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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 with *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 persistant infection in the southern islands and Okinawa.[1,2] 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.[3,4]

Complete eradication of strongyloidiasis by chemotherapy is difficult,[5,6] 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.[7] 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[8] 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.[9]

Therefore, to determine the mechanisms that influence the differential 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[10] 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.[7] 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),[11] which is endemic in Okinawa,[2,7] were examined by particle agglutination test (Serodia HTLV-I; Fujirebio, Tokyo, Japan) and also by an indirect immunofluorescence assay,[12] and were excluded from the study to avoid confounding of the data by a potentially immunosuppressed population.[13]

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.[14] 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 aminoethyl benzenesulfonylfluoride (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

TABLE 1
Results of treatment for infection with *Strongyloides stercoralis* in males versus females*

	Cured	Non-cured
Male (n = 29)	16 (55.2%)	13 (44.8%)
Female (n = 17)	14 (82.4%)	3 (17.6%)

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

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.[15] Briefly, enzyme-linked immunosorbent assay plates (Luminoplate; 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,[7] although the difference in our study was

TABLE 2
Comparison of *Strongyloides stercoralis*–specific antibody titers between males and females

	S. stercoralis-specific antibody titer ($\log_{10}$), median (range)				
	IgA	IgE	IgG1	IgG4	IgG
Male (n = 106)	1.26 (0.00–2.74)	0.63 (0.00–2.22)	1.04 (0.00–2.46)	1.42 (0.00–2.86)*	1.40 (0.13–2.52)
Female (n = 43)	1.29 (0.00–2.16)	0.43 (0.00–1.91)	0.94 (0.00–2.03)	0.83 (0.00–2.39)*	1.39 (0.00–2.26)

* 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

	S. stercoralis-specific antibody titer (log_{10}), median (range)				
	IgA	IgE	IgG1	IgG4	IgG
Cured (n = 16)	1.07 (0.00–1.97)	1.10 (0.00–2.08)	0.75 (0.00–2.05)	0.99 (0.00–2.48)*	1.25 (0.51–2.12)
Non-cured (n = 13)	1.17 (0.17–1.96)	0.43 (0.00–1.70)	1.10 (0.00–1.84)	1.89 (0.32–2.69)*	1.59 (0.85–1.75)

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

not significant. This might have been because the number of patients in our study (n = 46) was less than that in theirs (n = 78). To identify the factors involved in the therapeutic effect, it is important to examine the host immunity because the cases of *S. stercoralis* with immunosuppression, such as patients with acquired immunodeficiency syndrome, are more difficult to cure,[5] implying that host protective immunity may act additively with the drug, such as praziquantel in the treatment of schistosomiasis.[8]

Specific antibodies, which have been reported to have important roles in host protective immunity in strongyloides infection,[16–18] were examined and significant elevation was observed only for the *S. stercoralis*-specific IgG4 antibody titer in males. The elevation of the parasite-specific IgG4 antibody level has been reported in some helminthic infections.[19–21] Specifically, the levels of specific IgG4 are positively correlated with intensity of infection of *Schistosoma mansoni*.[19] It has been suggested that IgG4 may block IgE-mediated protective effector functions in *Schistosoma mansoni*.[22] IgE antibody has also been reported to be involved in the protective immunity against strongyloides infection.[23,24] Therefore, if similar mechanisms operate in *S. stercoralis* infection, the increase of the *S. stercoralis*-specific IgG4 antibody titer may result in the modification of host protective immunity, resulting in the decrease of therapeutic efficacy in males.

To examine the relationship between the efficacy of treatment and the *S. stercoralis*-specific antibody titer, these parameters were evaluated in males and females. A significant difference in cured versus non-cured patients was observed only for the *S. stercoralis*-specific IgG4 antibody titer in males. Although they were not significant, IgE levels in the uncured patients were lower than those in the cured patients, in both males and females, and lower IgG4 levels in the uncured females were observed compared with those in the cured females. No significant differences were observed in females, but this may have been because the number of noncured females was too low to evaluate (n = 3). There is a similar tendency for IgG4 antibody titers to be greater in males than in females in other helminthic infections.[25,26] Our findings that the serum IgG4 levels were elevated in males unable to be cured of *S. stercoralis* suggest that the mechanism of blocking of IgE by IgG4 may operate in *S. stercoralis* infection. Increases in specific IgG4 levels have been reported in the improved group following the treatment of allergic rhinitis or asthma with specific hyposensitization.[27–30] These findings suggest that the specific IgG4 antibodies act as blocking antibodies against a series of allergic reactions through specific IgE antibody. The role of IgG4 antibodies in downregulating allergic responses is incompletely understood; however, inhibition of IgE-mediated degranulation of effector cells, alternative T helper 2 cell responses, and involvement of interleukin-10 have been suspected.[31,32] IgG4 might also act as such an inhibitory factor in *S. stercoralis* infection, although determination of the precise mechanism will require further analysis.

Sex-related differences of the susceptibility to parasitic infections have already been reported.[33,34] In strongyloides-infected rodent models, male-dominant susceptibility has been reported,[35] and one of the causes of this sexual-specific susceptibility has been shown to be gonadal hormones.[36,37] It was suspected that this sex-related difference was induced by testosterone and rendered animals susceptible to migrating larvae by modulating their macrophage function.[36,38,39] However, these previous studies of the susceptibility of rodents to strongyloides infection were examined at the stage of larval migration in the primary infection. In our study, patients had already been infected for a long period with *S. stercoralis,* and adults and larvae of parasites had already resided in the patients for many years due to autoinfection before treatment. Therefore, we considered it likely that factors other than protective immunity against migrating larvae by macrophages caused the sex-related difference of therapeutic effect. A decrease in the humoral immunity by testosterone has been reported.[40,41] However, the response of antibody titers varies depending on the type of helminthic infection.[9] In our study, an increase of *S. stercoralis*-specific IgG4 antibody titer was observed, similar to that reported in other helminthic infections,[9] and it may have resulted in a decrease of protective immunity against *S. stercoralis* infection in males. However, it was not clear whether this increase of *S. stercoralis*-specific IgG4 antibody titer was induced by testosterone. No distinct sex-linked differences in albendazole metabolism, which might have affected cure rates in this study, have been observed.[42,43]

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

	S. stercoralis-specific antibody titer (log_{10}), median (range)				
	IgA	IgE	IgG1	IgG4	IgG
Cured (n = 14)	0.65 (0.00–2.09)	0.27 (0.00–1.32)	0.84 (0.00–1.50)	1.01 (0.00–1.67)	1.28 (0.00–1.92)
Non-cured (n = 3)	1.30 (0.69–1.52)	0.00 (0.00–0.00)	1.45 (0.00–1.78)	0.60 (0.12–1.18)	1.45 (1.14–1.47)

* 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.

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