CD4+ T-CELL COUNTS, CD4+/CD8+ T-CELL COUNT RATIOS, AND ANTIBODY LEVELS IN MIGRANT FISHERMEN INFECTED WITH *SCHISTOSOMA JAPONICUM* IN THE DONGTING LAKE, CHINA

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Abstract. In this study, CD4+ and CD8+ T cells and antibody levels were measured in 94 migrant fishermen infected with *Schistosoma japonicum* from Dongting Lake, China. Prevalence among these fishermen was high (63.8%), with a mean infection intensity of 61.4 ± 3.8 epg, and included a high proportion of individuals (39.4%) with substantial parenchymal fibrosis (stages ≥ 2/3). The CD4+/CD8+ ratio in men (1.34 ± 0.11) was significantly lower than that of women (1.58 ± 0.15). CD4+ T cells and the ratio of CD4+/CD8+ were significantly decreased both in subjects infected with *S. japonicum* and those with parenchymal fibrosis. However, levels of total IgA, IgM, and anti-schistosome egg antigen IgG correlated positively with infection intensity and pathologic lesion number. These results suggest an imbalance between cell-mediated and humoral immunity in these fishermen, the precise cause of which remains undetermined.

INTRODUCTION

The main pathology induced by the intestinal schistosomes (*Schistosoma japonicum* and *S. mansoni*) is a Th2-driven fibrotic reaction against the parasite eggs. This host reaction is an immunopathological process, and the resulting periportal fibrosis is the consequence of the peri-ovular inflammatory (granuloma) reaction. The repair of these fibrotic lesions leads to scarring, progressive organ damage, serious fibro-obstructive pathology, portal hypertension, ascites, acute hepatosplenomegaly, and eventually fatal hematemesis. The immunologic mechanisms that bring about schistosome-induced granuloma formation and the resulting fibrogenesis are slowly being understood, with the majority of studies having been undertaken on murine models of schistosomiasis.[1] T cells are required and the response is mediated by major histocompatibility complex (MHC) class 2–restricted CD4+ Th lymphocytes specific for schistosome egg antigens (SEAs). An early Th1-type cytokine reaction to SEA normally transitions to a Th2-dominant response as the disease evolves. Fibrosis develops in the granulomas during the chronic phase of inflammation in mice in which a variety of molecules stimulate the differentiation of stellate cells that secrete extracellular matrix proteins, including collagens and fibronectin. These cells are recruited to the areas of injury usually by chemokines and become activated, taking on a myofibroblast-like phenotype, and secrete a collagen-rich matrix into the extracellular space.[1]

The human immune response is regulated by a network of cross-regulatory cytokines released by T helper (Th) cells. However, the exact role played by cytokines in regulating susceptibly or resistance remains unclear. Reports on the immune status of individuals with schistosomiasis at different stages of pathology (acute, chronic, and advanced) have shown that the phenomenon of cellular immune suppression exists among people with chronic and advanced schistosomia-

sis.[2–5] However, the majority of these studies mainly focused on patients in hospitals who were classified clinically. Population-based studies of human immune status are lacking, especially in populations at high risk of infection. This study aimed to explore the relationship between immune responses and infection intensity or pathologic changes in the liver among a group of migrant fishermen who live and work on boats in the Dongting Lake in Hunan Province, China; these fishermen have a very high level of water exposure and a high frequency of infection.[6] The completed analysis was based on infection studies, morbidity measurements, and estimates of the levels of CD4+ and CD8+ T cells and antibodies in infected individuals compared with controls.

MATERIALS AND METHODS

Study area and study subjects. The study site selected was a migrant fishermen village on the shores of the eastern section of Dongting Lake, Yueyang, Hunan, China. The study area is highly endemic for schistosomiasis but not for malaria. The geographic and population information and prevalence of schistosomiasis for this community were described in a previous report.[6] The study focused on individuals who live and work on fishing boats on the lake and who are highly exposed to *S. japonicum* because of their lifestyle and occupation. A total of 94 subjects (men = 59; women = 35) with an average age of 31 years participated in the survey in 2002. All accepted ultrasound examination and all provided blood samples for CD4+ and CD8+ counts, sera for antibody level measurement, and stools for parasitological assessment.

In addition, 10 subjects (men = 5; women = 5) with a mean age of 28 years from a non-endemic area for schistosomiasis were included as normal controls in this study. All 10 controls were Hunan University students and staff who had no previous history of water contact and who were negative for *S. japonicum* by fecal examination and serology.

Infection assessment. Individual infection with *S. japonicum* was assessed by both the quantitative Kato-Katz thick smear examination and the miracidium hatching test, a traditional method developed in China.[7] Both methods, based on one stool sample, were described in detail in a previous re-

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port.[6] Infection intensity (eggs per gram of stool [epg]) was determined by the Kato-Katz technique.

Morbidity assessment of liver and spleen. A portable ultrasonograph (EUB 200; Hitachi Medical Corp., Tokyo, Japan) was used in the field to assess hepatosplenic morbidity in each subject. Protocols for measurement, classification, standard scan positions, and views followed the World Health Organization (WHO) standard protocol established in Cairo in 1990.[8] To prevent observer measurement bias, only one experienced observer was invited to conduct all ultrasound measurements. The measurements taken were reported in detail in a previous report.[6]

Measurement of peripheral blood CD4$^+$ and CD8$^+$ T cells. CD4$^+$ and CD8$^+$ peripheral blood T-lymphocyte subset counts were measured by flow cytometry using a FACS-420 at the Tumor Institute of He Bei Medical University, China. Blood samples (0.5 mL for each subject) were collected in heparinized tubes and distilled water was added (4.5 mL/tube).[9,10] The tubes were left at room temperature for 1 minute, and 0.5 mL sodium chloride (0.9% wt/vol) was added. The supernatants were removed after centrifugation at 1,000 rpm for 5 minutes, and the pellets were washed in phosphate-buffered saline (PBS). The supernatants were removed again by centrifugation at 1,000 rpm for 5 minutes. Formaldehyde (4% vol/vol)-PBS solution was added to the remaining pellets and stored at 4°C. Before measuring CD4$^+$ and CD8$^+$ T cells, the pellets were washed by centrifugation, and mouse anti-human CD4$^+$ and CD8$^+$ monoclonal antibodies were added, together with isosulfocyanic acid fluorandiol. To cell counts of 4×10^6/mL, the same volume of methanol was added to the tubes and lightly mixed. The tubes were left at 4°C for 1 hour, and the cells were processed using ribonuclease and stained by iodide pyridine. The percentage of CD4$^+$/CD8$^+$ T lymphocytes was based on the measurement of 10,000 cells.

Detection of total IgA and IgM levels in serum. Diagnostic reagent kits, based on published procedures,[11] were purchased from Shanghai Ke Hua Biology Project Corporation (Shanghai, China), and IgA and IgM levels were assessed by immuno-turbidimetry, performed using an auto-biochemical analyzer at the Clinical Laboratory of Xiang Ya Hospital. Measured units are expressed as milligrams per milliliter.

Detection of specific anti-SEA IgG in serum. Indirect ELISA was used for measuring anti-SEA IgG antibodies. Coating concentration with SEA was 5 μg/mL. Three controls on the ELISA plates included a blank PBS and negative and positive reference sera. Primary human serum samples were diluted at 1:800, and the secondary antibody used was horseradish peroxidase-staphylococcal protein A (HRP-SPA). OD values (596 mm) were measured using an E960 microplate reader.

Statistical analysis. SPSS10.0 statistical software was used for data entry and analysis. Student t test was used to compare the means of two groups. The averages among multi-groups were compared by analysis of variance and others by standard 2×2 tables (χ^2). Differences with $P < 0.05$ were considered statistically significant.

Ethical considerations. An information sheet describing the aims of the study was issued to each participant. Signed consent was collected from the participants or their parents. All patients who were stool-egg positive, except pregnant women, were offered a single dose (40 mg/kg body weight) of praziquantel. All the examination outcomes of the study were reported to local physicians who visited the fishermen's village. Ethical clearance for this study was obtained from the Medical Ethics Committee of Hunan Province and The Queensland Institute of Medical Research.

RESULTS

Profiles of *S. japonicum* infection and morbidity assessed by ultrasound. Of the 94 participants, 63.8% ($N = 60$) were shown to be infected with *S. japonicum* by the Kato-Katz method and/or the miracidium hatching test. A geometric mean intensity of 61.4 ± 3.8 epg, based on the Kato-Katz quantitative method, was evident in this infected group. Among these, light infection intensity (epg 1–100) made up 53.3% ($N = 32$), and heavy infection intensity (epg > 400) was 15% ($N = 9$). The highest infection intensity (epg = 2,400) was seen in a 27-year-old male patient. Infection (72.2%, 39/54) and infection intensity (70.3 ± 4.5 epg) in men ($N = 54$) were significantly higher than those (52.5%, 21/40 and 51.3 ± 3.3 epg, respectively) in women ($N = 40$; $P < 0.01$).

Thirty-seven of 94 (39.4%) subjects were diagnosed with parenchymal fibrosis (stage 2 or 3) and 17.7% (17/94) of subjects had periportal fibrosis assessed by ultrasound. Of the 37 patients with parenchymal fibrosis stage 2 or 3, 70.3% ($N = 26$) were shown to be excreting *S. japonicum* eggs, and fibrosis appeared to increase with infection intensity. Of the subjects with no parenchymal fibrosis, 52.9% (18/34) also had *S. japonicum* infection.

CD4$^+$T cells, CD4$^+$/CD8$^+$ T-cell count ratios and antibody levels in fishermen compared with naïve controls. In the 10 control subjects from non-endemic areas, the percentage of CD4$^+$ T cells in the measured 10,000 cells was 49.8% and the ratio of CD4$^+$/CD8$^+$ was 1.62. These values were 39.5% CD4$^+$ T cells and a ratio of 1.37 for CD4$^+$/CD8$^+$ T cells measurable in the migrant fishermen. CD4$^+$ T cell numbers and the ratio of CD4$^+$/CD8$^+$ T cells in the migrant fishermen were significantly lower than those of controls from non-schistosomiasis endemic areas. In general, the levels of serum IgA, IgM, and specific anti-SEA-IgG in the fishermen were higher than those of the controls. There was no significant difference in the levels of serum IgA and IgM between the controls and the 34 egg-negative persons, but the levels of specific anti-SEA-IgG showed a statistically significant difference ($P < 0.01$) between the controls and the egg-negative individuals. In the migrant fishermen, the CD4$^+$/CD8$^+$ T-cell ratio (1.34 ± 0.11) in men was significantly lower ($P < 0.01$) than that (1.58 ± 0.15) of women. There was no significant difference in the ratios of CD4$^+$/CD8$^+$ T cells among the age groups (age 6–17 years, 1.37 ± 0.33 years; age 18–44 years, 1.37 ± 0.11; age > 44 years, 1.38 ± 0.10).

Implications of CD4$^+$, CD4$^+$/CD8$^+$ T-cell ratios, and antibodies in infection and intensity of infection with *S. japonicum*. CD4$^+$ T-cell numbers and CD4$^+$/CD8$^+$ ratios in the fishermen who were stool-egg positive were significantly lower than in those subjects who were stool-egg negative. However, the numbers of CD8$^+$ T cells were not significantly different in the two groups. The numbers of CD4$^+$ T cells and the CD4$^+$/CD8$^+$ ratios declined with infection intensity ($P < 0.05$; Table 1). The levels of total IgM and anti-SEA-IgG, but not IgA, were significantly higher in the stool-egg positive subjects than in the stool-egg negative (Table 2).

TABLE 1

Schistosoma japonicum egg counts (epg), the percentage of CD4$^+$ (mean ± SD) and CD8$^+$ T cells (mean ± SD), and CD4$^+$/CD8$^+$ T-cell ratios in the 94 migrant fishermen from the Dongting Lake region of China, 2002

	Infection intensity (epg)				
T-cell counts	0 (N = 34)	1–100 (N = 32)	101–400 (N = 19)	≥ 401 (N = 9)	P value*
CD4$^+$T%	46.8 ± 2.3	38.4 ± 2.4	34.6 ± 2.1	32.2 ± 2.4	< 0.05
CD8$^+$T%	31.7 ± 2.8	28.2 ± 3.3	27.2 ± 4.1	27.1 ± 3.4	> 0.05
CD4$^+$/CD8$^+$ ratio	1.47 ± 0.2	1.36 ± 0.2	1.27 ± 0.1	1.19 ± 0.2	< 0.05

*P value was compared for the four groups of infection intensity (epg = 0 to epg ≥ 401).

CD4$^+$ T cells, CD4$^+$/CD8$^+$ T-cell ratios, and antibodies in liver parenchymal fibrosis subjects detected by ultrasound. CD4$^+$ T-cell numbers and the ratio of CD4$^+$/CD8$^+$ T cells were significantly decreased in subjects with liver parenchymal fibrosis stage 2/3 (*P* < 0.05). Total IgA and anti-SEA-IgG levels, but not IgM, were increased with stages of parenchymal fibrosis (*P* < 0.05; Table 3).

DISCUSSION

Schistosomiasis is a serious global helminthic disease, in which the main immunopathology consists of a granulomatous and fibrosing reaction against tissue-trapped parasite eggs. Granuloma formation and resulting pathology is dependent on, and mediated by, CD4$^+$ T cells responding to egg antigens. In some patients, excessive inflammation leads to deposition of connective tissue and hepatic fibrosis. Different cellular immune response profiles have been shown in patients with differing clinical forms of schistosomiasis and regulation of the cellular immune responses has been hypothesized to affect the outcome of clinical disease.[12,13] The severity of the inflammatory process is markedly uneven, both in human patients and in the experimental murine model of schistosomiaisis.[14,15] CD4$^+$ and CD8$^+$ T cells play an important role in regulating host immunity, and the CD4$^+$/CD8$^+$ T-cell ratio can reflect host cellular immune status. A recent study showed that hepatic fibrosis caused by *S. mansoni* infections was associated with significantly decreased CD4$^+$ T cell counts.[15] A decline in the CD4$^+$/CD8$^+$ T-cell ratio may imply a weakening in the host's immune function.

Our study, focusing on migrant fishermen at high risk of schistosome infection, measured peripheral blood CD4$^+$ T cells, CD8$^+$ T cells, CD4$^+$/CD8$^+$ T-cell ratios, and antibodies in patients with different infection intensities and with different liver pathologic changes assessed by ultrasound. The results showed that CD4$^+$ T cells and CD4$^+$/CD8$^+$ T-cell ratios in the migrant fishermen were significantly lower than those in control subjects from non-schistosomiasis areas. In the pa-

tients infected with *S. japonicum*, the percent of CD4$^+$ cells in the measured 10,000 cells was markedly lower than those in subjects who were stool-egg negative. Liver parenchymal fibrosis increased with infection intensity. This study showed that the decline in immune response was caused by infection intensity and liver pathology. Others have shown that immunosuppression in schistosome infection may be caused by the activation of CD8$^+$ T-cell function suppressing the maturation of CD4$^+$ T cell subsets or T-cell production, which could account for the decline in CD4 + T cells and the decrease in the CD4$^+$/CD8$^+$ T-cell ratio.[16] The relationship between pathogenesis and cellular immuno-reactivity in hosts infected with *S. japonicum* has been linked to the release of components from the worms as they develop inside the host.[17,18] In this study, those patients with heavy infections or with stage 2/3 liver fibrosis had reduced CD4$^+$ T cells and a lower CD4$^+$/CD8$^+$ T-cell ratio. A previous study in China also showed that CD4$^+$/CD8$^+$ T-cell ratios were normal or higher in the acute or early stage of schistosomiasis, but in the chronic or advanced stages, the ratios declined.[19,20] A possible explanation is that those patients with heavy infections had increased release of antigens from developing worms.[21]

The immune response to schistosomes at the early stage of infection is determined by CD4$^+$ helper Th1 T-cell and CD8$^+$ T-cell increases, leading to pathologic liver changes. When pathologic changes develop in the liver, the host immune response is modulated by CD4$^+$ helper Th2 T cells and suppressive CD8$^+$ T cells. This modulation of the immune response ensures pathologic changes are kept in check with the onset of the fibrosis that is promoted by Th2 cell-excreted interleukin (IL)-13.[22]

The status of the humoral immune responses was measured by the levels of total IgA, IgM, and anti-SEA IgG antibodies. These assays showed that the levels of IgM and anti-SEA-IgG antibodies were increased with infection intensity and that IgA and anti-SEA-IgG antibodies, but not IgM, were increased with the staging of parenchymal fibrosis assessed by ultrasonography. It thus seems that the humoral immune re-

TABLE 2

Antibody levels (mean ± SD) and infection intensity (epg) of *S. japonicum* in the 94 migrant fishermen from the Dongting Lake region of China

	Infection intensity (epg)				
Antibody levels	0 (N = 34)	1–100 (N = 32)	101–400 (N = 19)	≥ 401 (N = 9)	P value*
IgA (mg/mL)	1.89 ± 0.36	1.88 ± 0.40	1.95 ± 0.52	2.06 ± 0.45	> 0.05
IgM (mg/mL)	3.79 ± 0.71	5.51 ± 0.72	10.18 ± 0.79	13.13 ± 1.09	< 0.01
Anti-SEA-IgG (mg/mL)†	0.31 ± 0.11	0.49 ± 015	0.71 ± 0.23	0.99 ± 0.29	< 0.01

* P value was compared for the four groups of infection intensity (epg = 0 to epg ≥ 401).
† Anti-soluble egg antigen (SEA) IgG.

TABLE 3

Antibody levels (mean ± SD), the percentage of CD4$^+$ T cells (mean ± SD), and CD4$^+$/CD8$^+$ T-cell ratios (mean ± SD) in the 94 migrant fishermen with different stages of liver parenchymal fibrosis assessed by ultrasound

T-cell and antibody levels	Liver parenchymal fibrosis (stage)			
	0 (N = 29)	1 (N = 28)	2/3 (N = 37)	P value*
IgA (mg/mL)	1.67 ± 0.37	1.83 ± 0.42	2.29 ± 0.44	< 0.01
IgM (mg/mL)	6.69 ± 0.76	7.04 ± 0.87	6.80 ± 0.22	> 0.05
Anti-SEA-IgG (mg/mL)†	0.61 ± 0.24	0.70 ± 0.15	0.78 ± 0.22	< 0.01–0.05
CD4$^+$%	44.6 ± 3.1	38.4 ± 2.4	34.7 ± 2.5	< 0.01
CD4$^+$/CD8$^+$ ratio	1.36 ± 0.1	1.22 ± 0.10	1.09 ± 0.2	< 0.01

* P value was compared for liver parenchymal fibrosis stages 0, 1, 2/3.
† Anti-soluble egg antigen IgG antibodies.

sponses to *S. japonicum* infection, especially in heavy infections, and with liver morbidity caused by *S. japonicum* infection, are increased, which contrasts with the cellular immune responses measured in the same subjects.

This study has shown that there is an imbalance between humoral and cellular immune function in this group of migrant fishermen from the Dongting Lake in China, reflected by a weakened level of cell-mediated immunity concomitant with an increase in humoral immunity. The imbalance is more pronounced with increased infection intensity and aggravation of liver morbidity, but the precise cause of this imbalance remains to be determined.

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