[Jpn. J. Parasitol., Vol. 39, No. 4, 376–383, August, 1990]

Seroepidemiological Studies on the Concomitance of Strongyloidiasis with T-cell Leukemia Viral Infection in Okinawa, Japan

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(Accepted for publication; July 2, 1990)

Abstract

A seroepidemiological surveys on the concomitance of HTLV-I and *Strongyloides* infections was conducted on 2,906 inhabitants in three areas of Okinawa Prefecture, Japan. The overall positive rate was 19.9% for anti-*Strongyloides* antibody and 25.5% for anti-ATLA antibody. The positivities of both anti-*Strongyloides* and anti-ATLA antibodies increased rapidly with age. The positive rate of anti-ATLA antibody, however, was significantly higher in the *Strongyloides*-seropositive group than in the seronegative group; the rate was 40.0% for the seropositive group, but was only 22.0% for the seronegative group. In contrast to an apparent familial clustering of anti-ATLA antibody, the disposition of anti-*Strongyloides* antibody among married couples could not be confirmed in the present study. On the other hand, the detection rate of fecal larvae among the *Strongyloides*-seropositive persons was significantly higher in the HTLV-I-seropositive group than in the seronegative group, suggesting a probable effect of concurrence of an HTLV-I infection on the course and intensity of a *Strongyloides* infection. In order to investigate the relationship between *Strongyloides* infection and ATL leukaemogenesis, a follow-up study for a longer period is being continued on the subjects.

Key words: Strongyloidiasis; *S. stercoralis*; HTLV-I; adult T-cell leukemia; seroepidemiology

Introduction

Strongyloides stercoralis is an opportunistic parasitic pathogen which produces a massive and often fatal disseminated invasion of larval parasites as a consequence of increasing auto-infection under the condition of depressed immune competence (Scowden *et al.*, 1987). An overwhelming fatal infection has often been observed among patients with malignant diseases, malnutrition or following administration of cytotoxic drugs or corticosteroids (Purtilo *et al.*, 1974). Okinawa Prefecture is known as an endemic area in Japan both for strongyloidiasis and adult T-cell leukemia (ATL). ATL is characterized by a unique T-lymphocyte malignancy which leads to severe deficiencies in immunological responses, and therefore the progression of asymptomatic strongyloidiasis to a fatal disseminated state has been frequently observed among the patients in Okinawa who developed ATL. It is well known that T-lymphotropic retrovirus (human T-cell leukemia virus type I: HTLV-I) is etiologically associated with ATL (Robert-Guroff and Gallo, 1983). Recently, it has also been demonstrated that persons with an asymptomatic *Strongyloides* infection frequently have an ATL viral infection as well. A probable explanation for the high frequency of such a complication, however, has not yet been offered. In the present study the authors investigated seroepidemiologically the relation of the *Strongyloides* and ATL viral infections

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among the inhabitants of Okinawa.

Materials and Methods

Subjects

A total of 2,906 inhabitants in three areas of Okinawa Prefecture, Japan (858 in Gushikawa Village, 849 in Nakazato Village and 1,199 in Sashiki Town) were investigated in the present study. The subjects were composed of 788 males and 2,118 females ranging in age from 20 to 93 years (mean = 58.7 years). Their sera were examined for the presence of antibodies to *S. stercoralis* and to ATL-associated antigen (ATLA). Follow-up fecal examination to demonstrate actual infection with the parasite was performed on those who were positive for anti-*Strongyloides* antibody.

Fecal examination

The fecal examination was repeated for 3 consecutive days with a combination of two different methods. One method was the usual fecal concentration (a formalin-ether concentration method) and the other was a fecal culture using a primary agar plate (Arakaki *et al.*, 1988).

Detection of antibodies to S. stercoralis

An enzyme-linked immunosorbent assay (ELISA) was applied to detect the antibody to *S. stercoralis*. The antigen was prepared from *S. stercoralis* filariform larvae obtained from feces of strongyloidiasis patients. The procedures for the mass culture of feces to obtain large numbers of larvae and those for preparation of antigen from the larvae have been described elsewhere (Sato *et al.*, 1983). The procedures for the micro-ELISA using a microtiter plate were also the same as those described in a previous paper (Sato *et al.*, 1985). Each serum was tested at a single dilution of 1:50 and the results were expressed as the absorbance (OD) at 500 nm. In the present study, OD values over 0.5 were regarded as being an antibody-positive reaction.

Detection of anti-ATLA antibody

Healthy individuals having anti-ATLA antibody have been known to be the carriers of the HTLV-I in their peripheral lymphocytes (Gotoh *et al.*, 1982). A kit for the particle agglutination test (PA test) to detect the antibody to ATLA (Serodia-ATLA) was kindly supplied from Fujirebio Inc., Tokyo. A usual indirect agglutination test using gelatin particles sensitized with antigens prepared from culture fluid of the virus-producing cell line was done in U-bottomed wells of a plastic microplate (Ikeda *et al.*, 1984). The antigen-coated particles were mixed with serially diluted serum in the wells and the mixture was allowed to stand for 3 h at room temperature. The resulting agglutination patterns formed on the bottom were read and final serum dilution of 1:16 or higher showing agglutination was interpreted as positive.

Statistics

Statistical difference was analyzed using the X^2 (chi-square) test. A P value of more than 0.05 was considered not to be significant.

Results

Positive rates of anti-*Strongyloides* and anti-ATLA antibodies among the inhabitants in three areas are summarized in Table 1. A total of 577 persons (19.9%) were positive for anti-*Strongyloides* antibody and 742 (25.5%) were found positive for the anti-ATLA antibody in the present study. The positive rates of antibodies both to *Strongyloides* and ATLA were higher among the inhabitants in Sashiki T. than those in the other areas.

Age and sex distribution of the inhabitants in Sashiki T. is shown in Fig. 1(A). More than 90% of the subjects were in the middle and upper age brackets over 40 years of age. In all age groups, female subjects outnumbered males. Age- and sex-specific positive rates of anti-*Strongyloides* and anti-ATLA antibodies among the inhabitants are also shown in Fig. 1 (B and C). Among the under 30 age group, no serum antibody to *Strongyloides* was detected. The positivity of the anti-*Strongyloides* antibody, however, rapidly increased with age; the positive rate for the subjects over 80 years of age was almost 50%. The positive rates were consistently higher in male

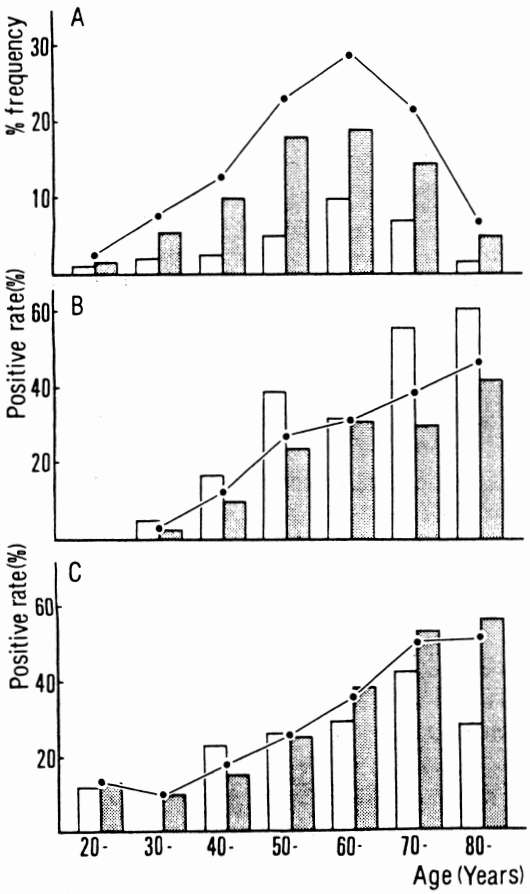
Table 1. Frequency of serum antibodies to *Strongyloides* and ATLA among the inhabitants in three areas of Okinawa

Area	Antibodies	
	anti-*Strongyloides*	anti-ATLA
Gushikawa V.	101/858 (11.8)	187/858 (21.8)
Nakazato V.	144/849 (17.0)	162/849 (19.1)
Sashiki T.	332/1,199 (27.7)	393/1,199 (32.8)
Total	577/2,906 (19.9)	742/2,906 (25.5)

Figures are numbers of persons positive for serum antibody/numbers of persons examined.

The percentages of the positivities are parenthesized.

subjects than in females. Similarly, a steady increase in the positivity of anti-ATLA antibody was observed along with the advance in age of the subjects. In the case of anti-ATLA antibody, however, the positive rate of female subjects was generally higher than that of males. Closely related results were also obtained in the other two areas.



In Sashiki T., a total of 164 married couples were included in the subjects examined. Eighty-seven (53.1%) out of the 164 pairs were found to be positive for anti-*Strongyloides* antibody in husbands and/or wives. Positive results in both the husband and wife of the married couples, however, were obtained only in 15 (17%) of the 87 pairs, suggesting no clustering of *Strongyloides* infection among the wedded pairs. On the other hand, the positive rate of anti-ATLA antibody among the same couples was 45.7% (75/164), and in 35 pairs (46.7%) anti-ATLA antibody was detected in both husband and wife. Among the remaining 40 couples in which anti-ATLA antibody was detected in either husband or wife, in 27 couples only the wives were found to be positive.

The positive rates of anti-ATLA antibody were compared between the two groups with or without anti-*Strongyloides* antibody. The results

Fig. 1. Age and sex distribution and positive rates of anti-*Strongyloides* and anti-ATLA antibodies among the inhabitants in Sashiki T. Figure (A) shows age and sex distribution of 1,199 inhabitants. Age- and sex-specific positive rates of anti-*Strongyloides* and anti-ATLA antibodies are represented in figures (B) and (C), respectively.
●—●: overall rate, □: males, ▨: females

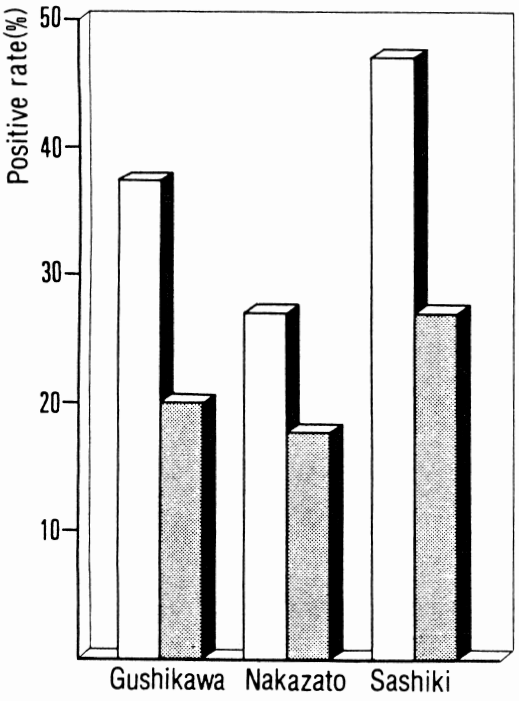


Fig. 2. Positive rates of anti-ATLA antibody in *Strongyloides*-seropositive and seronegative groups in three areas.
□: *Strongyloides*-seropositive,
▨: *Strongyloides*-seronegative
The positive rates of anti-ATLA antibody in the *Strongyloides*-seropositive group were significantly higher than those in the seronegative group ($P<0.001$ in Gushikawa V. and Sashiki T.; $P<0.02$ in Nakazato V.).

are shown in Fig. 2. In all areas, a significantly higher positive rate of anti-ATLA antibody was obtained among the persons who were positive for the anti-*Strongyloides* antibody; the overall rate was 40.0% (230/577) for the *Strongyloides*-seropositive group, but was only 22.0% (512/2,329) for the seronegative group. Similarly, when the positivities of the anti-ATLA antibody were compared by age group, higher positivities in the *Strongyloides*-seropositive group were also obtained in all age groups (Fig. 3).

For 434 persons whose sera showed positive antibody responses to *Strongyloides*, follow-up fecal examinations were performed to detect *Strongyloides* larvae and actual infections were demonstrated in 222 (51%) of them. The positive rate of anti-ATLA antibody among the persons with a documented *Strongyloides* infection was also significantly higher than the overall rates in each respective area (Fig. 4).

In order to examine whether or not the concomitance with HTLV-I infection may affect the intensity of a *Strongyloides* infection, the actual demonstration rate for fecal larvae was compared in relation to the positivity of the anti-ATLA antibody (Fig. 5). Although a higher positive rate of fecal larvae among the persons positive for anti-ATLA antibody was demonstrated in Gushikawa V. and Sashiki T., the rate in the HTLV-I-seropositive persons was lower in Nakazato V. In a district of Nakazato V., the positive rate of anti-*Strongyloides* antibody was as high as 32.5% (37/114). On the other hand, the positivity of anti-ATLA antibody in the same district was extremely low (9.6%), suggesting that the low concurrency of *Strongyloides* and HTLV-I infection in the district may cause the relatively low detection rate of fecal larvae among the HTLV-I-seropositive persons in Nakazato V. The total detection rate of fecal larvae, however, was 57.2% (103/180) in the persons who were seropositive for HTLV-I infection and was 45.7% (116/254) in the HTLV-I-seronegative persons; the difference was statistically significant ($P<0.02$).

Similarly, when the ELISA value to *Strongyloides* in persons with a documented *Strongyloides* infection was compared in relation to the presence of anti-ATLA antibody, no significant difference of the antibody levels could be observed between the HTLV-I-seropositive and the seronegative groups; the mean ELISA value ($\pm$SD) was 0.928 ($\pm$0.211) for the seropositive group and 0.913 ($\pm$0.198) for the seronegative group.

Discussion

Strongyloidiasis and ATL are presently highly prevalent in Okinawa Prefecture. The prevalence levels appear to be 5 to 10% for *Strongyloides* infection (Sato, 1986) and about 20% for HTLV-I infection (Clark *et al.*, 1985). Under these high prevalence conditions, the progression

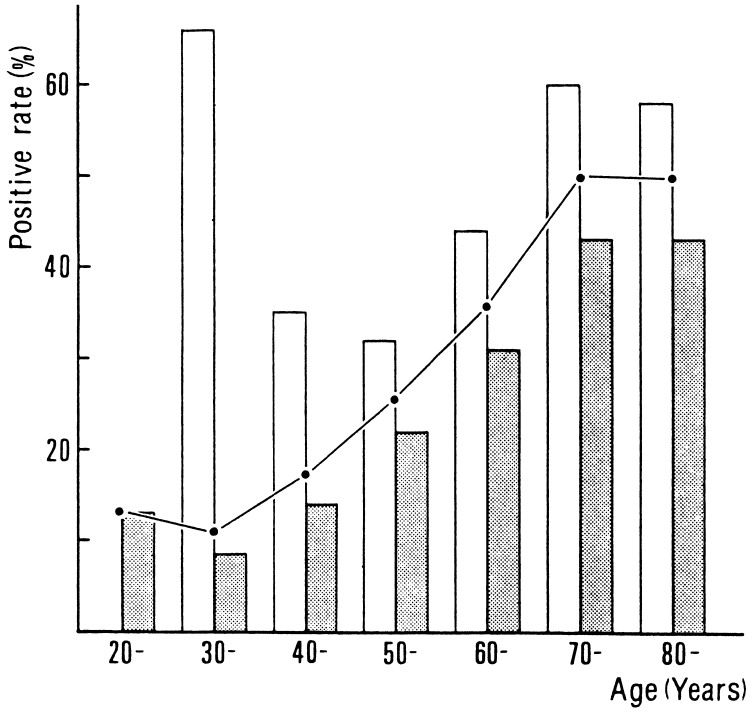
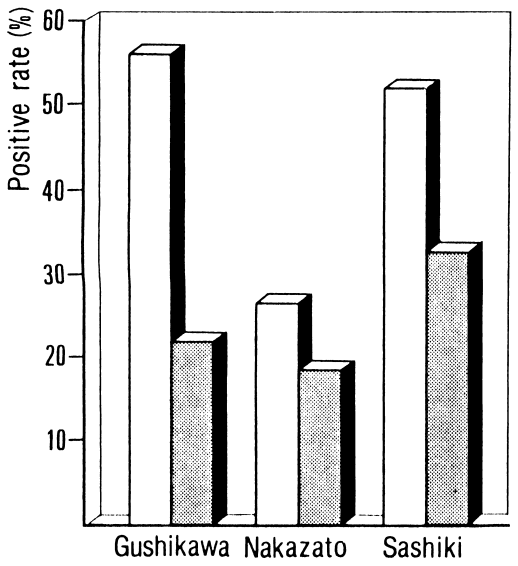


Fig. 3. Age-specific positive rates of anti-ATLA antibody in *Strongyloides*-seropositive and seronegative groups in Sashiki T.
●—●: overall rate, ▭: *Strongyloides*-seropositive, ▨: *Strongyloides*-seronegative
The positive rates in the *Strongyloides*-seropositive group were higher than those in the seronegative group in all age groups, except for the group aged under 30 in which no persons with serum antibody to *Strongyloides* was detected in the subjects examined.



of asymptomatic *Strongyloides* infection to a fatal disseminated state has often been observed among the patients who developed malignant lymphoma, including ATL (Takara *et al.*, 1980; Matsui *et al.*, 1982; Oura *et al.*, 1986). On the other hand, it has been pointed out by several researchers that the HTLV-I carriers in Okinawa are frequently accompanied by a chronic, asymptomatic *Strongyloides* infection (Nakada *et al.*, 1984; Fujita *et al.*, 1986). In the previous study,

Fig. 4. Positive rate of anti-ATLA antibody among the inhabitants with documented *Strongyloides* infection.
▭: rate in the group with *Strongyloides* infection, ▨: overall rate
The differences were statistically significant in all areas ($P<0.001$ in Gushikawa V. and Sashiki T.; $P<0.05$ in Nakazato V.).

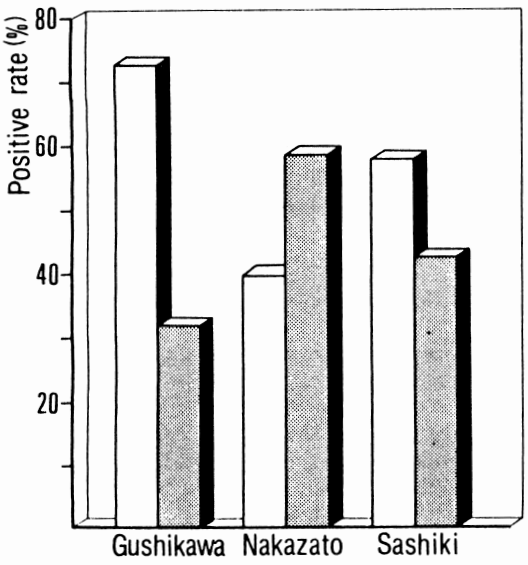


Fig. 5. Effect of concurrent HTLV-I infection on actual demonstration of *Strongyloides* larvae among the *Strongyloides*-seropositive persons. A total of 434 seropositive persons were examined for the presence of the larvae in their feces and the detection rates of the HTLV-I-seropositive (□) and seronegative (▨) groups were compared. The detection rates in the HTLV-I-seropositive group were significantly higher in Gushikawa V. and Sashiki T. Although the rate in the seropositive group was lower (not significant) in Nakazato V., the overall rate was significantly higher ($P<0.02$) in the seropositive group.

the authors have also reported that anti-ATLA antibody was detected with high frequency among persons with chronic strongyloidiasis in Okinawa; the positive rate of anti-ATLA antibody in the *Strongyloides*-positive persons was as high as 61% which was significantly higher than the 18% for the *Strongyloides*-negative group (Sato & Shiroma, 1989). In the present study, we confirmed further a higher frequency of anti-ATLA antibody among the persons whose sera showed positive antibody responses against *Strongyloides*, suggesting again a close relationship between the parasitic and viral infections. That there might be a possible participation of some antigenic components common to *Strongyloides* and ATLA, a supposition helpful in explaining the high concurrency of anti-*Strongyloides* and anti-ATLA antibodies, has already been excluded in the previous study (Sato & Shiroma, 1989). The age- and sex-specific positive rates of anti-*Strongyloides* antibody among the subjects were very similar to those of persons with a documented *Strongyloides* infection in previous studies in Okinawa (Sato, 1986). Although the increase in the positivities of anti-*Strongyloides* antibody paralleled that of anti-ATLA antibody as the age of the subjects increased, the positivities of anti-ATLA antibody in the respective age groups were always higher in the *Strongyloides*-seropositive group than in the seronegative group. The results indicate that the high complication of HTLV-I infection with *Strongyloides* infection may not be due to the age clustering of the two infections. On the other hand, it has been revealed that HTLV-I is transmitted naturally from husband to wife after marriage and subsequently from mother to child. Consequently, the prevalence of HTLV-I infection in families in a given area is greater than that in the general population in the same area (Tajima *et al.*, 1982; Ichimaru *et al.*, 1982). It can also be speculated that the similar familial disposition of *Strongyloides* infection may explain the high concomitance with the viral infection. The frequency of anti-*Strongyloides* antibody among 164 married couples was examined in the present study, but the results were inconclusive concerning the familial clustering of the *Strongyloides* infection.

It is also supposed that HTLV-I infection may be responsible for the long persistence and the severity of *Strongyloides* infection through the depressed immune competence of the host during the viral infection. Therefore, another possibility for the severe complication of *Strongyloides* and HTLV-I infection is that, as a consequence of the intensified *Strongyloides* infection due to the concurrent viral infection, the fecal larvae of the parasite may tend to be frequently detectable among the virus carriers. This is reasonable because of the opportunistic nature of the parasitic pathogen. In the present study, it was considered that the actual demonstration rate of fecal *Strongyloides* larvae may be affected by the concurrency of the viral infection. However,

when the ELISA values of the HTLV-I-seropositive and the seronegative groups were compared, we could not obtain any evidence for depressed antibody response specific for *Strongyloides* in the HTLV-I-seropositive group. Although Fujita *et al.* (1985) reported a significant decrease of anti-*Strongyloides* titers in the HTLV-I-seropositive group, they could not obtain a similar result of depressed antibody response in another survey in a different district (Fujita *et al.*, 1986). In the previous study in which the lymphoproliferative responses in strongyloidiasis patients were examined in relation to the concurrency of HTLV-I infection, we also failed to detect a depressed cellular response to *Strongyloides* antigen in the HTLV-I-seropositive group (Sato & Shiroma, 1989).

Alternatively, it can be supposed that the chronic parasitism may promote natural infection of HTLV-I and sero-conversion against the HTLV-I infection. In the previous study in which peripheral lymphocyte subsets and their response potential were investigated on 64 patients with strongyloidiasis, the authors suggested that the immunostimulation or immunodepression provided by chronic strongyloidiasis may set the stage for the acquisition and the flourishing of the viral infection (Sato & Shiroma, 1989). Furthermore, Shiroma and his co-workers have experienced treating several cases of adult T-cell leukemia/lymphoma which became overt during the course of chronic strongyloidiasis (Takara *et al.*, 1980). More recently, Nakada *et al.* (1987) also reported that monoclonal integration of ATL proviral DNA in peripheral lymphocytes is frequently observed among the patients with strongyloidiasis in Okinawa. These reports suggest that the parasitic infection may be an important co-factor leading to the development of ATL. In order to confirm this, the chronological observations on the relationship between *Strongyloides* infection and ATL manifestation should be continued for an extended period.

Acknowledgments

The authors are grateful to Fujirebio Inc., Tokyo, Japan (Mr. S. Hanzawa and Mr. Y. Miura) for supplying a kit for the PA test (Serodia-ATLA). The authors also thank Professor Dr. A. Saito, Department of Internal Medicine, School of Medicine, University of the Ryukyus, for supplying the specialized plastic dishes for the fecal culture method in the present study. This study was supported in part by a grant from the Okinawa Medical Science Research Foundation.

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