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High Levels of TNF, Soluble TNF Receptors, Soluble ICAM-1, and IFN-γ, but Low Levels of IL-5, Are Associated with Hepatosplenic Disease in Human Schistosomiasis Mansoni

Joseph K. Mwatha,* Gachuhi Kimani,* Timothy Kamau,* Gabriel G. Mbugua,* John H. Ouma,† Jasper Mumo,‡ Anthony J. C. Fulford,§ Frances M. Jones,§ Anthony E. Butterworth,§ Morven B. Roberts,§ and David W. Dunne§

In a case-control study based in two areas of Kenya, hepatosplenic schistosomiasis mansoni was shown to be linked with low levels of IL-5 and with correspondingly high IFN-γ, TNF, and circulating soluble TNF receptor I (sTNFR-I), sTNFR-II, and sICAM-1. PBMC from the hepatosplenic cases responded to in vitro Ag stimulation with significantly higher levels of IFN-γ and TNF, but lower levels of IL-5, compared with nonhepatosplenic controls matched for age and infection intensity. Most of these correlations were confounded by differences between geographical areas. However, principle component analysis identified a high IFN-γ and TNF, and low IL-5 axis in the data as the first principle component; this was significantly associated with hepatosplenomegaly (p < 0.0005) even after controlling for area. High plasma levels of sTNFR-I (p < 0.001), sTNFR-II, (p < 0.0001), and sICAM-1 (p < 0.009) were also significantly associated with hepatosplenomegaly, independently of area, in the case of the soluble forms of both TNF receptors. These parameters were negatively related to IL-5. These results suggest that proinflammatory cytokines are involved in the hepatosplenic disease process in infected individuals who have low anti-inflammatory Th2 responses and that sTNFR may be a useful circulating marker for this disease process, perhaps reflecting the level of TNF activity in hepatic tissues. The Journal of Immunology, 1998, 160: 1992–1999.

Schistosomiasis is caused by infection with parasitic trematodes of the genus Schistosoma. Adult Schistosoma mansoni worms live in the mesenteric blood vessels around the gut, and the hepatosplenic form of the disease is thought to be associated with the host’s immune response to parasite eggs lodged in the presinusoidal capillary beds of the liver (1). Most infected individuals living in schistosomiasis endemic areas do not suffer severe liver damage, but a minority go on to develop hepatosplenic morbidity characterized by hepatosplenomegaly, hepatic periportal fibrosis, and portal hypertension, which may lead to the formation of esophageal varices and hematemesis. The prevalence of hepatosplenic schistosomiasis varies significantly between different endemic areas (2–4). Although the risk of developing severe hepatosplenic disease is higher in individuals who experience higher intensities of infection (5), communities from the same tribal group in different parts of Kenya suffer markedly different prevalences of hepatosplenomegaly despite having similar intensities of infection (6), which suggests that factors other than infection intensity may influence the progression from uncomplicated infection to severe hepatosplenic disease.

Most of the available information on the immune mechanisms underlying the schistosome egg granuloma is derived from murine models and has been interpreted in terms of the Th1/Th2 paradigm, which subdivides CD4+ T cells on the basis of their characteristic patterns of cytokine production (7). The granuloma is a cell-mediated, CD4+ T cell-dependent response (8), controlled by a complex pattern of often counter-regulating cytokines, chemotactic factors, and cell adhesion factors, which successively initiate, maintain, and immunomodulate the granuloma (7). Tissue egg deposition in S. mansoni-infected mice initiates a switch of host cytokine responses from those characteristic of a Th1 or Th0 to a Th2-like pattern (9–11). Numerous experiments have demonstrated the central role of IL-4 in murine schistosomiasis granuloma formation (12–15) and hepatic fibrosis (16), and the counteracting down-regulatory influence of IFN-γ (15, 17, 18), which can be initiated by IL-12 (17–19). Despite the antimorbidities of Th1 cytokines, TNF may also be an important mediator of murine granuloma formation and hepatic fibrosis (20–23); part of the influence of TNF is associated with the up-regulation of ICAM-1 expression (24).

The paucity of information on human anti-egg granulomatous responses is such that it is difficult to judge how mechanisms delineated in murine models are applicable to the human disease process. In addition to differences that may occur between the responses of any two host species to the same parasite, there are particular differences between murine models and human infections. For example: the Th1/Th2 subgrouping of human CD4+ T cells is less well defined than in the mouse; human hepatosplenic schistosomiasis develops at lower intensities of infection than it is possible to achieve on the basis of number of worms per unit of host body weight in the mouse; and human disease takes a number

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of years of infection to develop (25), whereas murine infections are usually studied over a few weeks or months. Recently few studies of Ag-specific cytokine expression and human hepatosplenic schistosomiasis have been conducted. Of those that have been reported, only one (26) examined hepatosplenic patients alongside infected, nonhepatosplenic controls matched for age and intensity of infection, factors that affect immune responses in endemic populations (27). Significant associations between specific cytokine expression and human hepatosplenic disease have yet to be established (28–30). Generally, studies of inflammatory markers in sera or plasma from hepatosplenic patients have also been uncontrolled, but have shown that infection results in raised levels of TNF-α (31–34), ICAM-1, and endothelial leukocyte adhesion molecule (ELAM)-1 (35). In one study that was well controlled, hepatosplenic patients had significantly raised levels of serum TNF, but their peripheral blood monocytes released less TNF when treated with mitogen (31).

In a case-control study, we examined TNF, IFN-γ, and IL-5 responses of PBMC to in vitro stimulation with Con A as well as S. mansoni worm and egg Ags in groups of S. mansoni-infected patients, with and without hepatosplenomegaly, who were carefully matched for age and intensity of infection. In addition, in the same individuals, we measured plasma levels of immunologically detectable TNF-α, soluble TNF receptor I (sTNFR-I), sTNFR-II, and sICAM-1. The relationships between these parameters and their associations with the presence or absence of hepatosplenomegaly were analyzed.

Materials and Methods

Patients

The patients in this study came from two areas of Kenya endemic for S. mansoni, Kambu and Kangundo, described in a previous paper (6). Both are almost exclusively populated by members of the same tribe (the Kamba) and have similar mean S. mansoni infection intensities but very different rates of hepatosplenomegaly (6).

The present study followed a case-control design: 20 patients from Kamba (a high morbidity area) with hepatosplenomegaly were each matched with two controls, one from Kambu and one from Kangundo (a low morbidity area), of the same age and with similar numbers of eggs in their stools but no detectable hepatosplenomegaly.

Organomegaly was assessed by palpation by an experienced clinician (G.G.M.). Palpable livers were measured in the midsternal and midclavicular lines. Palpable spleens were measured in the midclavicular and midaxillary lines. All hepatosplenic cases had both spleen and liver enlargement of at least 2 cm, while neither organ was palpable in the controls.

Ages were matched ± 1 yr, and egg counts ± 10% as estimated from two Kato smears were prepared from a single stool. Matches could not be found for all 20 cases: 19 were matched with Kangundo controls and 17 with Kambu controls. Too few hepatosplenic cases were found in Kangundo to balance the study with respect to area. Characteristics of the patients in the study are summarized in Table I.

Study members were admitted to the clinical research wards at the Kenya Medical Research Institute in batches of six (two sets of three matched individuals) where they were bled (20 ml venous blood) and treated with Praziquantel (40 mg/kg body weight; Bayer AG, Leverkusen, Germany). Cellular assays were likewise performed in balanced batches of six.

Isolation and stimulation of PBMC

PBMC were isolated from heparinized blood by density gradient centrifugation on Histopaque 1077 (Sigma-Aldrich, Poole, Dorset, U.K.). PBMC were washed in RPMI 1640 (ICN Flow Biomedicals, Thame, Oxfordshire, U.K.) and resuspended at 1.5 × 10^6 cells/ml in Iscove’s medium (ICN Flow) supplemented with 5% AB serum (final concentration), 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 mM l-glutamine. The cells were stimulated with soluble worm Ag (SWA) and soluble egg Ag (SEA) prepared from respective stages of the S. mansoni life cycle as previously

3 Abbreviations used in this paper: sTNFR-I, soluble TNF receptor I; SWA, S. mansoni soluble worm Ag; SEA, S. mansoni soluble egg Ag; ANOVA, analysis of variance; MSS, moderate splenomegaly syndrome; HSS, hypersplenomegaly syndrome.

Table I. Characteristics of the patients in the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Without hepatosplenomegaly</th>
<th>With hepatosplenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (N)</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>12.89</td>
<td>12.47</td>
</tr>
<tr>
<td>Age range (yr)</td>
<td>8–16</td>
<td>8–16</td>
</tr>
<tr>
<td>Sex</td>
<td>Female 9</td>
<td>Female 9</td>
</tr>
<tr>
<td></td>
<td>Male 13</td>
<td>Male 13</td>
</tr>
<tr>
<td>Mean eggs per gm</td>
<td>705</td>
<td>1002</td>
</tr>
<tr>
<td>Range (egg count)</td>
<td>50–2730</td>
<td>80–2760</td>
</tr>
</tbody>
</table>

Cytokine and soluble receptor assays

IFN-γ and IL-5 were measured as marker cytokines for, respectively, Th1 and Th2-type patterns of cytokine response. In general, cytokine assays on PBMC culture supernatants were conducted as previously described (37). The murine IL-5 dependent cell line B13 (American Type Culture Collection (ATCC), Rockville, MD) was used to determine the levels of IL-5. The fibroblastic cell line L929 (ATCC) was used to detect TNF in a rapid cytotoxicity assay. The B13 cell line was regularly checked for IL-5 dependency, while L929 was checked for TNF susceptibility. Briefly, 100 μl of B13 cells at 5 × 10^6 cells/ml were incubated with 100 μl of culture supernatant in 96-well tissue culture plates in a humidified incubator at 37°C in an atmosphere of 5% CO2 in air. After 2 days, cytokine-dependent cell growth was assessed using the mononetozatrim (MTT) colorimetric assay (38). To detect TNF, 100 μl of supernatant was incubated with L929 cells at 2.5 × 10^5 cells/ml in the presence of actinomycin D (2 μg/ml; Sigma-Aldrich) to a final volume of 200 μl in 96-well tissue culture plates. After overnight incubation, the medium was poured off and the cells were stained with 100 μl of 0.5% crystal violet in methanol. The stain was poured off, the plates were washed and then allowed to dry. One hundred microliters of 10% acetic acid was used to solubilize the stain. Color release was read on a spectrophotometer at 540 nm.

To determine the levels of IFN-γ in supernatants, a capture ELISA was used. Briefly, Maxisorp ELISA plates (Nunc; Life Technologies, Paisley, U.K.) were coated with mouse anti-IFN-γ-Ab (Interferon, New Brunswick, NJ) in carbonate/bicarbonate buffer overnight. Plates were then washed in PBS containing 0.05% Tween-20 (PBS-T) and blocked for 1 h at 37°C. IFN-γ was detected by use of rabbit anti-human IFN-γ at 1:800 (Department of Pathology, Cambridge University) and a goat anti-rabbit peroxidase conjugate at 1:5000 (Dako, High Wycombe, Buckinghamshire, U.K.). Finally, ABTS substrate (2-2-azino-bis [3-ethylbenzthiazoline 6-sulfonic acid) diammonium) was added, and the absorbance was measured at a wavelength of 405 nm. Cytokine values for each test sample were determined by interpolation from standard curves obtained with recombinant cytokines that were included in each assay plate.

Assays of the plasma concentrations of immunologically detectable TNF-α, sTNFR-I, sTNFR-II, and sICAM-1 were all conducted using commercially available sandwich ELISA assays (all from Genzyme Diagnostics, Cambridge, MA) according to the manufacturer’s instructions. Plasma samples from 10 healthy European donors, with no history of schistosomiasis, were used as uninfected plasma controls in these assays.

Statistical methods

The distributions of cytokine responses were highly skewed so that cytokine levels were categorized as either positive or negative (according to whether cytokine production of the stimulated cells was greater than the nonstimulated cells) for analysis by logistic regression.
Nonparametric tests (Kruskal-Wallis) on the raw data were used to confirm the one-way analyses. Further analysis was performed on the first principle component (MINITAB; Minitab, Inc., PA) derived from the correlation matrix of the logarithms, log(1 + 1), of the six in vitro Ag-stimulated PBMC cytokine production assays, TNF, IFN-γ, and IL-5 production after stimulation with either SWA or SEA. The first principle component, which followed a reasonably normal distribution, was analyzed by ANOVA.

The relationship between the PBMC cytokine responses and the circulating soluble receptors/sICAM-1 was examined using Spearman’s rank correlation coefficients.

Two “contrasts” between the groups, hepatosplenic cases vs nonhepatosplenic controls and Kambu vs Kangundo, were examined. These contrasts were somewhat confounded by the lack of hepatosplenic cases from Kangundo. The carefully balanced design with respect to age and intensity of infection (fecal egg counts) eliminated any influence of these variables on the analysis; the inclusion of these variables altered none of the conclusions, and they are considered no further in this report.

The significance in terms of the logistic regression models were tested using $\chi^2$, the difference in deviance, which was assumed to follow a $\chi^2$ distribution under the null hypothesis that the term was unimportant.

Logistic regression was performed using GLIM (Numerical Algorithms Group Ltd., Oxford, U.K.), while MINITAB was used for principle component calculation, analysis of variance and Kruskal-Wallis tests. Both software packages were run on Macintosh Quadra 800 microcomputers.

Results

In vitro cytokine production by PBMC

The results of TNF and IL-5 bioassays and IFN-γ ELISA of PBMC culture supernatants after stimulation with SEA, SWA, or Con A are plotted in Figure 1 as geometric means along with the 95% confidence intervals for each assay and each morbidity group. The results of logistic regression on morbidity group and geographical area are summarized in Table II. Little difference was seen between the groups (or between morbidity or area contrasts) for any cytokine in response to Con A. Hepatosplenic cases had higher TNF and IFN-γ but lower IL-5 than the controls when cells were stimulated with either SWA or SEA. These differences were significant by logistic regression (Table II) in the case of TNF for both Ags, IFN-γ for SWA and IL-5 for SEA. The relationship between higher levels of SWA-stimulated IFN-γ remained significant even when the possible influence of different geographical areas was taken into account.

Table III shows the rank correlation coefficient for the associations between the cytokine response to each Ag/mitogen. From this comparison, it can be seen that TNF and IFN-γ are positively correlated with each other, and both are negatively correlated with IL-5.

Table IV shows the correlation coefficients between the first principle component and the SWA- and SEA-stimulated PBMC responses (TNF, IFN-γ, IL-5) from which it was derived. Since both of the IL-5 coefficients are positive, while those for the TNF and IFN-γ are all negative, the first principle component appears to correspond to a spectrum of response between Th1 and Th2. The first principle component was considerably lower (i.e., more “Th1-like”) among the hepatosplenic cases than the nonhepatosplenic controls ($p < 0.0005$ by ANOVA; Fig. 2), a difference that was undiminished after controlling for differences between areas.

Circulating inflammatory markers

Plasma from the same individuals in each morbidity group was assayed in specific TNF-α ELISA. These assays detected little or no TNF-α in any of the samples tested (data not shown). Serum levels of the sTNFR-I and -II can be better markers for tissue TNF production than serum levels of TNF itself, perhaps because of the instability or short half-life of TNF (39). Therefore, the individuals from each morbidity group were tested in ELISA to assess the levels of sTNFR-I and sTNFR-II in their plasma. These results are shown in Figure 3 as geometric means for each soluble receptor for each group along with their 95% confidence intervals. Multiple regression analysis showed that both soluble receptors remained significantly associated with hepatosplenomegaly after controlling for differences between geographical areas (sTNFR-I: $F = 15.98$ on 1 and 47 df, $p < 0.001$; sTNFR-II: $F = 31.45$ on 1 and 48 df).
Levels of sICAM-1 were measured in each individual plasma by ELISA. The hepatosplenic group had higher levels of sICAM-1 (356 ng/ml ± 182) than the two matched nonhepatosplenic control groups (Kangundo: 226 ng/ml ± 110; Kambu: 250 ng/ml ± 115). The uninfected European controls had levels of circulating sICAM-1 (245 ng/ml ± 85) similar to the nonhepatosplenic, infected controls. The difference between infected individuals with or without hepatosplenomegaly was statistically significant (F = 7.46 on 1 and 48 df, p = <0.009); however, this result just failed to reach a statistically significant level when area was allowed for in logistic regression analysis. Statistical correlations between sTNFR-1, sTNFR-2, and ICAM-1 were examined alongside the TNF, IFN-γ, and IL-5 responses of PBMC to SWA, SEA, and Con A. Table V shows that there were statistically significant positive relationships between all three circulating soluble markers. No correlations between Con A-induced cytokines and circulating sTNFR/sICAM-1 were found (results not shown). However, the levels of all the circulating markers were found to be generally positively correlated to Ag-induced IFN-γ and TNF responses (often this was statistically significant), but negatively related to IL-5 induced by the same Ags (Table V).

### Table II. Summary of logistic regression of proportion of responders vs morbidity and area

<table>
<thead>
<tr>
<th>Ag/Mitogen</th>
<th>TNF</th>
<th>IFN-γ</th>
<th>IL-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWA</td>
<td>4.28*</td>
<td>6.92**</td>
<td>0.70</td>
</tr>
<tr>
<td>% Positive</td>
<td>Nonhepatosplenic</td>
<td>Hepatosplenic</td>
<td>Hepatosplenic</td>
</tr>
<tr>
<td>χ² for effect of morbidity</td>
<td>1.09</td>
<td>7.66**</td>
<td>0.04</td>
</tr>
<tr>
<td>SEA</td>
<td>5.94*</td>
<td>1.99</td>
<td>6.00*</td>
</tr>
<tr>
<td>% Positive</td>
<td>Nonhepatosplenic</td>
<td>Hepatosplenic</td>
<td>Hepatosplenic</td>
</tr>
<tr>
<td>χ² for effect of morbidity</td>
<td>2.82</td>
<td>2.29</td>
<td>2.84</td>
</tr>
<tr>
<td>Con A</td>
<td>0.04</td>
<td>1.55</td>
<td>0.18</td>
</tr>
<tr>
<td>% Positive</td>
<td>Nonhepatosplenic</td>
<td>Hepatosplenic</td>
<td>Hepatosplenic</td>
</tr>
<tr>
<td>χ² for effect of morbidity</td>
<td>0.03</td>
<td>0.73</td>
<td>0.02</td>
</tr>
</tbody>
</table>

χ² is the difference in deviance between hierarchical models and is assumed, under the null hypothesis, to follow a chi-square distribution on 1 degree of freedom; *p < 0.05; **p < 0.01.

### Discussion

High levels of TNF and IFN-γ, and low levels of IL-5, produced by PBMC stimulated specifically with schistosome Ags SWA and SEA, but not with Con A, were significantly associated with the presence of hepatosplenomegaly. These are the first statistically significant relationships between Ag-specific cytokine responses and hepatosplenic schistosomiasis to be reported from a study controlled for patient age and intensity of infection. However, most of these relationships were confounded by the difference between the two areas, Kangundo and Kambu. The populations of these two areas suffer strikingly different levels of hepatosplenic disease, despite having the same ethnic background and similar intensities of infection (6). Environmental factors could influence both the prevalence of the hepatosplenic manifestation of schistosomiasis and immune responses. Such influences could be important cofactors involved in a causal linking of elevated IFN-γ and TNF with disease, or merely confounding factors, having an independent effect on both cytokine profile and the occurrence of hepatosplenic involvement. We have found that the high morbidity Kambu area had a higher prevalence of malaria and that the population had a poorer nutritional status (6, 40). It is worth noting that this study was deliberately conducted outside of the malaria transmission season and that blood smears were taken from all the individuals who participated and none were found to have a detectable malaria parasitaemia. Thus, while an active malaria infection is unlikely to be a contributor to either the observed organomegaly or cytokine pattern, the possibility of a previous history of malaria having an influence cannot be eliminated by this study.

A factor that might have contributed to the Kambu nonhepatosplenic controls being less significantly different from the Kambu hepatosplenic cases than were the Kangundo controls was that, on reexamination, some Kambu controls were found to have slight hepatosplenomegaly, which was not detected in the original physical examination used to define the subjects as being hepatosplenic or nonhepatosplenic. Reexamination of the Kangundo controls did not...

### Table IV. Correlations between the first principle component and the Ag-specific PBMC responses from which it was derived

<table>
<thead>
<tr>
<th>Ag</th>
<th>Cytokine</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWA</td>
<td>TNF</td>
<td>-0.606</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>-0.486</td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>0.406</td>
</tr>
<tr>
<td>SEA</td>
<td>TNF</td>
<td>-0.335</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>-0.711</td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>0.520</td>
</tr>
</tbody>
</table>

### Table III. Associations between cytokine responses to SWA, SEA, and Con A

<table>
<thead>
<tr>
<th>Response to</th>
<th>Cytokine</th>
<th>TNF</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWA</td>
<td>IFN-γ</td>
<td>0.289*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>-0.202</td>
<td>-0.301*</td>
</tr>
<tr>
<td>SEA</td>
<td>IFN-γ</td>
<td>0.257*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>-0.105</td>
<td>-0.491***</td>
</tr>
<tr>
<td>Con A</td>
<td>IFN-γ</td>
<td>0.416***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>-0.118</td>
<td>-0.343*</td>
</tr>
</tbody>
</table>

*Values tabulated are the Spearman rank correlation coefficients between the cytokine levels to each of the Ags and to Con A. *p < 0.05; ***p < 0.001.
mainly produced by activated macrophages (41). TNF is generally associated with Th1-like responses, since Th1 cells can produce TNF directly and, by producing IFN-γ, they enhance the production of TNF through its macrophage-activating function (42). In addition, a number of Th2 cytokines have been shown to limit the expression and activity of Th1 cells. IL-4 is known to cause a reduction in the production of TNF, possibly by regulating TNF gene expression (43).

Accumulated evidence from many studies, using a variety of mouse models, strongly supports the hypothesis that Th2-type cytokine responses, and IL-4 in particular, are critical in promoting the schistosome egg granuloma. It has been shown that in vivo neutralization of IL-4 suppresses egg granuloma formation, while administration of rIL-4 enhances the granuloma response and decreases Th1-like cytokine responses, including IFN-γ (12–15). Anti-IL-4 treatment also significantly suppresses hepatic collagen formation and expression of IL-5 and IL-13 mRNA in the liver (16). Neutralization of endogenous IFN-γ has been shown usually to decrease murine granuloma size (13, 15, 17, 44), while granuloma formation occurs normally in IFN-γ knockout mice (18). Thus the observations reported here, that in vitro stimulation with SEA and SWA induce PBMC from patients with hepatospleno-megaly to produce more IFN-γ and less IL-5 than nonhepato-splenic controls, would not be predicted from the well-established association between Th2 cytokine responses, egg granuloma formation, hepatic fibrosis, and portal hypertension seen in the mouse. However, the significantly higher levels of Ag-induced TNF in hepatosplenic patients can be more easily related to a number of murine schistosomiasis studies. Anti-TNF serum reduced, and treatment with TNF-α increased, hepatic granuloma size in S. mansoni-infected mice (20). Similarly, rTNF-α has been shown to reconstitute granuloma formation in S. mansoni-infected SCID mice (21), and worm-induced TNF-α has been shown to mediate the immune priming necessary for hepatic granuloma formation after injection of S. mansoni eggs into naive mice (23).

A particularly interesting S. mansoni-mouse model has been described by Henderson and colleagues (45). In this model, CBA/J mice chronically infected (10 to 20 wk in the mouse) with S. mansoni, develop two distinct syndromes designated moderate splenomegaly syndrome (MSS) and hypersplenomegaly syndrome (HSS), which are similar to, respectively, the nonhepatosplenic (also termed "intestinal") and hepatosplenic forms of schistosomiasis. HSS occurs in about 20% of infected mice and is a strikingly more severe form of the infection, characterized by massive splenomegaly, ascites, thymic atrophy, severe anemia, and cachexia. In addition, HSS mice suffer increased perivascular and hepatic portal fibrosis (45). Recently, it has been shown that CBA/J mice that go on to develop HSS fail to modulate the production of TNF-α mRNA in their livers and suffer...
greater liver fibrosis than MSS mice (22). Interestingly, IL-4 knockout mice also suffer severe TNF-α-mediated morbidity when infected with *S. mansoni* (46). As TNF-α is associated with the induction and maintenance of fibrotic reactions (47–49), and as large amounts are produced by IFN-γ-activated macrophages (41), it is possible that chronically elevated levels of IFN-γ and TNF (and low levels of counter-regulating Th2 cytokines) might predispose some schistosome-infected individuals to the development of hepatic fibrosis and hepatosplenic disease.

Some previous human studies have pointed toward the involvement of TNF in hepatosplenic schistosomiasis. In a well-controlled study, Zwingenberger and colleagues (31) found higher TNF levels in serum of patients with hepatosplenic disease compared with patients without organomegaly. Similar observations were made on Egyptian schistosomiasis patients who were not controlled for age and intensity of infection (33, 34). In addition, TNF secreted by nonstimulated monocytes and serum TNF was found to be elevated in *S. haematobium*-infected patients with carcinoma of the urinary bladder (32).

Our failure to detect significant levels of plasma TNF-α may have been because the methods used to separate PBMC for in vitro studies were not optimal for the preservation of plasma TNF. However, in a variety of infectious diseases, it has been shown that circulating levels of TNF are increased only transiently in the early stages of infection, while circulating concentrations of sTNFR remain elevated for longer periods (39). Circulating levels of sTNFR-I or sTNFR-II have been found to be more useful than circulating TNF for monitoring the course of disease or morbidity in a variety of infections such as, for example, leishmaniasis (50), tuberculosis (51), HIV infections, (52) and rickettsiosis (53). In addition, circulating levels of sTNFR were found to be useful indicators of disease activity and prognosis for cancer (54) and rheumatoid arthritis (55). Here, we have found that elevated plasma levels of sTNFR-I and sTNFR-II are strongly and significantly correlated with the presence of hepatosplenic disease. These correlations are significant, irrespective of geographical area, and are supportive of the observation that elevated Ag-specific release of TNF is a characteristic feature of the hepatosplenic form of human schistosomiasis.

In experimental animal models, the administration of sTNFR can neutralize the pathologic effects of TNF (56). TNFR:Fc has been shown to neutralize the biologic activity of TNF-α in mice (57) and in human transplantation patients (58), and it reduces granuloma size in murine schistosomiasis (24). It might have been expected, therefore, that high levels of sTNFR would be negatively associated with hepatosplenic disease, rather than the positive relationship found in this study. However, it also appears that sTNFR can stabilize TNF-α and may facilitate its biologic activity (59). The positive correlations shown in this study (Table V) between circulating sTNFR and in vitro TNF production by Ag-stimulated PBMC, in particular the statistically significant correlation between the TNF response to SEA and circulating sTNFR-I, suggest that sTNFR may be directly related to up-regulated TNF and, therefore, may be acting as a circulating marker for the local production of TNF in host tissues. It is interesting to note that Josimovic-Alasevic and colleagues observed that elevated concentrations of soluble 55 kDa IL-2R subunit are detectable in the sera of hepatosplenic schistosomiasis patients (60).

In addition to proinflammatory cytokines and their antagonists, intercellular adhesion molecules may be important in the murine anti-egg granulomatous response. Elevated ICAM-1 expression is associated with murine acute phase granuloma formation (61). Anti-ICAM-1 Ab treatment suppresses murine *S. mansoni* egg granuloma formation, and injection of sTNFR:Fc down-regulates both granuloma formation and ICAM-1 expression (24). Secor and colleagues (35) have found that the circulating, soluble form of ICAM-1 was significantly higher in hepatosplenic Brazilian patients than in nonhepatosplenic infected individuals but, unfortunately, because of the age-distribution of infection intensity and morbidity in the study population, they were unable to directly match their subjects for age and intensity of infection. Our results support those of Secor and colleagues, in that circulating levels of sICAM-1 were significantly higher in Kenyan hepatosplenic patients than in the matched nonhepatosplenic controls, although this relationship fell just below the level of statistical significance when geographical area was taken into account.

In the present study, there was a consistent pattern of positive, often statistically significant, correlations between Ag-induced TNF and IFN-γ and circulating sTNFR-I/II and sICAM-1; and there were negative associations between these parameters and IL-5 (Table V). This pattern, together with the relationship between these response patterns and hepatospleno-megaly, strongly suggests a link, perhaps causal, between Th1 responses to schistosome Ags, up-regulated proinflammatory cytokines, and adhesion molecules, and the development of hepatosplenic schistosomiasis.

A particular strength of this study design is that age and intensity of infection, which are important influences on immunologic responses in schistosome-infected populations, have been fully controlled for, thereby greatly increasing the chances of the identification of any immune correlations of hepatosplenic disease. However, the corollary is that the influence of these factors on morbidity cannot be assessed in this study, and therefore, caution should be exercised in extrapolating these results to the relationship between immunologic responses and hepatosplenic disease in older, perhaps less heavily infected individuals. Similarly, the current study groups were selected on the basis of hepatosplenomegaly rather than ultrasonography-detectable hepatic fibrosis, and it is important to acknowledge that the detailed relationship between these two aspects of schistosomiasis-associated morbidity remains largely undefined. We are, therefore, currently undertaking cross-sectional population studies, in addition to further case-control studies, to evaluate the relationship between immune responses, hepatosplenomegaly, and hepatic fibrosis. Cross-sectional studies of schistosomiasis endemic populations will complement the case-control study design, as no preselection of study subjects is involved, and therefore, the influences on both age and intensity of infection can be examined by multiple regression analysis.

The immune correlates of schistosomiasis-associated hepatosplenic disease reported in this study appear to contrast with the established role of Th2 responses in murine granuloma formation and hepatosplenic morbidity. The *S. mansoni* egg is a powerful stimulus to Th2-type cytokine responses. However, in evolutionary terms, it would be unfortunate if the predominant response of the natural host (man) to a common parasite was an entirely immunopathologic one. Indeed, most schistosome-infected individuals in endemic regions do not develop severe hepatosplenic disease. This being so, it is possible that a dominant Th2-like response to the egg may actually represent a normal, relatively nonpathologic, anti-inflammatory response in human schistosomiasis. In that case, it would be the minority of infected individuals, with a poor Th2 response, who fail to regulate their proinflammatory responses and consequently risk suffering severe hepatosplenic disease. While this conclusion is necessarily speculative, it does underline the need for further, carefully controlled studies of the interactions between human immune responses and schistosomiasis-induced hepatosplenic disease.
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References


