ASSOCIATION OF A SEX-RELATED DIFFERENCE OF STRONGYLOIDES STERCORALIS–SPECIFIC IgG4 ANTIBODY TITER WITH THE EFFICACY OF TREATMENT OF STRONGYLOIDIASIS

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Abstract. It is difficult to completely eradicate strongyloidiasis, a human intestinal nematode infection caused by Strongyloides stercoralis with drugs, especially in males. To find host factors involved in the response to treatment, patients infected with S. stercoralis were examined for S. stercoralis-specific antibody titers and the effect of treatment with albendazole on these titers were determined. The cure rate was slightly but not significantly lower in males than in females ($P = 0.108$). However, a significantly higher titer of $S$. stercoralis-specific IgG4 antibody was observed in males than in females ($P = 0.0097$), and the $S$. stercoralis-specific IgG4 antibody titer was significantly higher in the male non-cured group than in the cured group ($P = 0.035$). These results suggest that elevation of the $S$. stercoralis-specific IgG4 antibody titer is associated with resistance to treatment of $S$. stercoralis infection, especially in males.

INTRODUCTION

Strongyloidiasis is a human intestinal nematode infection caused by Strongyloides stercoralis. There are many patients infected with $S$. stercoralis in tropical and subtropical areas, and in Japan, there are many patients with persistent infection in the southern islands and Okinawa. Chronic, usually asymptomatic, gastrointestinal infections are found in most healthy infected individuals; however, in immunocompromised hosts or patients receiving immunosuppressive therapy, extreme multiplication of the parasite occurs with dissemination of larvae in the body, resulting in serious illness.

Complete eradication of strongyloidiasis by chemotherapy is difficult, perhaps because of the systemic migration of $S$. stercoralis larvae due to its unique life cycle of autoinfection. In addition, the efficacy of treatment of $S$. stercoralis infection has been reported to be lower in males than females. However, the sex-related factors involved in the efficacy to treatment of $S$. stercoralis infection remain unknown.

The purpose of this study was to determine the mechanisms involved in the sexual difference in resistance to $S$. stercoralis treatment. Fallon and others have reported on the relationship between host immunity and the effect of treatment on schistosomiasis. In patients infected with Schistosoma mansoni, specific antibody titers against the parasite were higher in males than in females, and sexual differences were suspected to be involved in the difference of antibody titers.

Therefore, to determine the mechanisms that influence the different effects of treatment of $S$. stercoralis infection between males and females, $S$. stercoralis-specific antibody titers were evaluated. In this study, an increased $S$. stercoralis-specific IgG4 antibody titer was found to be associated with resistance to treatment in males.

MATERIALS AND METHODS

Study population. $S$. stercoralis–specific antibody titers were determined in 149 patients (106 males and 43 females, with mean ± SD = 65.0 ± 9.24 and 65.4 ± 9.37 years, respectively), including 46 patients who were evaluated for efficacy of treatment (29 males and 17 females, mean ± ages = 66.0 ± 7.98 and 67.0 ± 10.4 years, respectively). All patients in this study were diagnosed as being infected with $S$. stercoralis by agar plate fecal culture from 1994 to 1998 in health examinations performed in the Okinawa Prefecture of Japan. Presently, since new infections from the environment rarely occur among inhabitants in this area, the majority of recent cases are suspected to be due to long-standing chronic infection by autoinfection.

Informed consent was obtained from all patients. Protocols involving human subjects were reviewed and approved by the regional review boards of the University of the Ryukyus. Individuals seropositive for human T cell leukemia virus type 1 (HTLV-I), which is endemic in Okinawa, were examined by particle agglutination test (Serodia HTLV-I; Fujirebio, Tokyo, Japan) and also by an indirect immunofluorescence assay, and were excluded from the study to avoid confounding of the data by a potentially immunosuppressed population.

Treatments. Because of its relatively low side effects and its availability, albendazole was used in this study. Albendazole was administered after the diagnosis at a dosage of 400 mg a day for three consecutive days. The same therapeutic course was repeated after two weeks. The efficacy of treatment was assessed by stool examination three times: at two weeks, six months, and one year after treatment. Cured cases were free of parasites in all three stool examinations (at two weeks, six months, and one year). All other cases were assessed as non-cured.

Antigen. Somatic $S$. stercoralis filariform larval antigen was prepared as previously described with minor modifications. Briefly, third-stage filariform larvae were collected from fecal cultures obtained from parasite-free laboratory-reared beagles experimentally infected with a human strain of $S$. stercoralis. The larvae were washed five times in phosphate-buffered saline (PBS) with an antibiotic-antimycotic mixture (1/100; Gibco, Grand Island, NY), gentamicin reagent solution (1/200; Gibco), washed again three times in sterile PBS, and frozen for storage at −70°C. After sufficient numbers of larvae were collected, they were thawed and resuspended in sterile PBS containing 0.2 mM aminothyl benzenesulfonfluoride (Calbiochem, San Diego, CA), 1.0 mM EDTA (Wako Pure Chemical, Osaka, Japan), 1.0 mM leupeptin (Wako Pure Chemical), and 1.0 mM pepstatin A (Wako Pure Chemical).
Chemical). The suspended larvae were then homogenized with a teflon homogenizer and fragmented by a two-minute sonication at 4°C in wet ice. The suspension of fragmented larvae was stirred in PBS for 18 hours at 4°C to extract antigenic components. The supernatant was collected by centrifugation at 8,000 g for one hour, filtered through a 0.45-μm pore size membrane filter (Acrodisc; Gelman Sciences, Ann Arbor, MI), and stored at −70°C until use. The protein concentration was determined with a Micro bicinchoninic acid kit (Pierce, Rockford, IL).

**Determination of specific antibody titer to S. stercoralis.** Specific antibodies to S. stercoralis antigen were measured as previously described with minor modification. Briefly, enzyme-linked immunosorbent assay plates (Lumino-plate; Labsystems, Helsinki, Finland) were coated overnight at 4°C with S. stercoralis antigen (5 μg/mL) and blocked with 0.2% blocking reagent (Boehringer Mannheim, Mannheim, Germany) in 0.1% Tween 20 (Wako Pure Chemical) in PBS for two hours at 37°C. Plates were incubated with serum at an optimal dilution for each antibody class or subclass (IgA: 1/15,000, IgE: 1/100, IgG1: 1/10,000, IgG4: 1/15,000, IgG: 1/60,000) overnight at 4°C. All sera for IgE determinations were preabsorbed with protein G Sepharose (Pharmacia, Piscataway, NJ). Horseradish peroxidase (HRP)—conjugated mouse anti-human IgG1 and IgG4 (Southern Biotechnology Associates, Birmingham, AL) and HRP-conjugated goat anti-human IgA, IgE, or IgG (Biosource International, Camarillo, CA) was added to each well and the plates were incubated for one hour at room temperature. After the wells were washed, substrate (Super Signal Substrate; Pierce) was added and the luminescent intensity was read with a microplate reader (Luminoskan; Labsystems). For each isotype-antigen combination, a standard serum was selected and its titer was determined. The antibody levels of the samples were expressed as units relative to the local standard serum calculated by the following formula: serum antibody units = counts per minute (CPM) of the sample/CPM of the local standard serum (appropriately diluted) × 100.

**Statistical analysis.** The statistical significance of the differences was analyzed by the Mann-Whitney U test and Fisher’s exact probability test.

**RESULTS**

**Results of S. stercoralis treatment in males versus females.** We first compared the results of treatment of S. stercoralis infection in males and females to examine the influence of the sexual difference. Of the 29 male patients infected with S. stercoralis and treated with albendazole, 16 patients (55.2%) were cured. Fourteen of 17 female patients infected with S. stercoralis (82.4%) were cured with the same treatment. The cure rate of female patients was thus higher than that of male patients, although the difference was not significant by Fisher’s exact probability test (P = 0.108; Table 1).

**S. stercoralis—specific antibody titers in males versus females.** To examine the influence of sex on the S. stercoralis—specific antibody titers, the titers were compared between males and females. Only the male S. stercoralis—specific IgG4 antibody titer (median = 1.42, range = 0.00–2.86) was significantly higher than that of the corresponding female titer (median = 0.83, range = 0.00–2.39) by the Mann-Whitney U test (P = 0.0097; Table 2).

**S. stercoralis—specific antibody titers in patients treated for strongyloidiasis.** To examine the relationship between S. stercoralis—specific antibody titers and the efficacy of treatment, S. stercoralis—specific antibody before treatment were compared between the cured and non-cured groups. The male S. stercoralis—specific IgG4 antibody titer in the non-cured group (median = 1.89, range = 0.32–2.69) was significantly higher than that in the cured group (median = 0.99, range = 0.00–2.48) by the Mann-Whitney U test (P = 0.035). No significant differences were observed in other antibody titers (Table 3). There were no significant differences in females; however, the lack of significance might have been due to the small number of non-cured individuals (Table 4). The S. stercoralis—specific IgG4 antibody titer was therefore suggested to influence the efficacy of treatment, especially in males.

**DISCUSSION**

A sex-related difference in the efficacy of treatment of S. stercoralis infection has been reported; however, the factors responsible for this difference remain unknown. In this study, increased S. stercoralis—specific IgG4 antibody titer and decreased efficacy of treatment were observed in males. We therefore suspect that the sex-related difference of the efficacy of treatment of S. stercoralis is due at least in part to the influence of the S. stercoralis—specific IgG4 antibody titer.

In this study, the cure rate of the treatment of S. stercoralis infection in males was lower than that in females, and this result supports the findings of the previous study by Kobayashi and others, although the difference in our study was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of Strongyloides stercoralis—specific antibody titers between males and females</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td>Male (n = 29)</td>
<td>16 (55.2%)</td>
</tr>
<tr>
<td>Female (n = 17)</td>
<td>14 (82.4%)</td>
</tr>
</tbody>
</table>

* No significant difference between males and females was observed (P = 0.108, by Fisher’s exact probability test).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>S. stercoralis—specific antibody titer (log10), median (range)</th>
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<tbody>
<tr>
<td>IgA</td>
<td>1.26 (0.00–2.74)</td>
</tr>
<tr>
<td>IgE</td>
<td>0.63 (0.00–2.22)</td>
</tr>
<tr>
<td>IgG1</td>
<td>1.04 (0.00–2.46)</td>
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<tr>
<td>IgG4</td>
<td>1.42 (0.00–2.86)*</td>
</tr>
<tr>
<td>IgG</td>
<td>1.40 (0.13–2.52)</td>
</tr>
<tr>
<td>Male (n = 106)</td>
<td></td>
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<tr>
<td>Female (n = 43)</td>
<td></td>
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</tbody>
</table>

* Significant difference in IgG4 antibody titer between males and females (P = 0.0097, by Mann-Whitney U test).
Table 3
Comparison of Strongyloides stercoralis–specific antibody titers between cured and non-cured male patients before treatment

<table>
<thead>
<tr>
<th></th>
<th>IgA (log10)</th>
<th>IgE (log10)</th>
<th>IgG1 (log10)</th>
<th>IgG4 (log10)</th>
<th>IgG (log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured (n = 16)</td>
<td>1.07 (0.00–1.97)</td>
<td>1.10 (0.00–2.08)</td>
<td>0.75 (0.00–2.05)</td>
<td>0.99 (0.00–2.48)*</td>
<td>1.25 (0.51–2.12)</td>
</tr>
<tr>
<td>Non-cured (n = 13)</td>
<td>1.17 (0.17–1.96)</td>
<td>0.43 (0.00–1.70)</td>
<td>1.10 (0.00–1.84)</td>
<td>1.89 (0.32–2.69)*</td>
<td>1.59 (0.85–1.75)</td>
</tr>
</tbody>
</table>

* Significant difference in IgG4 antibody titer between cured and non-cured male patients (P = 0.035, by Mann-Whitney U test).

Table 4
Comparison of Strongyloides stercoralis–specific antibody titers between cured and non-cured female patients before treatment

<table>
<thead>
<tr>
<th></th>
<th>IgA (log10)</th>
<th>IgE (log10)</th>
<th>IgG1 (log10)</th>
<th>IgG4 (log10)</th>
<th>IgG (log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured (n = 14)</td>
<td>0.65 (0.00–2.09)</td>
<td>0.27 (0.00–1.32)</td>
<td>0.84 (0.00–1.50)</td>
<td>1.01 (0.00–1.67)</td>
<td>1.28 (0.00–1.92)</td>
</tr>
<tr>
<td>Non-cured (n = 3)</td>
<td>1.30 (0.69–1.52)</td>
<td>0.00 (0.00–0.00)</td>
<td>1.45 (0.00–1.78)</td>
<td>0.60 (0.12–1.18)</td>
<td>1.45 (1.14–1.47)</td>
</tr>
</tbody>
</table>

* No significant differences between cured and non-cured female patients.
In conclusion, the current study demonstrated that a higher S. stercoralis-specific IgG4 antibody titer was observed in males, and this elevated IgG4 antibody titer was suggested to enhance the resistance of S. stercoralis to treatment. This may enable the evaluation of male patients with S. stercoralis before treatment to determine the likelihood of a therapeutic effect.

Received November 14, 2003. Accepted for publication March 10, 2004.

Acknowledgments: We thank all patients for participating in the study and all of our colleagues of the Department of Parasitology, School of Medicine, University of the Ryukyus and Izumizaki Hospital for their cooperation.

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