and 0.674 for Pakistan (P < .001). A significant association between the strength of the IgG1 response to Pfs and the strength of the IgG1 response to schistosomal antigens was only found in the Brazilian cohort (for SWA, R = 0.316 and P < .01; for SEA, R = 0.202 and P < .05).

Antigen blocking. Cross-reactivity was also examined by preincubation of serum samples from Kenyan IgG3 and IgG1 cross-responders (subjects who had IgG3 and IgG1 responses to both malarial and schistosomal antigens) with either SWA, SEA, or Pfs and subsequent measurement of IgG1 and IgG3 reactivity to all 3 antigen preparations by ELISA (figure 2). Preincubation with each antigen resulted in a marked reduction in IgG1 and IgG3 reactivity to the homologous antigen in ELISA. However, SWA, SEA, and Pfs differed in their capacity to block reactivity to heterologous antigens. Preincubation with SWA decreased IgG3 reactivity to SEA but did not significantly affect IgG1 reactivity to SEA or IgG1 and IgG3 reactivity to
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Cross-reactivity was confirmed by Western blots used to examine IgG3 recognition of SWA and Pfs in serum samples from 4 coinfected patients from Kenya, 4 patients with schistosomiasis only from Brazil, and 4 patients with malaria only from Pakistan (figure 3). The serum samples were selected because ELISA showed them to have high IgG3 responses to the parasites endemic in their area of origin. There was individual variation in IgG3 binding to different components of SWA. Samples from Pakistani subjects (who had never been exposed to schistosomiasis), although they recognized multiple distinct bands, nonetheless recognized fewer SWA bands than did samples from the S. mansoni–infected Kenyan and Brazilian subjects. A molecule close to the 29-kDa marker band appeared to be recognized by serum from all of the coinfected Kenyan subjects, 2 of the Brazilian subjects, and 3 of the Pakistani subjects. Similarly, a molecule above the 58.1-kDa marker band appeared to be recognized by serum from 2 of the Kenyan subjects, 3 of the Brazilian subjects, and 3 of the Pakistani subjects, and a molecule just below the 40-kDa marker band appeared to be recognized by serum from all of the Kenyan subjects and 2 of the Pakistani subjects. IgG3 recognition of Pfs was quite similar among the malaria-exposed Kenyan and Pakistani subjects. Serum samples from the Brazilian subjects (who had never been exposed to malaria) recognized a very restricted number of Pfs molecules, of which molecules of ~29 kDa and ~58–59 kDa were most conspicuous and were recognized by serum samples from all of the Kenyan and Pakistani subjects, as well as by 2 and 3, respectively, of the Brazilian subjects.

**DISCUSSION**

Concurrent infection with malaria and schistosomiasis is common among people living in areas where the infections are coendemic, and it may affect the immune responses to and clinical outcomes of these infections. It is possible that the immunological skewing effect of helminth infection may have a “bystander” effect on antimalarial responses or that the down-regulation and anti-inflammatory mechanisms reported to occur during either infection may have an additive or synergistic effect in coinfected individuals. IgG1 and IgG3 are dominant and biologically significant antibody responses in human Plasmodium infections. Specific IgG3 responses to crude blood-stage malarial antigens, which increase with age, are associated with a reduced risk of malaria attacks and with recovery from a severe attack [14, 27–29]. Specific antibody responses to the malaria vaccine candidate antigen MSP-2 are predominantly of the IgG3 subclass [13, 20], with a shift from IgG1 to IgG3 that is age dependent and is associated with protective immunity [15, 27, 30]. Antibodies, especially IgG3 to MSP-2, are associated with fewer fever episodes and less anemia [31].

Schistosomiasis-specific IgG1 and IgG3 antibodies from infected individuals can mediate the in vitro antibody-dependent cell-mediated cytotoxicity (ADCC) killing of S. mansoni schistosomula by human eosinophils [32]. IgG1 and IgG3 in serum from schistosome-infected individuals have been found to recognize recombinant schistosomal antigens, such as recombinant S. mansoni 28-kDa glutathione S-transferase [33], recombinant S. mansoni glyceraldehyde-3-phosphate dehydrogenase...
Subjects with IgG3 response to antigen in areas where these infections are coendemic. This was observed relationships between IgG3 responses to the two parasites or bind IgG3 antibodies may be sufficient to explain the cross-reactive epitopes that preferentially in.

Although immune cross-regulation may occur, the presence in these parasites of cross-reactive epitopes that preferentially induce or bind IgG3 antibodies may be sufficient to explain the observed relationships between IgG3 responses to the 2 parasites in areas where these infections are coendemic. This was confirmed by examining antimalarial and anti-schistosomal responses in cohorts from a malaria-free area in Brazil where *S. mansoni* was endemic and from an area in Pakistan where schistosomiasis is not endemic but malaria is. Data from these cohorts showed that anti-Pfs IgG3 from *P. falciparum*-exposed individuals who had never been exposed to schistosomiasis was able to recognize *S. mansoni* SEA and SWA. Conversely, serum samples with *S. mansoni*–infected individuals reacted to Pfs. Serum samples from healthy European control subjects did not react with antigen preparations from either parasite.

Cross-absorption experiments confirmed that cross-reactivity was present and that it was a predominantly IgG3-associated phenomenon.

The widespread occurrence of cross-reactive antibodies to *P. falciparum* and *S. mansoni* infections has not, to our knowledge, been reported elsewhere, although Moser et al. [37] showed that patients with malaria were able to recognize the *S. mansoni* heat shock protein 70 and although both malaria and schistosomiasis are known to induce antibodies to cross-reactive epitopes present on other pathogens. For example, antibodies from *S. mansoni*-infected patients who did not have fascioliasis recognized *Fasciola hepatica* antigens [38], whereas antibodies from individuals infected with *S. mansoni* who did not have cholera recognized cholera toxin [39]. Similar observations were described for reactions in serum from *S. mansoni*-infected patients to *Trichinella spiralis* [40], hookworm, and *Ascaris lumbricoides* [41]. Similar observations have also been reported for individuals infected with *P. falciparum* who were presumed to be seronegative for human immunodeficiency virus (HIV) but who had serologic reactions to HIV antigens [42], and antibodies from Sudanese and Danish malaria patients who did not have malaria were able to recognize the *S. mansoni* heat shock protein 70 [43].

Table 2. Results of comparison, using Student’s *t* test, of the differences in IgG1 and IgG3 responses to *Schistosoma mansoni* and *Plasmodium falciparum* antigens among *S. mansoni*-infected individuals from Brazil and *P. falciparum*-infected individuals from Pakistan.

<table>
<thead>
<tr>
<th>Geographic area, subject groups</th>
<th>Subjects with IgG1 response to antigen</th>
<th>Subjects with IgG3 response to antigen</th>
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<tbody>
<tr>
<td></td>
<td>SWA  F  P</td>
<td>SEA  F  P</td>
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<tr>
<td>Brazil</td>
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<tr>
<td>Subjects with IgG3 response to SWA (n = 37) vs. nonresponders (n = 66)</td>
<td>7.13 &lt;.009</td>
<td>5.19 &lt;.025</td>
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<td>Subjects with IgG3 response to Pfs (n = 9) vs. nonresponders (n = 93)</td>
<td>NS NS NS NS</td>
<td>NS NS</td>
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<tr>
<td>Pakistan</td>
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<tr>
<td>Subjects with IgG3 response to SWA (n = 9) vs. nonresponders (n = 41)</td>
<td>NS NS NS NS</td>
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<tr>
<td>Subjects with IgG3 response to Pfs (n = 12) vs. nonresponders (n = 38)</td>
<td>NS NS NS NS</td>
<td>7.08 .011</td>
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**NOTE.** The cutoff point between subjects who had a response to antigen and those who did not (“nonresponders”) was determined by calculating the mean optical density (+ 4 SD) of the responses of 20 healthy European control subjects. NS, not significant; Pfs, *P. falciparum* schizont antigen; SEA, *S. mansoni* soluble egg antigen; SWA, *S. mansoni* soluble worm antigen.

[34], and recombinant *S. mansoni* 14-kDa fatty acid–binding protein [35]. During *S. mansoni* infection, human IgG1 responses to adult worm antigens (SWA) positively correlate with intensity of infection, whereas IgG1 responses to soluble antigens from the parasite egg (SEA) sharply decrease with age, independent of infection intensity [16–18]. In contrast to these distinct age-related patterns of IgG1 antischistosomal responses, none of these studies reported significant associations between antischistosomal IgG3 responses and any host or parasitological parameter. However, Mutapi et al. [36] reported that children with schistosomiasis who are also infected with malaria parasites have higher levels of schistosome-specific IgG3 than do children who are free of malaria and suggested that a plausible explanation for this would be that malaria infection influences the cytokine environment and generally favors the production of IgG3.

We have examined specific IgG1 and IgG3 responses to *P. falciparum* schizont antigen and *S. mansoni* worm and egg antigen in 767 individuals in Kenya, Uganda, and the Sudan who were exposed to both parasites concurrently. Although these areas differ both in the genetic backgrounds of their inhabitants and the patterns of transmission of both parasites, we identified strong correlations between antischistosomal and antimalarial IgG3 responses in the human populations of all of these areas. However, the association between these responses does not appear to depend on immunological cross-regulation or specific regulatory mechanisms induced by either or both parasites. Although immune cross-regulation may occur, the presence in these parasites of cross-reactive epitopes that preferentially induce or bind IgG3 antibodies may be sufficient to explain the observed relationships between IgG3 responses to the 2 parasites in areas where these infections are coendemic. This was confirmed by examining antimalarial and anti-schistosomal responses in cohorts from a malaria-free area in Brazil where *S. mansoni* was endemic and from an area in Pakistan where schistosomiasis is not endemic but malaria is. Data from these cohorts showed that anti-Pfs IgG3 from *P. falciparum*-exposed individuals who had never been exposed to schistosomiasis was able to recognize *S. mansoni* SEA and SWA. Conversely, serum samples with *S. mansoni*–infected individuals reacted to Pfs. Serum samples from healthy European control subjects did not react with antigen preparations from either parasite. Cross-absorption experiments confirmed that cross-reactivity was present and that it was a predominantly IgG3-associated phenomenon.

The widespread occurrence of cross-reactive antibodies to *P. falciparum* and *S. mansoni* infections has not, to our knowledge, been reported elsewhere, although Moser et al. [37] showed that patients with malaria were able to recognize the *S. mansoni* heat shock protein 70 and although both malaria and schistosomiasis are known to induce antibodies to cross-reactive epitopes present on other pathogens. For example, antibodies from *S. mansoni*-infected patients who did not have fascioliasis recognized *Fasciola hepatica* antigens [38], whereas antibodies from individuals infected with *S. mansoni* who did not have cholera recognized cholera toxin [39]. Similar observations were described for reactions in serum from *S. mansoni*-infected patients to *Trichinella spiralis* [40], hookworm, and *Ascaris lumbricoides* [41]. Similar observations have also been reported for individuals infected with *P. falciparum* who were presumed to be seronegative for human immunodeficiency virus (HIV) but who had serologic reactions to HIV antigens [42], and antibodies from Sudanese and Danish malaria patients who did
Figure 2. Antigen blocking of specific IgG1 and IgG3 antibodies to *Schistosoma mansoni* soluble worm antigen (SWA) and soluble egg antigen (SEA) as well as *Plasmodium falciparum* schizont antigen (Pfs) in serum samples (*n* = 6) from individuals from an area in Kenya where schistosomiasis and malaria are coendemic who had strong antibody responses to *S. mansoni* and *P. falciparum* antigens. Results are expressed as the mean of the overall absorbance values (optical density at 490 nm) detected by ELISA. They were measured in unblocked serum samples and compared with aliquots of the same samples that were incubated with SWA (solid lines), SEA (dotted lines), or Pfs (dashed lines) before testing. Error bars represent 95% confidence intervals.

not have leishmaniasis recognized *Leishmania donovani* antigens [43].

The pattern seen in Western blot analysis of IgG3 cross-reactivity between the 2 parasites showed that serum from individuals who had not been exposed to malaria recognized a number of Pfs bands and that serum from individuals who had not been exposed to schistosomiasis recognized SWA bands. However, in both cases, individuals who had been exposed to the homologous parasite recognized significantly more Pfs and SWA bands than did those who had been exposed only to heterologous parasites. This suggests that IgG3 cross-reactivity is not the result of a single dominant antigen or a generalized low-affinity unrestricted interaction. Rather, it seems to be a property of a diverse, if restricted, number of parasite molecules that may share ≥1 cross-reactive epitope. We have no data on the molecular nature of the cross-reactive epitope(s). However, it would appear to be present in organisms as zoologically diverse as *Plasmodium*, *Schistosoma*, and *Onchocerca*. Malarial antigens, in particular, are known to undergo significant post-translational processing, so that a single precursor protein can generate many different molecules that may contain the relevant cross-reactive epitope. For example, MSP-2 is a major inductor of IgG3 in *P. falciparum* infections, and it contains a glycosyl-phosphatidylinositol (GPI) anchor. Interestingly, it appears that
GPI anchors represent the major carbohydrate modification in intraerythrocytic-stage P. falciparum proteins, with relatively low levels of O-linked and N-linked glycosylation [44]. However, we have no information at present about cross-reactivity between schistosome and Plasmodium GPIs or any other potentially cross-reactive common molecular motifs. An alternative explanation for the observed cross-reactivity of IgG3 that cannot be eliminated at this stage would be that, rather than sharing common antigenic epitopes, both parasites possess molecules (e.g., an unknown lectin) that bind human IgG3 via an isotype-specific component of this antibody. The biological implications of an unusual parasite-antibody isotype interaction of this kind are unclear.

Although preincubation of cross-reacting serum with Pfs was very effective in blocking antischistosomal IgG3 activity on ELISA, preincubation with SWA was much less effective in blocking anti-Pfs IgG3 activity. This suggests that some of the anti-Pfs IgG3 activity we observed is Plasmodium specific, whereas most of the IgG3 that binds to SWA is cross-reactive with Plasmodium antigens (figure 2). In addition, relatively few Pfs bands were recognized by IgG3 from Brazilian subjects who had been exposed to schistosomiasis but not to malaria. These observations, together with the facts that, in areas where these infections are coendemic, children will be exposed to malaria earlier in life than to schistosomiasis and that IgG3 is a dominant isotype in malaria-only infections but not schistosomiasis-only infections, suggest to us that P. falciparum is more influential than S. mansoni in driving the production of cross-reactive IgG3 in areas where these infections are coendemic. We reinterpret our earlier observation that immigrants who previously have been unexposed to schistosomiasis developed a striking and unusually high level of anti-S. mansoni IgG3 during the first 2 years of their residence in an area of Kenya where schistosomiasis is endemic [16]. Because this population was exposed to malaria for the first time concurrently, we may well have been observing a cross-reactive IgG3 response induced by the initial exposure to malaria. However, the fact that the anti-Pfs IgG3 response in the immigrant population was higher than that among residents of the area is somewhat unexpected. Although there does appear to be an increase in some of the antimalarial IgG3 responses that corresponds with age in both the immigrant and resident populations (figure 1), the finding of higher IgG3 responses among the immigrants differs from previous reports [15], in that IgG3 responses to Pfs and MSP-2 have been found to be both age dependent and dependent on longer exposure to malaria infection. We do not know whether a primary coexposure to schistosomiasis could have exerted a reciprocal influence that would alter the expected early low IgG3 responses of a naive population to malaria exposure.

Why cross-reactivity appears predominantly to be restricted to the IgG3 isotype remains an interesting question. Both IgG1 and IgG3 recognize peptide, as well as carbohydrate, epitopes on S. mansoni SEA [45]. They also have similar immunological functions, including complement-fixing ability, the promotion of phagocytosis, ADCC, and antibody-dependent cell-mediated inhibition. However, IgG3 is the more effective isotype in terms of fixing complement [46], its hinge region is more flexible [47], and it has superior activity in ADCC and complement-mediated cell lysis at limited antigen loads or low target cell concentrations [48]. IgG1 has a longer half-life than IgG3 [49] and is more effective in inducing mediator release from monocytes [50]. The restriction of cross-reactivity to IgG3 suggests that epitope recognition by this isotype is restricted, presumably.
by a mechanism involving specific isotype switching. Because the IgG3 response is dominant and biologically important in P. falciparum infections, this is now an important area of ongoing research. Although malaria appears to be a potential inducer of IgG3 isotype switching, the Brazilian subjects with schistosomiasis, who were not exposed to malaria, also had some anti-Plasmodium IgG3 activity, which suggests that cross-reactive schistosomal antigens also are capable of inducing IgG3 isotype switching, albeit not as effectively as malaria infections.

In conclusion, we observed cross-reactivity between P. falciparum schizont and S. mansoni worm in diverse human populations living in areas where the 2 infections are coendemic. This cross-reactivity is largely limited to the IgG3 isotype response. The molecular basis for the observed cross-reactivity is unknown. The biological and immunological implications of these findings need further examination.

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