# Production of a high level of specific IgG4 antibody associated with resistance to albendazole treatment in HLA-DRBI*0901-positive patients with strongyloidiasis

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# PRODUCTION OF A HIGH LEVEL OF SPECIFIC IgG4 ANTIBODY ASSOCIATED WITH RESISTANCE TO ALBENDAZOLE TREATMENT IN *HLA-DRB1*0901*-POSITIVE PATIENTS WITH STRONGYLOIDIASIS

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*Abstract.* Strongyloidiasis, a human intestinal infection with *Strongyloides stercoralis,* is difficult to treat with drugs. The factors influencing this phenomenon remain unclear. To determine the host factors involved in response to treatment, 46 patients with strongyloidiasis were treated with albendazole, followed-up for 1 year, and separated into two groups: cured and non-cured. Serum levels of specific IgA, IgE, IgG, IgG1, and IgG4 antibodies were estimated using *S. stercoralis* antigen. Significantly higher titers of IgG4 antibody were observed in the non-cured group than in the cured group ($P = 0.016$). A total of 88 patients were typed for HLA-DRB1 alleles and analyzed for serum levels of antibody. The *S. stercoralis*-specific IgG4 antibody titers were significantly higher in the *HLA-DRB1*0901*-positive group than in the negative group (corrected $P = 0.044$). These results suggest that *HLA-DRB1*0901* is a possible genetic marker for resistance to treatment of *S. stercoralis* that is associated with elevation of *S. stercoralis*-specific IgG4 antibody titer.

Strongyloidiasis is a human intestinal nematode infection caused by *Strongyloides stercoralis.* It causes chronic bowel problems, especially in immunocompromised hosts, in which systemic migration of larvae provokes a serious illness due to the unique life cycle of autoinfection of this nematode.[1] It is difficult to completely disrupt this process with drugs against *S. stercoralis.*[1,2] In Japan, there are many patients with persistent infection in the Southern Islands and Okinawa.[2,3] The factors involved in resistance to treatment remain unknown. Several protocols for treatment have been used, but the efficacy of chemotherapy is not always sufficient to achieve complete treatment.[2,3] In general, there are 2 factors determining the effectiveness of antimicrobial drugs. The first is its pharmacologic effect, including specific cytotoxicity and pharmacokinetics. The other factor is host immunity.

The purpose of this study was to determine the factors related to host immunity that influence resistance to treatment of *S. stercoralis* infection. We examined the HLA-DRB1 gene because its shows the most genetic polymorphism in HLA. Here we show that an increased *S. stercoralis*-specific IgG4 antibody titer is associated with resistance to treatment, and that patients with *HLA-DRB1*0901* produce higher levels of specific IgG4 antibody.

## MATERIALS AND METHODS

**Study population.** The efficacy of treatment was evaluated in 46 patients with *S. stercoralis* infection (29 males and 17 females). The mean ages were 66.4 and 67.1 years, respectively. The frequency of HLA-DRB1 alleles were determined in 88 patients (59 males and 29 females, mean ages = 64.9 and 65.1 years, respectively), including 29 patients who were evaluated for efficacy of treatment. All patients in this study were diagnosed with *S. stercoralis* infection by an agar plate fecal culture as described[4] at the 1994 annual regional health examination performed in Okinawa prefecture, Japan. Informed consent was obtained from all patients. Protocols involving human subjects were approved by the regional review boards of University of the Ryukyus. Individuals seropositive for human T-cell leukemia virus type-1 (HTLV-1), which is endemic in Okinawa,[2,3] were examined by a particle agglutination test (Serodia HTLV-1: Fujirebio, Tokyo, Japan) and were excluded from the study to avoid a possible immunosuppressive effect.[2,5]

**Treatments.** Because of few side effects and its availability, albendazole was used in this study. It was administered at a dosage of 400 mg/day for 3 consecutive days. The same therapeutic course was repeated after 2 weeks. The efficacy of treatment was assessed by stool examination 3 times: at 2 weeks, 6 months, and 1 year after treatment. Cured cases were free of parasites in all 3 stool examinations. All other cases were assessed as non-cured.

**Antigen.** Somatic *S. stercoralis* filariform larval antigen (*S.s.* Ag) was prepared as previously described with minor modifications.[6] 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 5 times in phosphate-buffered saline (PBS) with antibiotic-antimycotic (1/100; Gibco/BRL, Gaithersburg, MD), gentamicin reagent solution (1/200; Gibco/BRL), washed again 3 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 aminoethylbenzsulfonylfluoride (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). The suspended larvae were then homogenized with a teflon homogenizer and fragmented by a 2-min sonication at 4°C in wet ice. The suspension with fragmented larvae was stirred in PBS for 18 hr at 4°C to extract antigenic components. The supernatant was collected by centrifugation at 8,000 × *g* for 1 hr, filtered through a 0.45-mm pore size membrane filter (Acrodisc; Gelman Sciences, Ann Arbor, MI), and stored at −70°C until use. The protein concentration was determined with the Micro bicinchoninic acid kit (Pierce, Rockford, IL).

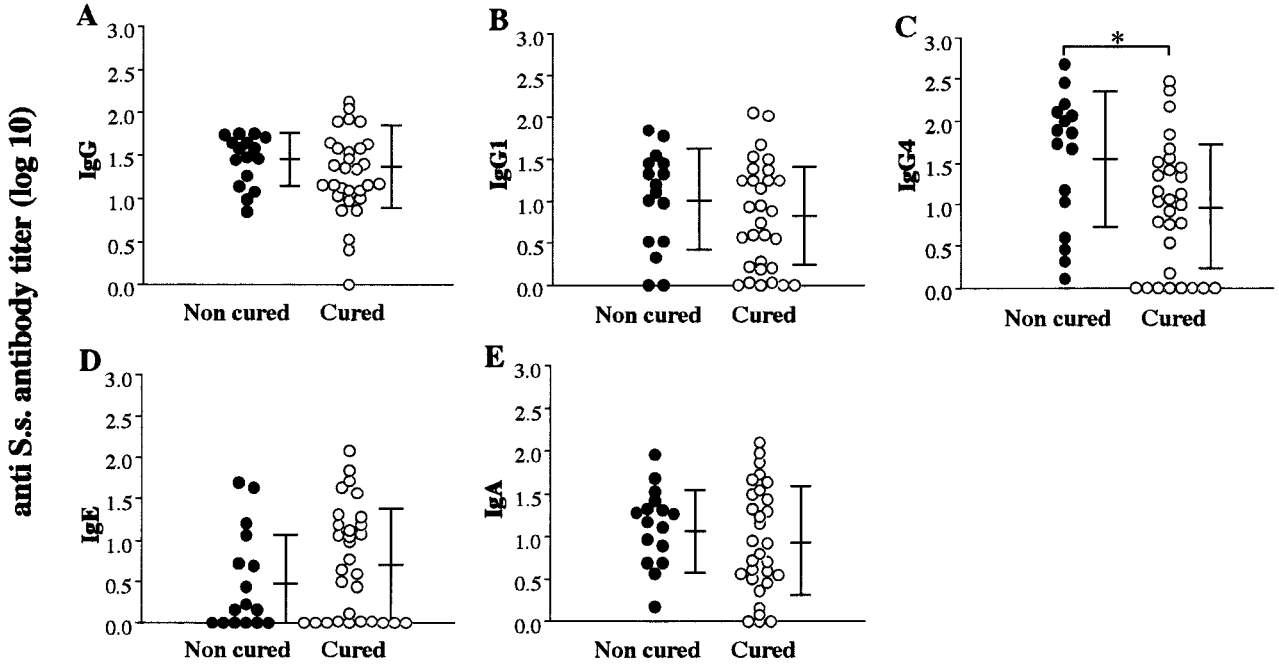


FIGURE 1. Comparison of *Strongyloides stercoralis* (S.s.)–specific antibody titers between cured (**open circles**) and non-cured (**solid circles**) groups. Horizontal lines indicate the mean ± SD. *$P$ = 0.016, by the Mann-Whitney U test.

**Determination of specific antibody titer to *S. stercoralis*.** Specific antibodies to *S.s.* Ag were measured as previously described with minor modifications.[7] Briefly, ELISA plates (Luminoplate; Labsystems, Helsinki, Finland) were coated overnight at 4°C with *S.s.* Ag (5 μg/ml) and blocked with 0.2% blocking reagent (Boehringer Mannheim, Mannheim, Germany) in 0.1% Tween 20 (Wako Pure Chemical), PBS for 2 hr at 37°C. Plates were incubated with plasma at an optimal dilution for each antibody class or subclass (IgA: 1/15,000, IgE: 1/100, IgG: 1/60,000, IgG1: 1/10,000, and IgG4: 1/15,000) overnight at 4°C. Horseradish peroxidase (HRP)–conjugated mouse anti-human IgG1, 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 1 hr at room temperature. *Strongyloides stercoralis*–specific IgM, IgG2, and IgG3 antibodies were not examined because the patients in this study were chronically infected with *S. stercoralis*, and the functions of IgG2 and IgG3 antibodies are not clearly understood. The plates were then washed with 0.1% Tween 20 in PBS, substrate (Super Signal Substrate; Pierce) was added, and the luminescent intensity was read with a microplate reader (Luminoskan; Labsystems). For each isotype-antigen combination, standard plasma were selected and assigned units. The antibody levels of the samples were expressed as units of the local standard plasma calculated by the formula: serum antibody units = counts per minute (cpm) of the sample/cpm of the local standard plasma (appropriately diluted) × 100.

**HLA typing.** Typing of HLA-DRB1 was performed using the polymerase chain reaction (PCR) sequence-specific oligonucleotide (PCR-SSO) probe method as previously described.[8] Briefly, DNA was isolated from white blood cells with a DNA extractor kit (Wako Pure Chemical) following the manufacturer's instructions. The polymorphic portion of the HLA-DRB1 gene was amplified by the PCR using sets of primers and conditions specified by the 11th HLA Workshop.[9] Amplicons spotted onto a nylon membrane were probed with specific oligonucleotides. Positive hybridization signals were visualized by chemiluminescent assay (Southern Light CSPD; Tropix, Bedford, MA). Alleles were determined by reading the pattern generated by specific probes.

**Statistical analysis.** The statistical significance of the difference in *S. stercoralis*-specific antibody titers was analyzed by the Mann-Whitney U test. The $P$ values were multiplied by the number of independent comparisons, i.e., the number of alleles tested (30), to obtain a corrected $P$ value ($Pc$) by the Bonferroni inequality method.[10]

## RESULTS

***Strongyloides stercoralis*–specific antibody titer.** Of the 46 patients with *S. stercoralis* infection treated with albendazole, 30 were cured. The *S. stercoralis*-specific antibody titers in plasma before treatment were compared between the cured and non-cured groups (Figure 1). The IgG4 antibody titers in the non-cured group (n = 16, mean ± SD = 1.53 ± 0.80 units) were significantly higher than that in the cured group (n = 30, mean ± SD = 0.95 ± 0.76 units) ($P$ = 0.016; Figure 1C). No significant differences were observed in other antibody titers (Figure 1A, B, D, and E).

**Typing of HLA-DRB1.** To examine the host factors that control the plasma levels of *S. stercoralis*-specific IgG4 antibody in patients with persistent infection, HLA-DRB1 DNA typing was performed (n = 88). As shown in Table 1, *S. stercoralis*-specific IgG4 antibody titers were significantly higher in the *HLA-DRB1*0901*-positive individuals than in

TABLE 1

Comparison of *Strongyloides stercoralis*–specific IgG4 antibody titers ($log_{10}$) between *HLA-DRB1*0901*-positive and -negative groups.

| Mean ± SD of specific IgG4 titers ($log_{10}$) | | | |
|---|---|---|---|
| *DRB1*0901* positive (n = 27) | *DRB1*0901* negative (n = 61) | *P* | *Pc*† |
| 1.53 ± 0.76 | 0.97 ± 0.75 | 0.0015 | 0.044 |

† *Pc* = corrected *P*.

the negative group (*Pc* = 0.044). We observed no associations between the other HLA-DRB1 alleles and *S. stercoralis*-specific IgG4 antibody titers.

## DISCUSSION

Panzani and others[11] reported an increase in specific IgG4 levels in the improved group following treatment of allergic rhinitis with specific hyposensitization. There is a report that the effectiveness of anti-asthmatic hyposensitization is associated with an elevation of antigen-specific IgG4 antibody levels in the serum.[12] These findings suggest that the specific IgG4 antibodies act as blocking antibodies against a series of allergic reactions through specific IgE antibody.

The elevation of parasite-specific IgG4 antibody levels is also observed in helminthic infections, including strongyloidiasis,[6] schistosomiasis,[13,14] and filariasis,[15] that are all characterized by high titers of specific and non-specific IgE in the serum. In fact, the levels of specific IgG4 are positively correlated with intensity of infection of *Schistosoma mansoni*.[13] It has been suggested that IgG4 may block IgE-mediated protective effector functions in *Schistosoma mansoni*.[16] Our findings that serum IgG4 levels are elevated in patients unable to be cured of *S. stercoralis* infection suggest that similar mechanisms may operate in *S. stercoralis* infection. Host protective immunity was modulated in those with higher levels of *S. stercoralis*-specific IgG4 antibody, and the efficacy of treatment was decreased in patients with higher *S. stercoralis*-specific IgG4 antibody titers.

Although albendazole has been reported to induce inhibition of fumarate reductase and microtubular formation,[17] the interaction between host immunity and albendazole treatment against *S. stercoralis* has yet to be completely elucidated. However, cases with immunosuppression, such as acquired immunodeficiency syndrome patients infected with *S. stercoralis,* are more difficult to cure.[1] Thus, host protective immunity may act additively with the drug, similar to praziquantel in the treatment of schistosomiasis.[18] Genta and others[6] reported that serum factors capable of competing with IgE for *S. stercoralis* antigens are present on the basophil surface, and suggested that such a mechanism could block the IgE-basophil system. Therefore, in our treated patients, IgG4 might be acting as such an inhibitory factor, although determination of the precise mechanism will require further analysis.

To investigate host genetic factors related to individual responses of *S. stercoralis*-specific IgG4 antibody, HLA-DRB1 allele typing was performed. The patients with *S. stercoralis* infections who had *HLA-DRB1*0901* produced increased levels of *S. stercoralis*-specific IgG4 antibody. Because of the limited number of patients, we could find any significant association between the HLA-DRB1 alleles and the cured or non-cured groups. The frequency of *HLA-DRB1*0901* in the non-cured group (2 of 7, 28.6%) appeared slightly higher than that in the cured group (4 of 22, 18.2%); however, this was not statistically significant.

In conclusion, the current study demonstrated that elevated levels of specific IgG4 antibody were associated both with the resistance of *S. stercoralis* to treatment and with *HLA-DRB1*0901*. This may enable patients infected with *S. stercoralis* to be evaluated before treatment for the likelihood of a therapeutic effect. The *HLA-DRB1*0901* allele has been reported to be associated with the promotion of a juvenile onset of rheumatoid arthritis.[19] To our knowledge, however, there have been no reports concerning elevation of IgG4 levels and resistance to treatment in parasitic infection. Further investigation of the role of HLA or other immune factors, such as cytokines, may reveal important information about this unique parasitic infection.

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