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Effect of Concurrent HTLV-I Infection on the Efficacy of Pyrvinium Pamoate Treatment of Strongyloidiasis

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Abstract

The effect of concurrent HTLV-I infection on the efficacy of pyrvinium pamoate treatment was examined in 176 patients with *Strongyloides* infection. The cure rate assessed by faecal examination 6 months or more after the treatment was significantly lower in the HTLV-I seropositive patients than in the seronegative patients; 25.0 to 35.5% for the seropositive group and 50.0 to 64.1% for the seronegative group. The difference in the efficacy of treatment became more significant when complete cure was further evaluated serologically after exclusion of the equivocal cases in which the faecal larvae were negative but no marked decrease in anti-*Strongyloides* antibody level was observed after the treatment. Complete cure in the HTLV-I seropositive group was as low as one-third that of the HTLV-I seronegative group. The low efficacy of anthelmintic treatment in cases of concurrent *Strongyloides* and HTLV-I infection was supposed as a causal explanation for the significant accumulation of patients with concurrent infection in Okinawa. The effect of immune dependence on the low efficacy is also discussed.

Key words: strongyloidiasis, *Strongyloides stercoralis,* ATL, HTLV-I, treatment, pyrvinium pamoate

Introduction

Strongyloidiasis caused by *Strongyloides stercoralis* infection is an opportunistic parasitic disease (Scowden *et al.*, 1978). The infection remains prevalent in Okinawa Prefecture, Japan, where other parasitic diseases have been almost entirely eradicated in recent years (Sato, 1986). Okinawa Prefecture is also known as an endemic area for human T-cell leukemia virus (HTLV-I) infection (Clark *et al.*, 1985). The HTLV-I is aetiologically associated with adult T-cell leukemia (ATL) which leads to severe deficiencies in immunological responses. Recently, a high rate of HTLV-I infection has been demonstrated in patients with asymptomatic *Strongyloides* infection in Okinawa (Nakada *et al.*, 1984; Fujita *et al.*, 1985; Sato and Shiroma, 1989). Under the condition of concurrent *Strongyloides* and HTLV-I infection, the progression of asymptomatic *Strongyloides* infection to a fatal disseminated state has been often observed among the patients who developed ATL (Takara *et al.*, 1980; Matsui *et al.*, 1982; Oura *et al.*, 1986).

An explanation why strongyloidiasis is so frequently complicated by HTLV-I infection in Okinawa has not yet been offered. It is possible, however, that HTLV-I infection may intensify the parasitic infection and also may produce a resistance to anthelmintic treatment through impairment of the immunity of the host.

In the present study, we have examined the effect of concurrent HTLV-I infection on the treatment with pyrvinium pamoate in patients with asymptomatic *Strongyloides* infection.

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Materials and Methods

Subjects:

A total of 176 individuals were found to be harbouring the parasite by stool examination at mass screening in three areas (Sashiki Town, Nakazato Village and Gushikawa Village; Okinawa Prefecture, Japan). The numbers, age and sex of the sample population are summarized in Table 1. Among them, positive rates for antibodies against ATL-associated antigen (ATLA) were 50.0% in Sashiki, 33.3% in Nakazato and 59.3% in Gushikawa, which were significantly higher than those for the *Strongyloides*-negative persons in the same areas; 30.3% in Sashiki, 17.4% in Nakazato and 19.9% in Gushikawa, respectively.

Stool examination:

Stool examination before and after the treatment was performed repeatedly for 3 consecutive days by three different methods (direct smear, formol-ether concentration method and faecal culture). For faecal culture, an agar-plate culture method by Arakaki *et al.* (1988) was applied, in which a faecal mass (of about 3 g) was placed in the center of a primary agar plate and incubated at 28°C for 3 days. *Strongyloides* larvae which emerged from the faecal mass on the surface of the agar-plate were examined microscopically. By this method, distinctive alignments of bacterial colonies formed along the tracks of the wandering larvae were also observed on the surface, indicating the presence of the larvae. When only the colony alignments were observed, further appropriate examinations were repeated to determine diagnosis.

Detection of antibodies to S. stercoralis:

Serum antibodies to *S. stercoralis* were compared before and after the treatment to assess serologically the efficacy of treatment.

The serum antibodies were detected by an enzyme-linked immunosorbent assay (ELISA). The antigen was prepared from *S. stercoralis* filariform larvae obtained from faeces of strongyloidiasis patients (Sato *et al.*, 1983). The conventional technique for the micro-ELISA using a microtitre plate has been described in a previous paper (Sato *et al.*, 1985). Sera were collected from each patient at mass screening, treatment and follow-up examination. The sera were tested at a single dilution of 1:50 and the intensity of antibody response was expressed as the absorbancy (OD) at 500 nm. The antibody levels at treatment and follow-up were compared as an antibody ratio against the antibody levels recorded at the mass screening. The antibody ratio was calculated as follows:

$$\text{Antibody ratio} = \frac{\text{OD value at treatment or at follow-up examination}}{\text{OD value at mass screening}}$$

Table 1 Positive rates of anti-ATLA antibody among the subjects with strongyloidiasis

Area	No. subject	Age in years (Mean)	Sex	Positive rate (%) of anti-ATLA antibody
Sashiki	78	41–88 (67.6)	M35 F 43	50.0
Nakazato	39	47–83 (66.0)	M19 F 20	33.3
Gushikawa	59	43–83 (64.4)	M38 F 21	59.3

M: male, F: female

Detection of antibodies to ATL virus:

Individuals having anti-ATLA antibody have been known to harbour HTLV-I in the peripheral lymphocytes (Gotoh *et al.*, 1982). A kit for the particle agglutination test to detect antibodies to ATLA (Serodia-ATLA) was kindly supplied by 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 as previously described (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 hr at room temperature. The resulting agglutination patterns formed on the bottom of the wells were read, and a final serum dilution of 1:16 or higher showing agglutination was interpreted as positive.

Anthelmintic treatment:

Subjects found at mass screening to be harbouring the parasite were treated with pyrvinium pamoate suspension (Poquil; Warner Lambert). The drug was administered at the dosage of 5 mg per kg of body weight daily for 3 consecutive days. This treatment schedule has long been used in Okinawa for the mass treatment of strongyloidiasis patients.

The subjects in Sashiki were treated 3 months after diagnosis and received follow-up faecal examination 12 months later. In Nakazato, treatment and follow-up examination were performed 2 months after diagnosis and 15 months after the treatment, respectively. In Gushikawa, however, 26 of the 59 subjects were treated a month after diagnosis and assessed 6 months later, while the remaining 33 subjects were left without treatment as a control and received follow-up examination 17 months after diagnosis.

Statistics:

Statistical difference was analysed using the Student's t test and X^2 (chi-square) test as appropriate. A P value of more than 0.05 was considered not to be significant.

Results

The results of stool examination after treatment are shown in Table 2. The total cure rates, as estimated by faecal examination, were 50.0% in Sashiki, 48.7% in Nakazato and 34.6% in Gushikawa. On the other hand, more than 87% of the control subjects in Gushikawa were still positive for faecal larvae. The cure rates were lower in the HTLV-I seropositive group than in the seronegative group in all areas.

In order to further evaluate the efficacy of the treatment, serum antibodies to *S. stercoralis* were compared before and after the treatment. The mean ELISA values at each examination are summarized in Table 3. The ELISA values detected at treatment are not significantly different from those at mass screening, whereas the antibody levels decreased significantly at follow-up examination. In Gushikawa, the control subjects showed no significant difference in antibody values at follow-up examination 17 months after diagnosis. In the same table, the mean ELISA values before and after the treatment are also compared between the HTLV-I seropositive and seronegative groups. The mean values, however, were not significantly different between the two groups, suggesting that there was no decrease in antibody responses against *Strongyloides* in the HTLV-I seropositive group.

Fig. 1 represents the distribution of ELISA values before and after treatment of subjects in Sashiki. Despite a significant decrease in mean ELISA values, high OD values of over 1.0 were still detected in many patients at follow-up examination. Therefore, the change in antibody level in relation to the result of follow-up faecal examination was determined for each patient (Fig. 2 and 3). A significant decrease in antibody ratios was observed in the group negative for faecal larvae, but not in subjects who were still positive at faecal examination. In the latter group, the antibody ratio at follow-up examination was more than 0.6 in all but one subject. From these results, if an antibody ratio of less than 0.6 (dashed line) was presumed as a criterion for successful cure, 17 and 8 subjects in the group negative for faecal larvae (43.6% in Sashiki and

Table 2 Effect of concurrent HTLV-I infection on mass treatment of strongyloidiasis with pyrvinium pamoate

Area	Anti-ATLA antibody	No. examined	No. cured (%)	Significance†
Sashiki	Positive	39	14 (35.9)	P<0.05
	Negative	39	25 (64.1)	
	Total	**78**	**39 (50.0)**	
Nakazato	Positive	13	4 (30.7)	N.S.
	Negative	26	15 (57.9)	
	Total	**39**	**19 (48.7)**	
Gushikawa	Positive	16	4 (25.0)	N.S.
	Negative	10	5 (50.0)	
	Total	**26**	**9 (34.6)**	
Gushikawa (Control)*	Positive	19	1 (5.3)	N.S.
	Negative	14	3 (21.4)	
	Total	**33**	**4 (12.1)**	

Treatment: pyrvinium pamoate, 5 mg/kg, for a consecutive 3 days
The duration between the treatment and the follow-up faecal examination was 12 months in Sashiki, 15 months in Nakazato and 6 months in Gushikawa.
*Control subjects: no treatment was administered and subjects were re-examined 17 months after the diagnosis.
†Although the cure rates in Nakazato and Gushikawa were not significantly different between HTLV-I seropositive and seronegative subjects, the total cure rate of these three areas was significantly lower ($P<0.001$) in HTLV-I seropositive group.

42.1% in Nakazato) were interpreted to be equivocal for effective treatment. In Sashiki, the equivocal cases were further examined for the presence of faecal larvae 1 month after the follow-up and 20.0% (3/15) were found to be harbouring the parasites.

The above serological assessment for effective treatment was further applied for HTLV-I seropositive and seronegative subjects who were negative at follow-up faecal examination. The results of 58 subjects in Sashiki and Nakazato are represented in Fig. 4. The equivocal cases were more frequently observed among the HTLV-I seropositive group than in the seronegative group; 66.7% (12/18) for the seropositive group and 35.0% (14/40) for the seronegative group.

The final cure rates determined by exclusion of the equivocal cases are summarized in Table 4. The cure rates became much lower in the HTLV-I seropositive group. The final cure rate in the seropositive group was as low as one-third that of the seronegative group in Sashiki, and only one subject was determined to be completely cured among the seropositive group in Nakazato. Four of the 33 control subjects in Gushikawa were negative for faecal larvae at follow-up examination. However, they showed high antibody levels at the follow-up examination and were determined to be equivocal for spontaneous cure.

Discussion

Strongyloidiasis and ATL are presently highly prevalent in Okinawa Prefecture, Japan. The prevalence levels appear to be 5 to 10% for *Strongyloides* (Sato, 1986; Sato *et al.*, 1990a) and about 20% for HTLV-I infection (Clark *et al.*, 1985). With this background of high prevalence, the progression from asymptomatic *Strongyloides*

Table 3 Comparison of anti-*Strongyloides* ELISA values before and after treatment

Area	Anti-ATLA antibody	Mean ELISA value (± SD) at: Mass screening[a)]	Treatment[b)]	Follow-up exam.[c)]	Significance*
Sashiki	Positive	1.024 ± 0.224	1.028 ± 0.239	0.871 ± 0.274	P<0.01
	Negative	1.090 ± 0.287	1.058 ± 0.283	0.751 ± 0.317	P<0.01
	Total	**1.057 ± 0.258**	**1.043 ± 0.260**	**0.811 ± 0.300**	**P<0.01**
Nakazato	Positive	0.925 ± 0.176	0.914 ± 0.198	0.869 ± 0.251	N.S.
	Negative	0.919 ± 0.172	0.917 ± 0.197	0.756 ± 0.279	P<0.05
	Total	**0.921 ± 0.171**	**0.916 ± 0.272**	**0.789 ± 0.272**	**P<0.05**
Gushikawa	Positive	0.830 ± 0.081	0.856 ± 0.112	N.T.	
	Negative	0.997 ± 0.209	0.910 ± 0.230	N.T.	
	Total	**0.932 ± 0.188**	**0.890 ± 0.192**	**N.T.**	
Gushikawa (Control)	Positive	0.971 ± 0.202	N.T.	0.897 ± 0.214	N.S.
	Negative	0.855 ± 0.123	N.T.	0.845 ± 0.172	N.S.
	Total	**0.922 ± 0.180**	**N.T.**	**0.875 ± 0.196**	**N.S.**

*Significance: a) or b) versus c); N.T.: not tested;
The duration from diagnosis to treatment was 3 months in Sashiki, 2 months in Nakazato and 1 month in Gushikawa, and that from treatment to follow-up examination was 12 months in Sashiki, 15 months in Nakazato and 6 months in Gushikawa.
The control subjects in Gushikawa were left without treatment.

infection to a fatal hyperinfected state has often been observed among the patients who developed ATL (Takara *et al.*, 1980; Matsui *et al.*, 1982; Oura *et al.*, 1986). Recently, several researchers have described the frequent concurrence of *Strongyloides* infection and HTLV-I infection in Okinawa. Nakada *et al.* (1984) first demonstrated that 60% of *Strongyloides* carriers were positive for anti-ATLA antibody. Subsequently, Fujita *et al.* (1985) and Sato and Shiroma (1989) reported positive rates as high as 57.8% and 73.6%, respectively. Furthermore, the authors have confirmed seroepidemiologically a higher frequency of anti-ATLA antibody among individuals whose sera showed positive antibody responses against *Strongyloides,* suggesting again a close relationship between the parasitic infection and viral infection.

Several explanations have been proposed for the high frequency of the concurrent infection. The participation of antigenic components common to *Strongyloides* and ATLA, a supposition attempting to explain the high concurrency of anti-*Strongyloides* and anti-ATLA antibodies, was excluded in a previous study (Sato and Shiroma, 1989) because no correlation was observed between the antibody titres to ATLA and *Strongyloides* and also because the antibody titres to ATLA were not affected by absorption with *Strongyloides* antigens.

Another possibility is that an epidemiological disposition of *Strongyloides* and HTLV-I infection may produce the concurrent infection. Possible sex and age clusterings have been excluded in a previous study (Sato *et al.*, 1990b). In Okinawa, the infection rate of *Strongyloides* was about 2-fold higher in male subjects, but, conversely, was higher in female subjects for HTLV-I infection. Although the positive rates for anti-*Strongyloides* and anti-ATLA antibodies increased in parallel to the increase in age of the subjects, the positivities of anti-ATLA antibody in the respective age groups were always higher in the *Strongyloides*-seropositive group than in the seronegative group. This result also provides an evidence that the high concurrence of HTLV-I

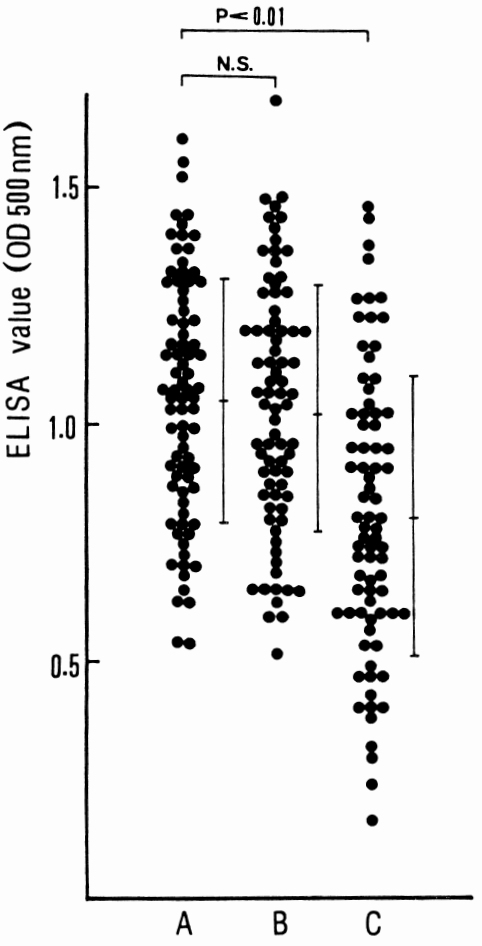


Fig. 1 Comparison of ELISA values before and after the treatment on 78 subjects in Sashiki who were positive for *Strongyloides* infection. The sera were collected three times at mass screening (A), treatment (B) and follow-up examination (C). The mean ELISA values were similar between (A) and (B) but decreased significantly at (C). The duration from mass screening to treatment was 3 months and from treatment to follow-up examination was 12 months. The vertical lines represent the mean ± SD.

infection and *Strongyloides* infection may not be attributed to the age clustering in the higher age-bracket. On the other hand, it has been revealed that HTLV-I is transmitted by intimate contact from husband to wife, and also from mother to child. Consequently, the prevalence of HTLV-I infection in families in a given area is greater than that among 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 rate of complication with HTLV-I infection. Another explanation for conjugal clustering may be that rural couples often work together at the same farm and therefore both come into contact with infested soil. A previous study on the conjugal clustering of *Strongyloides* infection, however, was inconclusive (Sato *et al.*, 1990b). Thus, there is no epidemiological background in Okinawa to explain the overlap of *Strongyloides* and HTLV-I infection.

Alternatively, it can be supposed that concurrent HTLV-I infection may be responsible for the severity of *Strongyloides* infection through the depressed immune competence of the host. Therefore, it is also possible that, as a consequence of the intensified *Strongyloides* infection due to the concurrent viral infection, the faecal larvae of the parasite may be more easily detectable. This is reasonable because of the opportunistic nature of the parasitic pathogen, and also because of the insufficient efficacy of faecal examination in detecting chronic *Strongyloides* infection. In a previous study, it was found that the actual demonstration of faecal *Strongyloides* larvae among the individuals who were positive for anti-*Strongyloides* antibody was relatively higher in the HTLV-I carrier group than that in the virus-negative group (Sato *et al.*, 1990b). The selective demonstration of *Strongyloides* infection among HTLV-I seropositive individuals may be one of the factors contributing to the high concurrency of the two infections. However, we were unable to obtain any evidence to support the depressed immune responses specific for *Strongyloides* in the HTLV-I seropositive group.

On the other hand, we have examined in a previous prognostic study whether the concurrent HTLV-I infection might affect the efficacy of past infection. The positive rate of anti-ATLA antibody among the patients who had been cured, however, did not differ from that in the patients of unsuccessful treatment, suggesting no depressed curative property in patients positive for HTLV-I infection (Sato and Shiroma, 1989).

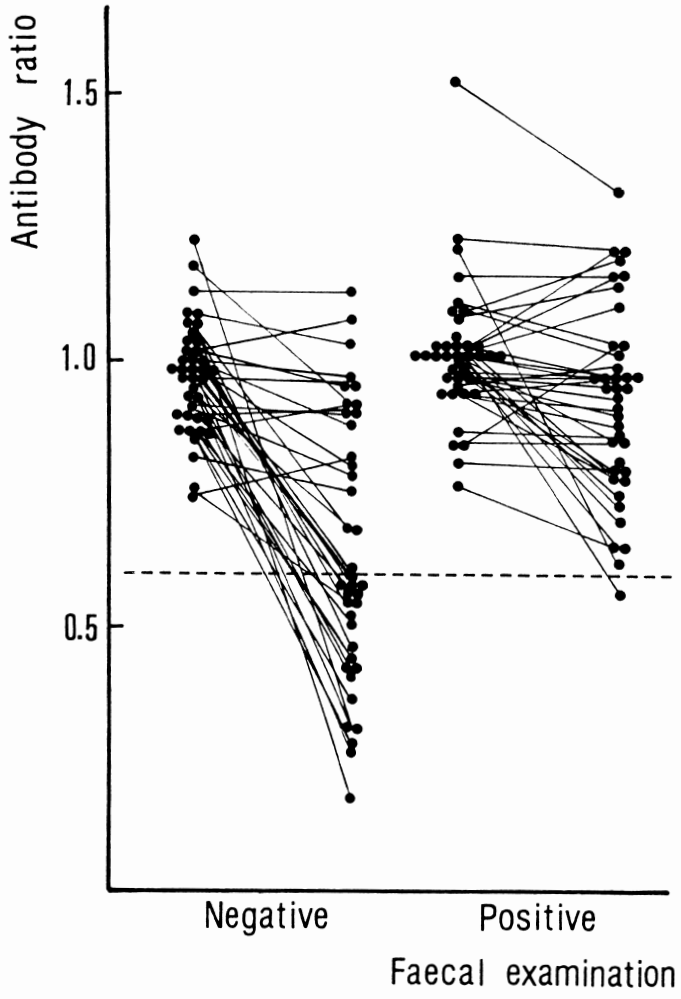


Fig. 2 Changes in the ELISA values at treatment and 12 months after the treatment in strongyloidiasis subjects in Sashiki. The data are represented as an antibody ratio at treatment and follow-up examination against those at the mass screening and the corresponding values of the same persons are connected. A significant decrease in the antibody ratio after treatment was observed in many cases who were negative for faecal larvae in the follow-up examination. The dashed line represents an antibody ratio of 0.6, which is the criterion in the present study for equivocal cases for complete cure.

Subsequently, when the complete cure was further assessed serologically on the same patients, it was noted that many of the patients who were negative at follow-up faecal examination were equivocal for complete cure because they were still positive for anti-*Strongyloides* antibody, and that the cure rate after exclusion of the equivocal cases was significantly low in the HTLV-I seropositive patients (unpublished data). We now present an evidence that the anti-parasitic effect of pyrvinium pamoate, which has long been used in Okinawa for strongyloidiasis, is greatly reduced in the patients with concurrent HTLV-I infection. In Okinawa, intractable cases,

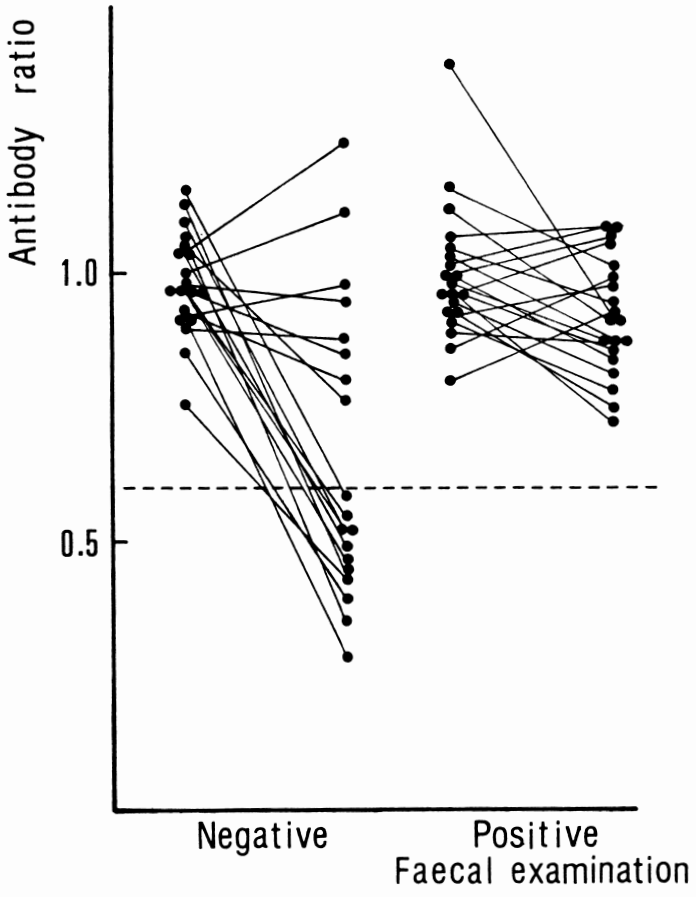
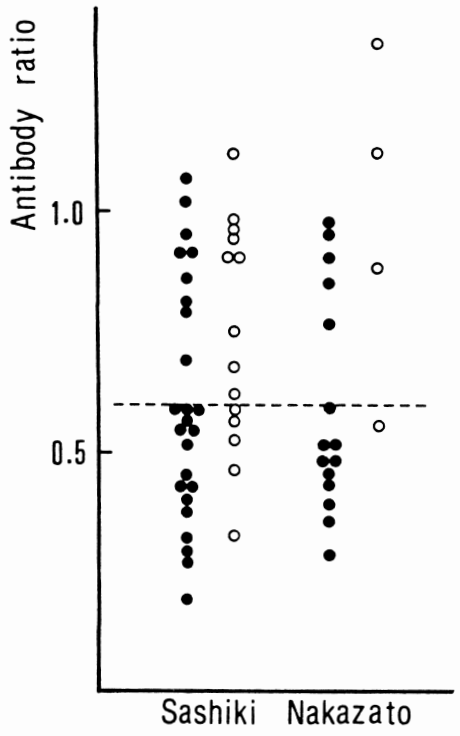


Fig. 3 The antibody ratios at treatment and 15 months after the treatment in strongyloidiasis subjects in Nakazato. The results are similar to those presented in Fig. 2.

in which relapse occurs repeatedly after various treatments over a period of many years, have been often observed (Shiroma *et al.*, 1990). Although the factor responsible for such a resistance to anthelmintic treatment is unclear, it can be postulated that the poor efficacy may be attributed to the depressed immune response which provided by the viral infection. As well documented, ATL is characterized by a unique T-lymphocyte malignancy which leads to severe deficiencies in immune response, and it is also known that the disorder to a T-cell mediated immune system has already begun in the stage of virus carrier (Imai and Hinuma, 1983; Yasuda *et al.*, 1986; Tanaka *et al.*, 1989; Prince *et al.*, 1990).

In the past decade, substantial evidence for the immune dependence of chemotherapy has been accumulated. Immunosuppression is now known to reduce the efficacy of chemotherapy in several parasitic diseases, such as rodent malaria (Lwin *et al.*, 1987), trypanosomiasis (Frommel, 1988), onchocerciasis (Bianco *et al.*, 1986) and schistosomiasis (Doenhoff and Bain, 1978). It is also well documented that severe cases of strongyloidiasis in the immunocompromised individuals often fail to respond to anthelmintic treatment, and repeated courses of treatment are necessary to obtain a complete cure (Shelhamer *et al.*, 1982; Scowden *et al.*, 1978; Morgan *et al.*,



1986; Weller *et al.*, 1981). Although the immune factor involved in influencing the drug efficacy is not yet determined, it has been reported that antibodies are synergystically involved in enhancing the efficacy of the drug against schistosomes and malaria parasites. When immune sera were administered simultaneously with the drug, greater cure rates could be achieved in immunosuppressed hosts with schistosomiasis and malaria (Brindley and Sher, 1987; Targett, 1985). In the case of schistosomiasis, it is considered that the drug-induced damage first occurs on the surface of the worm, resulting in exposure of antigens sensitive to damage by antibody (Doenhoff *et al.*, 1988). When the antibody titres to *Strongyloides* were compared in relation to the positivity of

Fig. 4 Comparison of antibody ratios after treatment between HTLV-I seropositive (○) and seronegative (●) subjects who were negative for faecal larvae at follow-up examination. The equivocal cases over the 0.6 criterion (dashed line) are observed frequently among the HTLV-I seropositive subjects.

Table 4 Efficacy of pryvinium pamoate treatment determined by both faecal examination and serum antibody levels after the treatment

Area	Anti-ATLA antibody	Stool examination			Significance*
		Positive (not cured)	Negative		
			Sero-positive† (equivocal)	Sero-negative (cured)	
Sashiki	Positive (n = 39)	25 (64.1)	9 (23.1)	5 (12.8)	
	Negative (n = 39)	14 (35.9)	9 (23.1)	16 (41.0)	P<0.01
	Total (n = 78)	**39 (50.0)**	**18 (23.1)**	**21 (26.9)**	
Nakazato	Positive (n = 13)	9 (69.2)	3 (23.1)	1 (7.7)	
	Negative (n = 26)	11 (42.3)	5 (19.2)	10 (38.5)	P<0.05
	Total (n = 39)	**20 (51.3)**	**8 (20.5)**	**11 (28.2)**	
Gushikawa (Control)	Positive (n = 19)	18 (94.7)	1 (5.3)	0 (0)	
	Negative (n = 14)	11 (78.6)	3 (21.4)	0 (0)	N.S.
	Total (n = 33)	**29 (87.9)**	**4 (12.1)**	**0 (0)**	

Complete cure was determined by serological examination on subjects who were negative for faecal larvae at follow-up examination.
The results of the subjects in Gushikawa whose serum antibody was not determined at follow-up examination were excluded.
The control subjects in Gushikawa were left without treatment and the presence of infection was evaluated 17 months after diagnosis.
*Significance between HTLV-I seropositive and seronegative groups.
†Antibody ratio of more than 0.6

anti-ATLA antibody in the present study, however, we could not find any evidence to suppose a depressed antibody response against *Strongyloides* in the HTLV-I seropositive group. The relationship between the efficacy of treatment and antibody response to the parasite is also still obscure. Murine studies have revealed that the drug efficacy in onchocerciasis reduced markedly in T-cell deprived mice but not in congenitally B-cell deficient mice, suggesting that cellular immune effector mechanism other than the serum antibody may contribute to efficacy of chemotherapy in the parasitic disease (Bianco *et al.*, 1986). With respect to strongyloidiasis, significance of cell-mediated immunity in controlling and preventing the infection has been suggested in many clinicopathological studies, as well as in studies using a murine model of infection (Purtilo *et al.*, 1974; Scowden *et al.*, 1978; Cohen and Spry, 1979; Abe and Nawa, 1988; Korenaga *et al.*, 1991). Further investigations on a possible connection between cell-mediated immunity and drug efficacy in strongyloidiasis are needed.

Finally, the reduced efficacy of drug treatment in strongyloidiasis may provide another explanation for the high concurrency of HTLV-I infection. Due to resistance to anthelmintic treatment, patients with concurrent HTLV-I infection might harbour the disease for many years, resulting in a significant accumulation of such patients for a long period.

Acknowledgments

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