Hepatosplenomegaly Is Associated with Low Regulatory and Th2 Responses to Schistosome Antigens in Childhood Schistosomiasis and Malaria Coinfection[▽]

Shona Wilson,^{1*} Frances M. Jones,¹ Joseph K. Mwatha,² Gachuhi Kimani,² Mark Booth,¹ H. Curtis Kariuki,³ Birgitte J. Vennervald,⁴ John H. Ouma,⁵ Eric Muchiri,³ and David W. Dunne¹

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, United Kingdom¹; Kenya Medical Research Institute, Nairobi, Kenya²; Division of Vector Borne Diseases, Kenyan Ministry of Health, PO Box 54840 Nairobi, Kenya³; DBL—Institute for Health Research and Development, Jægersborg Alle 1D, 2920 Charlottenlund, Denmark⁴; and Maseno University, Kisumu, Kenya⁵

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Hepatosplenomegaly among Kenyan schoolchildren has been shown to be exacerbated where there is transmission of both *Schistosoma mansoni* and *Plasmodium falciparum*. This highly prevalent and chronic morbidity often occurs in the absence of ultrasound-detectable periportal fibrosis and may be due to immunological inflammation. For a cohort of school-age children, whole-blood cultures were stimulated with *S. mansoni* soluble egg antigen (SEA) or soluble worm antigen (SWA). Responses to SWA were found to be predominantly Th2 cytokines; however, they were not significantly associated with either hepatosplenomegaly or infection with *S. mansoni* or *P. falciparum*. In comparison, SEA-specific Th2 cytokine responses were low, and the levels were negatively correlated with *S. mansoni* infection intensities and were lower among children who were coinfecting with *P. falciparum*. Tumor necrosis factor alpha levels in response to stimulation with SEA were high, and a negative association between presentation with hepatomegaly and the levels of the regulatory cytokines interleukin-6 and transforming growth factor β_1 suggests that a possible mechanism for childhood hepatomegaly in areas where both malaria and schistosomiasis are endemic is poor regulation of an inflammatory response to schistosome eggs.

Human schistosomiasis-associated severe pathology, which can result in portal hypertension and hepatosplenomegaly, is the consequence of periportal fibrosis, which is detectable by ultrasonography (33). Periportal fibrosis is thought to result from chronic immunological responses directed against *Schistosoma mansoni* eggs (12), and since exposure to infection over many years is associated with this pathology (6), it is detected mostly within adult members of populations in areas where *S. mansoni* is endemic (18, 25). However, a form of *S. mansoni*-associated hepatosplenomegaly that can extend into adulthood is common among children, and the incorporation of ultrasonography into *S. mansoni* epidemiological studies has shown that this *S. mansoni*-associated childhood hepatosplenomegaly can occur in the absence of ultrasound-detectable fibrosis (4, 41). It is therefore proposed that immunological inflammation, rather than periportal fibrosis, could be the more common cause of hepatosplenomegaly in this age group (15).

Evidence from mouse models suggests that the formation of granulomas around *S. mansoni* eggs, which are swept by the circulation into the liver, is CD4⁺ T cell dependent. These CD4⁺ T cells secrete predominantly Th2 cytokines, and the chronic phase of infection is typified by a continuing, but down-regulated, Th2 response (30). In human studies in Brazil, schistosome antigen-specific T-cell clones derived from *S. mansoni*-

infected individuals were found to be predominantly interleukin-4 (IL-4)-secreting CD4⁺ T cells (9), with mRNA levels indicating that *S. mansoni* egg antigen (SEA) stimulates a predominantly Th2 response, while *S. mansoni* worm antigen (SWA) stimulates a nonpolarized Th0 response (44). However, in African studies, both peripheral blood mononuclear cell (PBMC) cultures and whole-blood cultures stimulated with SWA have been shown to release higher levels of the Th2 cytokines IL-4, IL-5, and IL-13 than cultures stimulated with SEA (20, 34). These *S. mansoni* studies in Africa suggest that SWA, even in chronically infected individuals, stimulates a measurable Th2 response but that the Th2 response to SEA is downregulated to a much greater extent. How these observations from mouse and human studies relate to *S. mansoni*-associated childhood hepatosplenomegaly is, however, poorly understood, though an immunoepidemiological study of *S. mansoni*-associated hepatosplenomegaly among Kenyan schoolchildren indicates that high levels of the inflammatory cytokines tumor necrosis factor alpha (TNF- α) and gamma interferon (IFN- γ), in response to both SEA and SWA, and low levels of the Th2 cytokine IL-5 are associated with this morbidity (26).

Inflammation, characterized by reticuloendothelial and lymphoid hyperplasia, is also thought to be the cause of chronic hepatosplenomegaly that is associated with malarial infections (40, 42). Early studies concluded that schistosomiasis and malaria were confounding factors in relation to the etiology of hepatosplenomegaly (29, 38); however, it now appears that *S. mansoni* infection and chronic exposure to *Plasmodium* have either additive or synergistic effects, with hepatosplenomegaly

* Corresponding author. Mailing address: Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, United Kingdom. Phone: 44-1223333332. Fax: 44-1223333741. E-mail: sw320@cam.ac.uk.

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being more prevalent (14, 43) and more severe (5, 46) in cases where both parasites are transmitted. The immune mechanism of hepatosplenomegaly associated with chronic exposure to malaria has not been characterized. However, it is known that infection with *Plasmodium* can influence the immune response that is associated with *S. mansoni* infection, with mouse model studies showing the modulation of in vitro responses to schistosome antigens (17) and alterations in plasma cytokine levels being found in coinfecting humans, in comparison with individuals infected with one parasite but not the other (11, 32).

Here, the immunological basis of chronic, firm hepatosplenomegaly, to which both *S. mansoni* infection (in an intensity-dependent manner for hepatomegaly but not for splenomegaly) and chronic exposure to malaria contribute (46), is examined in Kenyan schoolchildren. A range of cytokines released in whole-blood cultures stimulated with schistosome antigens were measured, including Th1 proinflammatory cytokines, regulatory mediators, and Th2 cytokines. The influence of *Plasmodium* infection on these responses is also examined, as the modulation of these responses may suggest a mechanism for the exacerbation of hepatosplenomegaly among children who are exposed to both parasites.

MATERIALS AND METHODS

Study population. Seventy-nine children attending Matangini Primary School, Lower Mangelete, Makueni District, Kenya, were selected to participate in the study. They were selected because they met the criteria, in that they were proportionally representative of the clinical profile of all the children at the school who were infected with *S. mansoni* and their *S. mansoni* infections covered a range of intensities. Lower Mangelete is a fertile area, with permanent streams and a network of irrigation canals providing ecological conditions suitable for the transmission of *S. mansoni* and *Plasmodium* species. The ages of the selected cohort ranged from 4 to 17 years, with a mean age of 13.4 years. All of the selected children had *S. mansoni* infections detectable by the Kato-Katz method (21). The intensities of *S. mansoni* infection were calculated from the mean infection intensities of 10 Kato-Katz slides, 2 from each of the five stool samples collected. The intensities of infection ranged from 4 to 1,368 eggs per gram of fecal material. The median infection intensity was 68 eggs per gram of fecal material. The prevalence of microscopy-detectable *Plasmodium falciparum* infection was 27.8%. DNA for *Plasmodium* genus-specific PCR was extracted from 20 μ l of whole blood using a Qiagen DNA blood minikit (Qiagen, Crawley, United Kingdom) according to the manufacturer's instructions and amplified using a modification of a published nested PCR (37), in which the published reverse primer for nest one was replaced with the primer 5'-ATT TCT CAG GCT CCC TCT CC-3'. The prevalence of PCR-detectable *Plasmodium falciparum* infection was 74.7%. *Plasmodium falciparum* schizont antigen-immunoglobulin G3 (Pfs-IgG3) levels, a marker for the relative exposure levels to *Plasmodium* in school-age children but not related to current detectable *Plasmodium* infection, were measured by enzyme-linked immunosorbent assay (ELISA) as previously described (47). Clinical examinations for firm/hard palpable livers and spleens were carried out twice by three clinicians, as previously described (41), with organs greater than 2 cm below the costal margin being considered enlarged. No attempt was made to clinically define the upper margin, and therefore the span, of either organ. The children presented with a range of morbidities according to our criteria: 9 had enlargement of neither the liver nor the spleen, 10 had enlargement of the spleen only, 20 had enlargement of the liver only, and 29 had hepatosplenomegaly. A consensus could not be reached as to the clinical findings for the remaining 11 children. Clinical measurements were used to class left-liver-lobe enlargement and spleen enlargement into three categories: no enlargement (<2 cm for both), moderate enlargement (2 to 5 cm and 2 to 4 cm, respectively), and substantial enlargement (>5 cm and >4 cm, respectively). For analysis of the clinical findings, only those children for whom clinical findings were classifiable were included.

Whole-blood cultures. Venous blood was collected in heparin at 10 U/ml. SEA and SWA were prepared as previously described (13). SWA and SEA were filtered through sterile 0.22- μ m-pore-size filters and assayed for endotoxin using the *Limulus* amoebocyte lysate kit (QCL-1000; BioWhittaker, Inc., Walkersville,

MD). The levels of endotoxin in the antigens used in this study were 10.7 ng of endotoxin/mg of SEA and 25 ng of endotoxin/mg of SWA. Purified endotoxin at the concentrations found in 10 μ g of SEA or SWA preparations (<0.3 ng/ml) does not stimulate detectable production of any of the cytokines measured by ELISA in whole-blood cultures. Whole-blood cultures were stimulated with SEA, SWA, or phytohemagglutinin-M (PHA; Calbiochem, Darmstadt, Germany) in biological duplicates. Medium was added to control cultures. Each culture contained 1 ml of a 1:6 dilution of whole blood stimulated with 10 μ g of antigen in RPMI 1640 with 5 U penicillin, 50 μ g streptomycin, and 2 mM L-glutamine (Sigma, Poole, Dorset, United Kingdom). For each individual and each antigen, duplicate cultures were incubated for 48 h and 96 h at 37°C. The supernatants were harvested and immediately frozen at -20°C prior to transport. In the United Kingdom, the samples were treated with 0.3% tributyl phosphate (Sigma) to inactivate any enveloped viruses and stored at -80°C until use.

Cytokine assays. All cytokine levels were measured in 96-h culture supernatants, except for TNF- α and IL-4, which were measured in 48-h culture supernatants. TNF- α , IFN- γ , IL-4, IL-5, IL-13, and IL-10 were measured by capture ELISA, using matched-pair antibody sets (Pharmingen, San Diego, CA), as previously described (20). IL-6 was also measured using a matched-pair antibody set of the clones MQ2-13A5 and MQ2-39C3 (Pharmingen). Latent transforming growth factor β (TGF- β) was acid stripped prior to being assayed by treating the supernatants with 1 μ l 1 M HCl per 4- μ l sample, incubated at room temperature for 10 min and neutralized with 1 μ l 1.2 M NaOH-0.5 M HEPES per 1 μ l 1 M HCl added. TGF- β was assayed using a matched antibody pair: capture antibody clone 9016 and a chicken IgY polyclonal detecting antibody (R&D Systems, Minneapolis, MN). For each cytokine, all supernatants from each individual and culture were assayed simultaneously. Optical densities were calibrated to standard curves measured by the detection of known concentrations of recombinant cytokines, purchased from the same company as the antibody matched pairs, as previously described (20).

Treatment and ethical considerations. The purpose of the study was carefully explained to members of the community, and informed consent was obtained from the parents or guardians of the children who participated in the study. After the examinations were complete, all children were treated with a single dose of praziquantel. Any cases of acute clinical malaria were treated by a local nurse, as were minor ailments. The study was approved by the Kenya Medical Research Institute's national ethical review committee.

Statistical analysis. A nonparametric Friedman test for tied data, with Wilcoxon's post hoc analysis, was used to compare the individuals' cytokine responses to each of the antigens, including to the medium control cultures. If the levels of cytokine measured in response to a schistosome antigen were greater than those in response to the medium control, the spontaneously released cytokine level (in medium-stimulated cultures) was subtracted to obtain the antigen-specific response. Antigen-specific responses were skewed, and since log transformation failed to normalize a number of the responses, they were normalized by calculating Blom normal scores, a procedure based on ranks rather than on absolute values (3), prior to further analysis. Correlation matrices indicated that there was a high degree of covariance within the data set, allowing normalized cytokine responses to be entered into a principal-component analysis. Principal components with eigenvalues greater than 1 were extracted by regression analysis, with orthogonal varimax rotation. To be considered reliable, an extracted principal component had to have four or more of the original cytokine variables with factor loadings, which indicate the correlation between the extracted component and the original variable, of >0.6 (16). Extracted principal components were normally distributed, so comparisons of three or more groups were carried out using analysis of variance with Hochberg's GT2 post hoc analysis, and comparisons between two groups were carried out using Student's *t* test. The specific levels of individual cytokines of two groups were analyzed using Mann-Whitney U tests, and those of three groups were analyzed using the Kruskal-Wallis with Mann-Whitney U test post hoc analysis. Correlations with *S. mansoni* egg counts and Pfs-IgG3 levels were analyzed using Spearman's rank correlations.

RESULTS

Cytokine responses in whole-blood cultures. The levels of cytokines produced in response to SEA, SWA, and PHA and in control cultures are shown in Fig. 1. For all cytokine levels measured, the results of the Friedman test for tied data were highly significant ($P < 0.001$). The levels of all cytokines measured were significantly greater in the supernatants of SEA- and SWA-stimulated cultures than in the supernatants of un-

FIG. 1. Cytokine production by whole-blood cultures stimulated with *S. mansoni* antigens and PHA. The levels of the cytokines were measured in whole-blood culture supernatants left unstimulated (media) or stimulated with SEA or SWA or with the mitogen PHA. Shown are the medians and interquartile ranges of the cytokine levels, with whiskers representing 1.5 times the interquartile range. Outlying data points are represented by circles. *P* values are <0.05 (*), <0.01 (**), and <0.001 (***) for comparisons between cultures stimulated by SEA and cultures stimulated by SWA.

stimulated cultures, except for the levels of TNF- α in the supernatants of SWA-stimulated cultures ($P = 0.464$). The levels of TNF- α in cultures stimulated with SEA were significantly higher than they were in cultures stimulated with SWA ($P < 0.001$). The levels of TGF- β_1 and IL-6 measured in the supernatants of SEA-stimulated cultures were also significantly greater than the levels that were detectable in the supernatants of SWA-stimulated cultures ($P < 0.001$ for both cytokines). The levels of TGF- β_1 released in response to SEA stimulation were comparable with the levels measured in the supernatants of PHA-stimulated cultures ($P = 0.072$). The levels of IL-4, IL-5, and IL-13 were significantly greater in the supernatants of SWA-stimulated cultures than they were in the supernatants of SEA-stimulated cultures ($P = 0.04$, $P < 0.001$, and $P < 0.001$, respectively). The levels of IL-5 measured in the supernatants of SWA-stimulated cultures were also significantly higher than they were in the supernatants of PHA-stimulated cultures ($P < 0.001$).

Data reduction of in vitro cytokine responses. Preliminary analysis indicated there were strong correlations among in vitro cytokine responses to schistosome antigens (data not shown), and therefore, they could be entered into a principal-

component analysis. Immunological mechanisms involve a number of cytokines working together, rather than independently. By using data reduction by principal-component analysis, the covariance that results from a number of cytokines being produced by an immunological mechanism can be harnessed to allow interpretation to be at the level of the immunological mechanism, rather than at the level of individual cytokines. All specific responses to SEA and SWA were entered into the principal-component analysis, with the exception of SWA-specific TNF- α responses, the levels of which were not significantly higher than those detected in the control cultures and did not significantly correlate with any of the other responses (data not shown). Three components with eigenvalues greater than 1 were extracted, representing 34.28%, 17.03%, and 12.26% of the total variation in the data set, respectively. The factor loadings of the different variables onto the three components after rotation are shown in Table 1. The variables with the strongest loadings onto the first principal component were the SWA-specific responses of the Th2 cytokines IL-4, IL-13, and IL-5 and the SWA-specific IL-10 responses. The variables with the strongest loadings onto the second principal component were SEA- and SWA-specific IL-6 and TGF- β_1

TABLE 1. Factor loadings of in vitro cytokine responses in whole-blood cultures stimulated with *S. mansoni* antigens onto three components generated by principal-component analysis^a

Cytokine response	Antigen	Factor loading (correlation coefficient) on indicated principal component		
		First	Second	Third
TNF- α	SEA	-0.102	0.416	-0.014
IFN- γ	SEA	-0.283	0.337	0.747^b
	SWA	0.277	0.455	0.237
IL-10	SEA	0.358	0.517	0.449
	SWA	0.702	0.426	0.153
TGF- β	SEA	0.039	0.695	0.230
	SWA	0.431	0.610	-0.029
IL-6	SEA	-0.124	0.807	0.130
	SWA	0.167	0.769	-0.079
IL-4	SEA	0.263	-0.180	0.720
	SWA	0.814	-0.191	0.033
IL-13	SEA	0.181	0.277	0.802
	SWA	0.906	0.062	0.203
IL-5	SEA	0.381	-0.029	0.797
	SWA	0.862	0.115	0.283

^a Shown is the component matrix after rotation by varimax, with correlation coefficients between extracted principal components and the antigen-specific responses from which they were derived.

^b In bold are variables with a factor loading greater than 0.6, a cutoff value that indicates that the variable is making a substantial contribution to the component. A component is considered reliable if it has four or more of these variables.

responses. The variables with the strongest loadings onto the third principal component were the SEA-specific responses of the Th2 cytokines IL-4, IL-13, and IL-5 and the SEA-specific IFN- γ response.

In vitro cytokine responses and *S. mansoni* and malaria infection. Neither the scores of the first principal component (SWA-specific Th2) nor those of the second principal component (TGF- β_1 plus IL-6) were significantly correlated with *S. mansoni* infection intensities ($\rho = 0.143$ [$P = 0.208$] and $\rho = -0.047$ [$P = 0.683$], respectively). The third-principal-component scores (SEA-specific Th2 plus IFN- γ) were negatively correlated with *S. mansoni* infection intensities ($\rho =$

-0.371 , $P = 0.001$) (Fig. 2A). Neither the scores of the first principal component nor those of the second principal component were significantly different between children with and without microscopy-detectable *P. falciparum* infections ($t = -0.416$ [$P = 0.678$] and $t = -0.163$ [$P = 0.871$], respectively). However, children who had microscopy-detectable *P. falciparum* infections had significantly lower third-principal-component scores (Fig. 2B). There was a trend for the third-principal-component scores of children that had PCR-detectable malaria to be lower than those of children that did not have PCR-detectable malaria (Fig. 2C); however, this failed to reach a level of significance ($t = 1.571$, $P = 0.120$). None of the extracted principal-component scores were significantly correlated with Pfs-IgG3 levels (data not shown).

The four specific cytokine responses that significantly contributed to the third principal component were then analyzed separately to determine if any of the individual responses were associated with any of the parasitological parameters examined. The levels of all four of the individual cytokines were lower among children who tested slide or PCR positive for *Plasmodium* infections than among children who tested negative; however, none were significantly lower (Table 2). The levels of SEA-specific IFN- γ and the levels of SEA-specific IL-13 were significantly negatively correlated with *S. mansoni* infection intensities.

In vitro cytokine responses and hepatosplenomegaly. There were no significant relationships between the first-principal-component scores (SWA-specific Th2) and groups representing differing extents of liver ($F = 2.123$, $P = 0.128$) and spleen ($F = 0.891$, $P = 0.415$) enlargement, as measured by palpation. The second-principal-component scores (TGF- β_1 plus IL-6) did not differ significantly among groups of children with differing extents of spleen enlargement ($F = 1.115$, $P = 0.333$) but did differ significantly with the extent of the left-liver-lobe enlargement ($F = 4.746$, $P = 0.012$). Post hoc analysis indicated that children who had a substantial enlargement of the left liver lobe had lower second-principal-component scores than children who had no enlargement of the left liver lobe (Hochberg's GT2 post hoc analysis, $P = 0.009$) (Fig. 3A). None of the individual specific cytokine responses that substantially contributed to the second principal component were significantly associated with the extent of left-liver-lobe enlargement (data not shown).

The third-principal-component scores (SEA-specific Th2 plus

FIG. 2. Associations between cytokine responses in whole-blood cultures stimulated with *S. mansoni* antigens and *S. mansoni* infection intensity and malaria prevalence. (A) Scatter plot of *S. mansoni* infection intensities against the third-principal-component scores (SEA-specific Th2 plus IFN- γ) derived from whole-blood cultures stimulated with SEA or SWA. (B) Means $\pm$ 2 standard errors (SE) of the third-principal-component scores of children who did and did not have slide-detectable *Plasmodium* infections. *, $P < 0.05$. (C) Means $\pm$ 2 SE of the third-principal-component scores of children who did and did not have PCR-detectable *Plasmodium* infections.

TABLE 2. Associations between parasitological parameters and individual cytokine responses that contributed substantially to the second principal component

SEA-specific cytokine response	Median cytokine level (pg/ml) in children with indicated test result for <i>Plasmodium</i> infection ^a				Spearman's rho with <i>S. mansoni</i> infection intensity ^b
	Slide		PCR		
	Negative	Positive	Negative	Positive	
IL-4	3.02 (0, 8.80)	1.06 (0, 3.57)	4.56 (0.06, 9.29)	1.85 (0, 7.58)	−0.117
IL-5	27.15 (0, 195.81)	7.36 (0, 32.26)	27.12 (1.84, 355.41)	17.56 (0, 79.70)	−0.167
IL-13	0 (0, 343.96)	0 (0, 0)	0 (0, 510.2)	0 (0, 36.49)	−0.396
IFN-γ	2.968 (0, 7.49)	1.786 (0, 3.95)	3.51 (0.21, 6.86)	2.01 (0, 6.86)	−0.388

^a Values are given for children with (positive) and without (negative) *Plasmodium* species infection, diagnosed either by microscopy (slide) or PCR. Values in parentheses are interquartile ranges in pg/ml.

^b Shown are the Spearman rank correlation coefficients between specific cytokine responses and *S. mansoni* infection intensities. For the SEA-specific IL-13 and IFN- γ responses, the *P* values are <0.001.

IFN- γ) did not differ significantly among groups representing differing extents of liver enlargement ($F = 0.153$, $P = 0.859$) but did differ among children with differing extents of spleen enlargement ($F = 4.515$, $P = 0.014$). Hochberg's GT2 post hoc analysis indicated that the relationship was not linear, as children presenting with moderate enlargement of the spleen ($P = 0.012$) but not children with substantial enlargement of the spleen ($P = 0.223$) had significantly lower third-principal-component scores than children who had no enlargement of the spleen. However, there was a trend for the children presenting with substantially enlarged spleens to have lower third-principal-component scores than children with little to no enlargement of the spleen (Fig. 3B). Analysis of the individual cytokines that substantially contributed to the third principal component showed that neither SEA-specific IFN- γ ($P = 0.187$) nor SEA-specific IL-5 ($P = 0.063$) was significantly associated with the extent of spleen enlargement. SEA-specific IL-4 levels did differ significantly among the groups, with levels being significantly lower for the group with moderately enlarged spleens than for both those with no enlargement of the spleen ($P = 0.025$) and those with a substantial enlargement of the spleen ($P = 0.011$). SEA-specific IL-13 was also significantly lower for children with moderately enlarged spleens than for

children with no enlargement of the spleen ($P = 0.011$) but not for children with substantially enlarged spleens ($P = 0.088$).

DISCUSSION

The aim of the present study was to determine if cytokine responses of whole-blood cultures stimulated with SEA and SWA were related to hepatosplenomegaly in school-age children. Hepatosplenomegaly in the absence of periportal fibrosis can occur in adults but is more common in school-age children, among whom it has previously been shown to be associated with exposure to both *S. mansoni* and *Plasmodium* infections. It is important to increase our understanding of the mechanisms behind this chronic, subtle morbidity, which is thought to be due to immunological inflammation, as hepatosplenomegaly in the absence of periportal fibrosis has been associated with clinical consequences, including dilation of the portal vein, indicating increases in portal pressure (41), and the stunting of growth (8; S. Wilson, submitted for publication).

It is likely that the immunological reaction to *S. mansoni* eggs that have been swept by the host's circulation into the liver, where they become trapped, is a major component of any immunological inflammation involved in hepatomegaly. In the present study, the levels of SEA-specific Th2 responses were low, as they were also found to be in a Ugandan study (20). Additionally, the third-principal-component scores, substantially explained by variation in the responses of the SEA-specific Th2 cytokines IL-4, IL-13, and IL-5, along with SEA-specific IFN- γ , were negatively correlated with *S. mansoni* egg counts, indicating that the SEA-specific Th2 responses are downregulated in an infection intensity-dependent manner, although on an individual cytokine level this was true of IL-13 but not of IL-4 and IL-5. As SWA-specific Th2 responses were both greater than SEA-specific Th2 responses and not related to *S. mansoni* infection intensities, this infection intensity-related downregulation is an SEA-specific phenomenon. A similar phenomenon has been described among Brazilian patients, for whom proliferative responses to SEA were also more downregulated than SWA responses (7).

In the present study, SEA-specific Th2 responses were also lower in *Plasmodium*-coinfected children. In the mouse model of *S. mansoni* infection, it has been shown that splenocytes of mice coinfecting with *Plasmodium chabaudi*, stimulated with SEA, release significantly lower levels of IL-4 and IL-5 than those of mice infected with only *S. mansoni*. Although this occurs

FIG. 3. Associations between cytokine responses in whole-blood cultures stimulated with *S. mansoni* antigens and the extent of liver and spleen enlargement. (A) Means $\pm$ 2 SE of the second-principal-component scores (TGF- β_1 plus IL-6) derived from whole-blood cultures stimulated with SEA or SWA for children with no enlargement (<2 cm), moderate enlargement (3 to 5 cm), or substantial enlargement (>5 cm) of the left liver lobe below the costal margin. (B) Means $\pm$ 2 SE of the third-principal-component scores (SEA-specific Th2 plus IFN- γ) for children with no enlargement (<2 cm), moderate enlargement (3 to 4 cm), or substantial enlargement (>4 cm) of the spleen below the costal margin. *P* values are <0.05 (*) and <0.01 (**) for comparisons with no enlargement of the organ.

at the time during the course of *P. chabaudi* infection when parasitemia is at its greatest (17, 48), it has also been shown that infection of mice with *Plasmodium berghei* at the same time as the injection of *S. mansoni* eggs into the microvasculature of their lungs leads to smaller granuloma reactions around the eggs than in mice that are not infected with *P. berghei*. In this experiment, it was also noted that eosinophils were sparse in the granulomas that were formed in the coinfecting mice (1). These murine studies suggest that Th2 responses to SEA may be downregulated by concomitant malaria infection and may result in poor eosinophil recruitment to the egg granuloma.

Concurrent microscopy-detectable *Plasmodium* infections have not been reported to exacerbate childhood *S. mansoni*-associated hepatosplenomegaly, whereas chronic exposure to *Plasmodium* infections has, as indicated by the higher levels of Pfs-IgG3 among children with hepatosplenomegaly (5, 27, 46). None of the principal components extracted were found to be significantly correlated with Pfs-IgG3 levels. However, there was a trend for children with low-level, PCR-detectable *Plasmodium* infections to have lower third-principal-component scores (SEA-specific Th2 plus IFN- γ). As antibody responses to *Plasmodium* infections may be short-lived (23), low-level infections, not detectable by microscopy, could be responsible for the persistence of higher specific antibody responses (39), such as those measured here as a marker of relative exposure. Although not related to Pfs-IgG3 levels among the children in this study, low-level PCR-detectable *Plasmodium* infections were associated with a greater extent of spleen enlargement (S. Wilson, unpublished data).

In concurrence with the case control study that was previously carried out in this area of Kenya, in which the children who presented with hepatosplenomegaly had significantly lower levels of SEA-specific IL-5 in PBMC cultures than the children in the control groups (26), the third-principal-component scores were lower in children who had enlargement of the spleen. However, this is contradictory with the previous study with respect to IFN- γ ; one possibility is that factors released from granulocytes, which would be absent in PBMC cultures, have a downregulatory effect on IFN- γ in whole-blood cultures. However, this relationship between the extent of splenomegaly and the third-principal-component scores, as well as their constituent parts, SEA-specific IL-4 and IL-13, was not linear, since children who had moderate enlargement of the spleen, but not those with substantial enlargement of the spleen, had significantly lower third-principal-component scores than those who had no enlargement of the spleen. As this principal component was associated with both *S. mansoni* infection intensities and the presence of *P. falciparum* infections, this nonlinear association could reflect a relationship between the two infections and in vitro responsiveness, rather than a direct relationship between in vitro responsiveness and presentation with splenomegaly, particularly as the exacerbation of childhood splenomegaly by *S. mansoni* infection in this area of Kenya is not infection intensity dependent (46).

SEA induced particularly high levels of TNF- α . TNF- α can be released during an IL-12- and IFN- γ -driven Th1 response (19, 35) or via the IL-17 pathway that upregulates IL-6 and TNF- α (22). CBA mice, a high-pathology strain that has a proinflammatory skewed response to SEA, have been shown to have granulomas of reduced size 7 weeks postadministration of anti-IL-17 in compar-

ison with control mice (36). In this study, whole-blood cultures stimulated with SEA released high levels of TNF- α and IL-6; however, when measured retrospectively in the supernatants, little or no IL-17 was detectable in SEA-stimulated cultures (J. B. Houghton, unpublished data). The levels of TNF- α that were produced in SEA-stimulated cultures did, however, indicate that the response to SEA was proinflammatory.

None of the three principal components had a substantial loading for TNF- α , so no direct association between SEA-specific TNF- α and hepatosplenomegaly could be confirmed, but in a previous study, childhood hepatosplenomegaly caused by *S. mansoni* was positively related to SEA-specific TNF responses in PBMC cultures (26). In the present study, however, it was shown that a principal component with strong loadings for *S. mansoni* TGF- β_1 and IL-6 antigen-specific responses, two cytokines with Th1 downregulatory functions (2, 28), was negatively associated with clinical measurements of hepatomegaly. IL-6 production is often considered a proinflammatory response. However, in the *S. mansoni* mouse model, although it is induced by IL-12, IL-6 has been shown to downregulate IFN- γ and IL-12p40 release from lymph node and splenocyte cultures stimulated with anti-CD3 and to induce the production of IL-10 (24), suggesting a regulatory role for this cytokine during *S. mansoni* infections. In humans, IL-6 is elevated in the sera of individuals who have periportal fibrosis associated with *Schistosoma japonicum* infection (10), a clinical outcome thought to be due to prolonged anti-inflammatory responses to entrapped eggs, and IL-6, along with IL-5 and IL-10, is elevated in the serum 24 h after treatment with praziquantel, a time point when the plasma TNF- α level is significantly decreased compared with pretreatment levels (31). TGF- β_1 has been shown to downregulate vaccine-induced in vitro Th1 responses by splenocytes to schistosomulum antigen (45). If, as shown by the present study, two cytokines involved in the downregulation of proinflammatory responses are produced at low levels in response to schistosome antigen challenge, one explanation is that the observed hepatomegaly may be caused by the poor control of inflammatory responses to the eggs.

Therefore, this study indicates, in agreement with previous reports, that school-age children with hepatosplenomegaly, which is associated with *S. mansoni* infection and chronic exposure to malaria, have low levels of SEA-specific Th2 responses. The downregulation of these responses appears to be driven by *S. mansoni* infection, in an infection intensity-related manner, but is also modulated by the presence of *P. falciparum* infection. It was not possible to confirm that hepatosplenomegaly is associated with a high level of proinflammatory responsiveness to schistosome antigens. However, the proinflammatory cytokine TNF- α was one of the predominant responses to stimulation with SEA, and low levels of IL-6 and TGF responsiveness among the children with hepatomegaly indicate that these children may be unable to sufficiently control a proinflammatory response.

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