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Seroepidemiological Studies of Strongyloides Infection in Adult T-cell Leukemia Virus Carriers in Okinawa Island, Japan

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Abstract: Patients with *Strongyloides stercoralis* are commonly observed in Okinawa, the most southern prefecture in Japan, where adult T-cell leukemia/lymphoma (ATLL) is endemic. More than half (58%) of strongyloides-carriers, whereas only 21% of parasite-free adults had positive antibody to adult T-cell leukemia virus (ATLV) associated antigen (ATLA). A mean titer of antibody to strongyloides-antigen in 37 ATLV-carriers was significantly ($p<0.05$) lower than that in 27 non-carriers among strongyloides-carriers. However, there was no difference in the distributions of antibody titers against Epstein-Barr virus-specific antigens between them. These results suggest that some individuals, especially among ATLV-carriers, might have subclinical immunodeficiency, which may cause a low humoral response to strongyloides antigen and may lead to persistent infection with *Strongyloides stercoralis*.

Key words: Strongyloidiasis, Adult T-cell leukemia, Seroepidemiological study, Okinawa, Epstein-Barr virus specific antigen

INTRODUCTION

The Vital Statistics Japan Series in 1971–81 showed that the age-adjusted death rate for malignant lymphomas in Okinawa Prefecture was the highest (8.58 in males and 4.97 in females per 100,000) in the Kyushu districts where the incidence of adult T-cell

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leukemia/ lymphoma (ATLL) was remarkably high. Okinawa islands are located about 500km south of the main island of Kyushu and seem to be one of the typical endemic areas of ATLL in Japan. Recently, a retrovirus, adult T-cell leukemia virus (ATLV) (Hinuma *et al.*, 1981) which has been reported to be quite similar to human T-cell luekemia virus (HTLV) (Poietz *et al.*, 1980) isolated from patients with cutaneous T-cell malignancies in the U.S.A was identified from the tumor cells of ATLL. It was revealed that about 10 to 20 percent of healthy adults in Okinawa had antibody against ATLV-associated antigen (ATLA) (Hinuma *et al.*, 1982).

Okinawa has been known to be also an endemic area of strongyloidiasis caused by an intestinal parasites, *Strongyloides stercoralis*. Recently, it was reported that 60% of the strongyloides-carriers, whereas only 20% of non-carriers had positive antibody to ATLA (Nakada *et al.*, 1984). Furthermore, five cases of lymphoid malignancies mainly ATLL become overt during the course of this disease (Takara *et al.*, 1980). It is interesting to know whether persistent strongyloidiasis might play a role as causal factors for ATLL manifestation or it can be merely results of ATLL progression. In order to study the possible relation of strongyloides infection to ATLV infection and/or ATLL manifestation, the antibody titers against *Strongyloides stercoralis* and Epstein-Barr virus (EBV)-specific antigen were compared between positive and negative persons of anti- ATLA among groups of strongyloides-carriers and non-carriers in Okinawa.

MATERIALS AND METHODS

Blood specimen were collected from 64 adults of strongyloides-carriers and from 127 adults of non-carriers (healthy persons or patients without hematopoietic disorders) at Kyodou Hospital near Naha City in Okinawa Prefecture (Table 1). The mean age of strongyloides-carriers (ranged from 29 to 91 years) was mostly same as that of non-carriers (ranged 38 to 77 years). Carriers of *Strongyloides stercoralis* were determined by direct detection of rhabditi-form larvae in the fresh stool or by means of *in vitro* culture technique. The antibody titer of sera to strongyloides antigen was examined by means of an indirect

TABLE 1 Age and sex distribution of subjects in this study

Age (Years)	Strongyloides-carriers			Non-carriers		
	Males	Females	Total	Males	Females	Total
——49	11	10	21	13	17	30
50——64	13	5	18	35	37	72
65——	15	10	25	14	11	25
Total	39	25	64	62	65	127
Mean age	59.3	55.7	57.9	57.2	56.9	57.1

hemagglutination assay. Because of difficulties in collecting worms of *Strongyloides stercoralis* from human patients with strongyloidiasis, the filari-form larvae of *Strongyloides ratti* was used as a strongyloides antigen. Parasites were harvested from Wistar strain rat of adult female, weighing 180g infected with *Strongyloides ratti*. Antigen of parasites was prepared by means of a treatment as previously described by Fujita (1975).

Antibody to EBV capsid antigen (VCA) were determined by the indirect immunofluorescence technique described previously (Henle *et al.*, 1970). Antibody to EBV-associated nuclear antigen (EBNA) were determined by the anticomplement immunofluorescence technique (Reedman and Klein, 1973). Titers of antibody were expressed as reciprocals of the maximum dilution of serum (2-fold from the initials 1 : 5 dilution).

Antibody to ATLA were examined by the indirect immunofluorescence assay (Hinuma *et al.*, 1981). It was revealed that most, if not all, subjects with anti-ATLA titers more than 1 : 5 dilution of sera were regarded as ATLV-carriers, based on the observation that ATLV could be regularly formed in cultures of leukocytes from anti-ATLA positive individuals (Gotoh, Sugamura and Hinuma, 1982).

RESULTS

The positive rates of anti-ATLA were 57.8% among strongyloides-carriers and 20.5% among non-carriers. The age and sex specific positive rates of anti-ATLA among strongyloides-carriers were observed always higher than those among non-carriers, as shown in Table 2. Mean ages were 56.9 in ATLV-carriers and 62.2 in negatives of anti-ATLA among strongyloides-carriers, and were 57.6 and 56.9 respectively among non-carriers of strongyloides. It was shown in Table 3 that strongyloides-carriers had higher antibody titers against strongyloides antigen compared with non-carriers. The difference between them was highly significant ($P<0.001$). However, a mean titer of antibody to strongy-

TEBLE 2 Age and sex specific positive rates of anti-ATLA among strongyloides-carriers and non-cariers

Age (Years)	Strongyloides-carriers			Non-carriers		
	Males	Females	Total	Males	Females	Total
29——49	8/11 (72.7%)	8/10 (80.0%)	16/21 (76.2%)	2/13 (15.4%)	5/17 (29.4%)	7/30 (23.3%)
50——59	3/10 (30.0)	2/ 3 (66.7)	5/13 (38.5)	2/20 (10.0)	3/16 (18.8)	5/36 (13.9)
60——69	2/ 7 (28.6)	5/ 5 (100)	7/12 (58.3)	6/24 (25.0)	5/25 (20.0)	11/49 (22.4)
70——	5/11 (45.5)	4/7 (57.1)	9/18 (50.0)	2/6 (33.3)	1/ 6 (16.7)	3/12 (25.0)
Total	18/39 (46.2)	19/25 (76.0)	37/64 (57.8)	12/63 (19.0)	14/64 (21.9)	26/127 (20.5)

TABLE 3 Distribution of antibody titers to strongyloides-antigen among subjects of strongyloides-carriers and parasite-free controls by anti-ATLA positivity

Subjects	Anti-ATLA	Number tested	Antibody titers					Mean titers ($2^{n \pm SE}$)
			$\leqq 2^{0.5}$	$2^{1.0}$ | $2^{1.5}$	$2^{2.0}$ | $2^{2.5}$	$2^{3.0}$ | $2^{3.5}$	$2^{4.0} \leqq$	
Parasite-carriers	Positive	37	20	6	4	4	3	1.21 ± 0.54*
	Negative	27	8	4	5	4	6	2.06 ± 0.68*
	Total	64	28	10	9	8	9	1.57 ± 0.44**
Parasite-free controls[a)]	Positive	26	18	5	3	0	0	0.54 ± 0.28
	Negative	101	77	14	9	1	0	0.50 ± 0.15
	Total	127	95	19	12	1	0	0.51 ± 0.13**

a) Parasite was undetectable in the stool
*$P < 0.05$ --difference between anti-ATLA positive and negative groups
**$P < 0.001$ --difference between strongyloides-bearers and controls

TABLE 4 Distribution of antibody titers to VCA among subjects of strongyloides-carriers and non-carriers by anti-ATLA positivity

Subjects	Anti-ATLA	Number tested	Anti-VCA titers								GMT*
			<10	10	20	40	80	160	320	640≦	
Strongyloides -carriers	Positive	37	0	0	1	10	12	9	3	2	96
	Negative	27	0	0	2	4	12	6	2	1	91
	Total	64	0	0	3	14	24	15	5	3	94
Non-carriers	Positive	26	0	0	2	1	10	7	5	1	120
	Negative	101	0	0	1	14	34	35	11	6	120
	Total	127	0	0	3	15	44	42	16	7	120

*GMT: geometric mean titers

TABLE 5 Distribution of antibody titers to EBNA among subjects of strongyloides-carriers and non-carriers by anti-ATLA positivity

Subjects	Anti-ATLA	Number tested	Anti-EBNA titers								GMT*
			<10	10	20	40	80	160	320	640≦	
Strongyloides -carriers	Positive	37	1	4	9	10	7	4	1	1	41
	Negative	27	3	1	0	8	7	4	4	0	60
	Total	64	4	5	9	18	14	8	5	1	48
Non-carriers	Positive	26	1	0	8	4	9	4	0	0	47
	Negative	101	1	8	22	36	20	9	5	0	43
	Total	127	2	8	30	40	29	13	5	0	44

*GMT: geometric mean titers

loides antigen in ATLV-carriers was significantly ($P<0.05$) lower than that in negatives of anti-ATLA among strongyloides-carriers.

In order to compare the cellular immune status among these carriers, both antibody titers to VCA and to EBNA were assayed. As shown in Table 4, the geometric mean titer against VCA among strongyloides-carriers was lower than that among non-carriers, although there was no statistically significant difference. No difference the distribution of antibody titers to VCA between ATLV-carriers and negatives of anti-ATLA among both strongyloides-carriers and non-carriers was observed. Table 5 showed no significant difference in the distribution of anti-EBNA antibody titers among studied groups. However, four subjects among 64 strongyloides-carriers, whereas only two among 127 non-carriers had low titers ($<\times 10$) of anti-EBNA.

DISCUSSION

It was revealed that some patients with ATLL in Okinawa were admitted to a hospital with singns of severe disseminated strongyloidiasis (Takara *et al.*, 1980). It was recently reported that positive correlation between persistent strongyloidiasis and ATLV infection was apparently observed (*Nakada et al.*, 1984). In the present study, the proportion of ATLV-carriers among strongyloides-carriers was about three times as high as that of parasite-free adults.

It is natural that antibody titers against strongyloides antigen in strongyloides-carriers were higher than in non-carriers. However, a mean titer of antibody to strongyloides antigen in ATLV-carriers was significantly lower than that in negative hosts of anti-ATLV antibody among strongyloides-carriers. It was well known that most patients with ATLL had poor cellular immunity and also many patients with disseminated strongyloidiasis had severe infectious diseases and/or malignant neoplasiasis characterized by depressed cell-mediated immunity (Cummins, Suratt and Horwitz, 1976; Cohen and Spry, 1981). Furthermore, it was recently reported that cell-mediated immunity is also suppressed in 10 percent of healthy ATLV-carriers (Imai and Hinuma, 1983). In the present study, however, there was no significant difference in the distribution of antibody titers to VCA and EBNA between ATLV-carriers and negatives of anti-ATLA in both groups of strongyloides-carriers and non-carriers. Apparent impairment of cellular immunity in both groups of strongyloides-carriers and ATLV-carriers was not observed.

According to the sero-epidemiological studies on ATLV and filarial infection, positive correlation between ATLV infection and antibody titers against filarial antigen was observed (Tajima *et al.*, 1983). A mean titer of antibody to filarial antigen in ATLV-carriers was significantly higher than that in negatives of anti-ATLA antibody (Fujita *et al.*, 1983). It was revealed that filarial parasites impair the function of their host immune system (Weller, 1978), especially immune reactivity T-cell is strikingly suppressed in the hosts infected with filarial parasites (Piessens *et al.*, 1982). Thus, repeated filarial antigen

stimulation and filarial infection might have direct or indirect promoting effects on ATLV infection and/or ATLV proliferation in the hosts. In the case of strongyloides infection, however, inverse relationship between ATLV infection and antibody titers against strongyloides antigen was observed. These results suggested that some portion of ATLV-carriers might have subclinical immunodeficiency which may cause a low humoral response to strongyloides antigen and may lead to persistent infection with *Strongyloides stercoralis*. From these epidemiological view points, it seems very important for us to carefully follow up the ATLV-carriers with persistent strongyloidiasis as a high risk subject who may readily develop to clinical disease of ATLL in not distant future.

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沖縄における糞線虫感染と成人 T 細胞白血病ウイルス感染との関連についての血清疫学的研究

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糞線虫感染者の間には，高率に成人 T 細胞白血病ウイルス（ATLV）抗体陽性者が見出されることを我々は先に報告した．今回，我々は，沖縄協同病院を中心とした沖縄本島の糞線虫患者，29才から91才までの64例を選び，対照者として糞線虫感染がみられない同地区住民 127名を選んで，ATLV 感染におよぼす糞線虫感染の影響を血清疫学的手法を用いて研究した．すなわち，彼らの血清について ATLV 抗体価と糞線虫抗体価を測定し，あわせて，T 細胞機能として EB ウイルス核抗原に対する抗体（EBNA）と EB ウイルス・カプシッド IgG 抗体（VCA）を間接螢光抗体法で測定し，対照群と相互に比較した．
糞線虫感染者の ATLV 抗体陽性率は 57.8%であった．一方，対照群の陽性率は 20.5%で，糞線虫感染者がはるかに高い ATLV 抗体陽性率を示した．糞線虫感染者のうち，ATLV 抗体陽性者群の糞線虫抗体価は，陰性者群の抗体価に比べ，有意に低下していた．また EBNA 抗体価も有意ではないが低下していた．すなわち，糞線虫感染者のうち，ATLV 抗体陽性者群の血清抗体価および T 細胞機能が低下傾向にあることが観察された．

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