

## Primary Sjögren's syndrome with antibodies to HTLV-I: clinical and laboratory features

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### Abstract

The prevalence of antibodies to human T lymphotropic virus type I (HTLV-I) was studied in patients with primary Sjögren's syndrome. Thirteen of 36 serum samples were positive by enzyme linked immunosorbent assay (ELISA) and particle agglutination assay for antibodies to HTLV-I and were confirmed by western blotting. The presence of antibodies to HTLV-I may signify an HTLV-I carrier state. These patients had a high occurrence of extraglandular manifestations such as uveitis, myopathy, and recurrent high fever compared with patients who did not have antibodies to HTLV-I. Patients with antibodies to HTLV-I had an increased spontaneous proliferation of peripheral blood mononuclear cells compared with those without the antibodies. The proportions of activated and memory T cells (HLA-DR+CD3+, CD25+CD3+, and CD29+CD4+ cells) were higher in HTLV-I carriers than in non-carriers. The presence of antibodies to HTLV-I in some patients with primary Sjögren's syndrome suggests that HTLV-I may cause primary Sjögren's syndrome or its extraglandular manifestations, or both.

The human T lymphotropic virus type I (HTLV-I) is a retrovirus associated aetiologically with adult T cell leukaemia<sup>1</sup> and HTLV-I associated myelopathy/tropical spastic paraparesis.<sup>2,3</sup> Proviral DNA integration is monoclonal in adult T cell leukaemia<sup>4</sup> but occurs at random in the peripheral blood mononuclear cells and cerebrospinal fluid cells of patients with HTLV-I associated myelopathy/tropical spastic paraparesis.<sup>5,6</sup> Areas known to be endemic for HTLV-I include the Caribbean Islands<sup>7</sup> and southwestern Japan.<sup>1,2</sup> The Nagasaki prefecture, which is located in southwestern Japan, is one of the areas endemic for HTLV-I.

Sjögren's syndrome is an exocrinopathy of presumed autoimmune aetiology causing keratoconjunctivitis sicca or xerostomia, or both.<sup>8,9</sup> The characteristic histological findings are sialadenitis with lymphocytic infiltration of the salivary and lacrimal glands and proliferating nests of epithelial cells. Although many aetiologies have not been proposed, the pathogenesis of this syndrome has not been clarified. There has been great interest in the association between Sjögren's syndrome and retrovirus infection.<sup>10,11</sup> In fact, an association between Sjögren's syndrome and HTLV-I has been

suggested indirectly by case reports of HTLV-I infected patients with tropical spastic paraparesis who developed features of Sjögren's syndrome.<sup>12</sup> Clinical features of Sjögren's syndrome have been found in intravenous drug abusers who are at high risk for HTLV-I infection.<sup>13</sup> An exocrinopathy resembling Sjögren's syndrome is reported to be produced in HTLV-I tax transgenic mice.<sup>14</sup> In addition to being seen in those infected with HTLV-I, the clinical symptoms and signs of Sjögren's syndrome have been seen in some patients infected with human immunodeficiency virus (HIV-I).<sup>15-17</sup> In this study, we found that antibodies to HTLV-I are present in patients with primary Sjögren's syndrome and this paper presents in detail the clinical and laboratory features of primary Sjögren's syndrome which are associated with antibodies to HTLV-I.

### Patients and methods

#### SUBJECTS

The experimental group included 36 patients with primary Sjögren's syndrome (two men and 34 women; mean (SD) age 52.6 (10.5) years, range 21-75). The patients with Sjögren's syndrome attended the outpatient clinic of Nagasaki University School of Medicine. Sjögren's syndrome was diagnosed by the presence of objective evidence of lacrimal or salivary gland involvement, or both, as confirmed by Schirmer's test, sialography, or a minor salivary gland biopsy. Rheumatoid arthritis, systemic lupus erythematosus, and progressive systemic sclerosis were ruled out in each patient. Three of the 36 patients had been treated with prednisolone (less than 10 mg/day) and no other patient was receiving any systemic drugs. Carriers were defined as those who had antibodies to HTLV-I.

Two hundred and forty outpatients (36 men and 204 women; mean (SD) age 46.7 (10.3) years) in our hospital served as control subjects.

We studied immunologically 10 normal subjects with antibodies to HTLV-I (one man and nine women; mean (SD) age 39.6 (13.4) years) and 10 normal subjects without these antibodies (one man and nine women; mean (SD) age 48.5 (12.4) years).

#### CLINICAL LABORATORY DATA

Antinuclear antibodies were detected by an indirect immunofluorescence procedure using HEp-2 cells. Antibodies to SS-A (Ro) and SS-B (La) antigens were determined by gel double immunodiffusion using rabbit thymus extract.

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Accepted for publication  
19 November 1991

Antibodies to HTLV-I were measured by an enzyme linked immunosorbent assay (ELISA; Eitest-ATL kit, Eisai Inc, Japan) and the particle agglutination assay (Serodia-ATL kit, Fujirebio Inc, Japan). Western blot analysis was performed using HTLV-I antigens derived from the MT-2 cell line.<sup>18</sup>

#### SEPARATION OF MONONUCLEAR CELLS

Heparinised peripheral blood was obtained from patients with primary Sjögren's syndrome and from age and sex matched normal subjects. The method used to separate mononuclear cells has been reported in detail elsewhere.<sup>19</sup> Mononuclear cells were isolated from the peripheral blood by Ficoll-Conray gradient centrifugation (Daichi Pharmaceutical Co., Tokyo, Japan). After centrifugation, the cells were washed thoroughly with RPMI 1640 containing 2% fetal bovine serum (Gibco, Grand Island, NY, USA). The washed cells were resuspended at  $1 \times 10^6$ /ml in RPMI 1640 supplemented with 10% fetal bovine serum.

#### MONOCLONAL ANTIBODIES

The murine FITC or PE conjugated monoclonal antibodies, antiCD3, antiCD4, antiCD8, antiCD29, antiCD45RA, antiHLA-DR, antiCD25, and antiCD26 were purchased from Coulter Immunology (Hialeah, FL, USA). Their production and characterisation have been described elsewhere.<sup>20</sup> The antiCD3 antibody has been found previously to recognise all mature T cells. The antiCD4 antibody reacted with human helper-inducer T cells, antiCD8 with human suppressor-cytotoxic T cells, antiCD29 with the memory T cell subset, antiCD45RA with the virgin T cell subset, antiCD25 with interleukin 2 receptor, and antiCD26 with the T cell specific antigen or antigen triggering memory T cell antigen.

#### DUAL IMMUNOFLUORESCENT ANALYSIS

The method used for dual immunofluorescent analysis has been described previously elsewhere.<sup>21</sup> First, to prevent the non-specific binding by Fc fragments to FcR on mononuclear cells, the mononuclear cells were incubated with aggregated human IgG for 45 minutes at

4°C. The cells were then reacted with 5 µl of a FITC conjugated and 5 µl of a PE conjugated monoclonal antibody for 60 minutes at 4°C. After the cells had been washed, the percentage of positive cells was determined by flow cytometry (EPICS C Cell Sorter, Coulter Electronics, Hialeah, FL, USA).

#### PROLIFERATION ASSAY

Mononuclear cells ( $1 \times 10^5$ ) were cultured in quadruplicate in 96 well flat bottomed microtitre plates (Coster, Cambridge, MA, USA), with or without mitogens, in 0.2 ml of culture medium according to previously described methods.<sup>22</sup> The medium consisted of RPMI 1640 supplemented with 20% fetal bovine serum, 0.5% sodium hydrogen carbonate, 20 mM HEPES buffer, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Phytohaemagglutinin (Wellcome Foundation Ltd, Dartford, UK) was used at an optimum concentration of 20 µg/ml, concanavalin A (Sigma Chemical, St Louis, MO, USA) at 15 µg/ml, and pokeweed mitogen (Gibco) at 1:100 dilution. The cells were cultured in the presence of mitogen for four days at 37°C in a humidified atmosphere of 5% carbon dioxide in air. Wells were pulsed with 18.5 kBq of [<sup>3</sup>H]thymidine (New England Nuclear, Boston, MA, USA) for the last 24 hours of culture and the cells were harvested on glass filters with a semiautomatic cell harvester (Lab Mesh, Labo Science, Tokyo, Japan). The radioactivity of each sample was determined in a liquid scintillation counter, and the results expressed as mean counts per minute and stimulation index.

#### STATISTICS

Statistical analysis was performed using Student's *t* test,  $\chi^2$ , or Fisher's exact test, and *p* values <0.05 were considered significant.

## Results

### CLINICAL FEATURES OF HTLV-I SEROPOSITIVE PATIENTS

Thirteen of 36 patients with primary Sjögren's syndrome studied were positive for antibodies to HTLV-I (tables 1 and 2). In the western blot assay, all 13 serum samples reacted with HTLV-I gag (p19, p24, and p28) and with HTLV-I env (gp68) (fig 1). One patient was male and 12 female (mean (SD) age 51.7 (9.6) years). The mean (SD) age at the onset of clinical symptoms was 41.9 (9.8) years. The Nagasaki area was tentatively subdivided into the superendemic (first) and endemic (second) areas depending on the prevalence of antibodies to HTLV-I in subjects aged 40 years or more. All seropositive patients were born and had lived in the endemic (second) area. Antibodies to HTLV-I were also assayed in 240 age and sex matched control serum samples in our hospital. Positive serum samples were found in 15 (6%) subjects. One woman had high fever, joint pain, uveitis, xerophthalmia, and xerostomia. Her father and uncle had died of adult T cell leukaemia and her son, who had antibodies to

Table 1 Prevalence of antibodies to HTLV-I in patients with primary Sjögren's syndrome

	Patients with antibodies to HTLV-I (n=13 (36%))	Patients without antibodies to HTLV-I (n=23 (64%))
Sex (male:female)	1:12	1:22
Mean (SD) age (years)	51.7 (9.6)	53.5 (12.2)
Mean (SD) age at onset of clinical symptoms (years)	41.9 (9.8)	44.1 (11.0)
Place of birth (number (%))		
Pandemic area (ca. 20%)	0 (0)	6 (26)
Endemic area (ca. 10%)	13 (100)	14 (61)
Non-endemic area (5%)	0 (0)	3 (13)
Family history (number (%))		
Adult T cell leukaemia	1 (8)	0 (0)
HTLV-I associated myelopathy	1 (8)	0 (0)
Autoimmune diseases	5 (38)	3 (13)
Past history		
Blood transfusion	2 (15)	3 (13)

Table 2 Clinical manifestations of patients with Sjögren's syndrome with antibodies to HTLV-I and those without antibodies to HTLV-I

Clinical manifestations	Patients with antibodies to HTLV-I (number (%))	Patients without antibodies to HTLV-I (number (%))
<b>Glandular manifestations</b>		
Dryness of eyes	13 (100)	23 (100)
Corneal ulcer	4 (31)	2 (9)
Dryness of mouth	13 (100)	23 (100)
Enlargement of parotid glands	4 (31)	12 (52)
<b>Extraglandular manifestations</b>		
Photosensitivity	4 (31)	2 (9)
Swollen hand or sclerotic skin, or both	4 (31)	2 (9)
Raynaud's phenomenon	8 (62)	11 (48)
Joint pain	10 (77)	11 (48)
Lymphadenopathy	8 (62)	11 (48)
Uveitis	4 (31)*	0 (0)
Myopathy	8 (62)*	1 (4)
Recurrent high fever	9 (69)*	3 (13)
Interstitial pulmonary fibrosis	6 (46)	4 (17)
Renal disease	2 (15)	2 (9)
Peripheral neuropathy	2 (15)	0 (0)
Psychosis	1 (8)	0 (0)
Autoimmune thyroid diseases	2 (15)	7 (30)

\*p<0.05 versus patients without antibodies to HTLV-I.

HTLV-I, died of persistent Epstein-Barr virus infection. Another patient had Sjögren's syndrome and HTLV-I associated myelopathy and her aunt also had HTLV-I associated myelopathy. Two patients who were positive for antibodies to HTLV-I received a blood transfusion before the age at which they developed Sjögren's syndrome.

All seropositive patients had dryness of their eyes and mouth. Two of the four patients who had a history of corneal ulcers also had episodes of dry eyes. The prevalence of skin disease, including photosensitivity, swollen hands, and sclerosis, was higher in patients with antibodies to HTLV-I than in patients who did not have these antibodies, but this difference was not significant. Patients who had antibodies to

HTLV-I had slightly increased occurrences of joint pain, lymphadenopathy, and interstitial pulmonary fibrosis compared with those who were negative, but this difference was not significant. The patients who had antibodies to HTLV-I had significantly higher occurrences of extraglandular manifestations such as uveitis, myopathy, and recurrent high fever. A history of uveitis was found in four patients with antibodies to HTLV-I but was not found in any patient without the antibodies. The serum creatine kinase level was increased in eight patients who had antibodies to HTLV-I and one patient who was negative with a creatine kinase level of less than 250 IU/l. A man with antibodies to HTLV-I developed weakness of the arms and legs and was diagnosed as having polymyositis by electromyography and muscle biopsy. One woman had dryness of the eyes and mouth and spastic paraparesis with deep tendon hyperreflexia in her lower legs. She was diagnosed as having Sjögren's syndrome and HTLV-I associated myelopathy. The seropositive patients had a higher prevalence of recurrent high fever than did seronegative patients. Peripheral neuropathy was observed in two patients with Sjögren's syndrome and psychosis in one woman who had antibodies to HTLV-I.

#### CLINICAL LABORATORY DATA

The patients with Sjögren's syndrome had a polyclonal increase of their serum  $\gamma$  globulins. Table 3 shows that there was no significant difference in serum  $\gamma$  globulins and immunoglobulins between patients with and without antibodies to HTLV-I. The serum levels of C3 and C4 were normal. Antinuclear antibodies

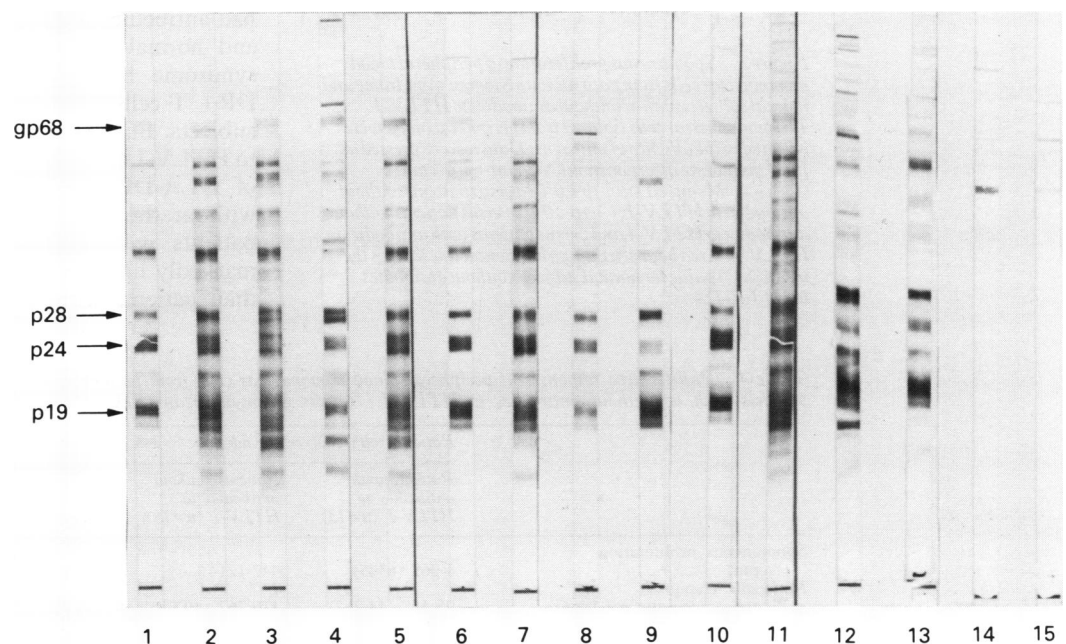


Figure 1 Immunoblot analysis of the reactions of patients with Sjögren's syndrome and normal subjects with human T lymphotropic virus I (HTLV-I) proteins (MT-2 cells). HTLV-I proteins were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred to a nitrocellulose membrane. Strips of the nitrocellulose membrane were exposed to serum samples from patients with Sjögren's syndrome (lanes 1-13) and normal subjects (lanes 14 and 15). Specifically bound IgG was visualised by western blotting.

Table 3 Laboratory data for patients with Sjögren's syndrome with antibodies to HTLV-I and those without antibodies to HTLV-I

Laboratory data	Patients with antibodies to HTLV-I (n=13)	Patients without antibodies to HTLV-I (n=23)
Mean (SD) $\gamma$ globulin (g/l)	23.8 (10.4)	22.5 (6.7)
Mean (SD) IgG (g/l)	28.8 (15.1)	28.4 (10.9)
Mean (SD) IgM (mg/l)	2310 (1630)	2040 (1050)
Mean (SD) IgA (mg/l)	4340 (1260)	4630 (2660)
Mean (SD) C3 (mg/l)	645 (82)	634 (193)
Mean (SD) C4 (mg/l)	252 (123)	258 (141)
No (%) positive for antinuclear antibodies	9/13 (69)	20/23 (87)
No (%) positive for antibodies to SS-A (Ro)	8/13 (62)	16/23 (70)
No (%) positive for antibodies to SS-B (La)	2/13 (15)	6/23 (26)

were found in nine of 13 patients who were carriers. Eight of the nine patients had a speckled antinuclear antibody pattern and one had a diffuse antinuclear antibody pattern. Antibodies to SS-A (Ro) antigen and SS-B (La) antigen were detected in eight and two respectively of the 13 patients with antibodies to HTLV-I. The prevalences of antinuclear anti-

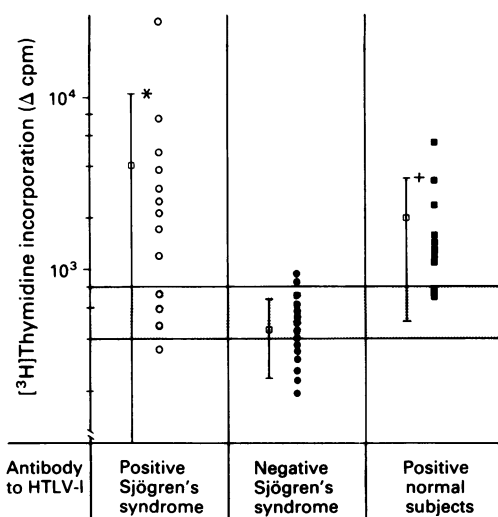


Figure 2 Spontaneous proliferation of peripheral blood mononuclear cells from patients with Sjögren's syndrome and normal subjects with or without antibodies to HTLV-I. The mononuclear cells isolated from peripheral blood were cultured in the absence of mitogens. Data were expressed as [<sup>3</sup>H]thymidine incorporation ( $\Delta$ counts/min (cpm)). (—□—) Mean (SD); (\*)  $p < 0.01$  versus patients without antibodies to HTLV-I; (+)  $p < 0.05$  versus patients without antibodies to HTLV-I and versus patients with antibodies to HTLV-I. Area between the two horizontal lines shows the mean (SD) value for normal subjects without antibodies to HTLV-I.

bodies and antibodies to SS-B (La) in patients who had antibodies to HTLV-I were less than those in patients who did not have these antibodies, but this difference was not significant.

#### PROLIFERATIVE RESPONSES OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH SJÖGREN'S SYNDROME WITH OR WITHOUT ANTIBODIES TO HTLV-I

The mononuclear cells isolated from peripheral blood were cultured with or without mitogens (fig 2 and table 4). Spontaneous proliferation of mononuclear cells from patients who were positive for these antibodies was markedly increased compared with that from patients who were negative for these antibodies. Furthermore, the spontaneous proliferation of mononuclear cells from normal subjects who were positive for these antibodies was significantly increased compared with that from normal subjects who were negative. Subjects who had antibodies to HTLV-I and those who did not had normal [<sup>3</sup>H]thymidine incorporation by their mononuclear cells in response to phytohaemagglutinin, concanavalin A, and pokeweed mitogen. As those patients with antibodies to HTLV-I had an increase in spontaneous mononuclear cell proliferation, the stimulation indices of mononuclear cells from patients with antibodies to HTLV-I responding to these mitogens were significantly less than those of cells from patients without these antibodies.

#### ACTIVATED T CELLS IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH SJÖGREN'S SYNDROME WITH OR WITHOUT ANTIBODIES TO HTLV-I

There was no significant difference in the percentage of CD3+ cells among patients who had antibodies to HTLV-I, those who did not, and normal subjects. Patients with Sjögren's syndrome had higher percentages of HLA-DR+ T cells and CD26+ T cells than normal subjects. Furthermore, patients with antibodies to HTLV-I had a significantly higher percentage of HLA-DR+ T cells compared with those without the antibodies (fig 3A). In addition, patients with antibodies to HTLV-I had a markedly higher percentage of CD25+ T cells than patients without the antibodies (fig 3B).

Table 4 Proliferative responses of peripheral blood mononuclear cells from patients with Sjögren's syndrome and normal subjects with or without antibodies to HTLV-I. Results given as mean (SD)

	Patients with Sjögren's syndrome		Normal subjects	
	Patients with antibodies to HTLV-I (n=13)	Patients without antibodies to HTLV-I (n=15)	Subjects with antibodies to HTLV-I (n=10)	Subjects without antibodies to HTLV-I (n=10)
Spontaneous proliferation ( $\Delta$ cpm)	4 065 (6545)*	447 (214)	1 959 (1468)†	587 (188)
Mitogenic response				
Phytohaemagglutinin ( $\Delta$ cpm) (SI)‡	95 415 (44 262) 92.8 (92.1)*	110 767 (40 010) 281.9 (140.5)	114 783 (29 668) 76.2 (44.4)†	127 260 (42 270) 217.1 (104.7)
Concanavalin A ( $\Delta$ cpm) (SI)	74 220 (40 568) 60.0 (64.4)*	62 386 (36 416) 143.5 (61.5)	129 630 (50 177) 80.2 (25.4)†	103 030 (52 450) 179.1 (101.8)
Pokeweed mitogen ( $\Delta$ cpm) (SI)	28 696 (18 620) 22.8 (26.0)*	20 800 (11 960) 53.0 (38.0)	54 445 (24 556) 33.6 (20.6)	22 541 (13 612) 38.8 (21.4)

\* $p < 0.05$  versus patients without antibodies to HTLV-I.

† $p < 0.05$  versus normal subjects without antibodies to HTLV-I.

‡SI=stimulation index.

No significant difference in the percentages of HLA-DR+ T cells and CD25+ T cells exists, however, between normal subjects with antibodies to HTLV-I and those without the antibodies (fig 3). Although the proportion of CD26+ T cells in patients with Sjögren's syndrome was markedly increased compared with that in normal subjects, the percentage of CD26+ T cells in HTLV-I positive patients was similar to that in those without the antibodies. The percentage of CD4+ cells in patients with Sjögren's syndrome with antibodies to HTLV-I was significantly less than that in patients without these antibodies. Furthermore, patients with Sjögren's syndrome had a high percentage of CD29+CD4+ cells and a low percentage of CD45RA+CD4+ cells among CD4+ cells

compared with those in normal subjects. The patients with antibodies to HTLV-I had a higher percentage of CD29+CD4+ cells and a lower percentage of CD45RA+CD4+ cells than patients without those antibodies.

### Discussion

We studied the presence of antibodies to HTLV-I in 36 patients with Sjögren's syndrome who live in Nagasaki, an area endemic for HTLV-I. The prevalence of serum antibodies to HTLV-I among normal adult subjects over 40 years of age was 10–25%. The prevalence in the pandemic area is high for HTLV-I (20–25%), that in the endemic area ranges from 10 to 15%,

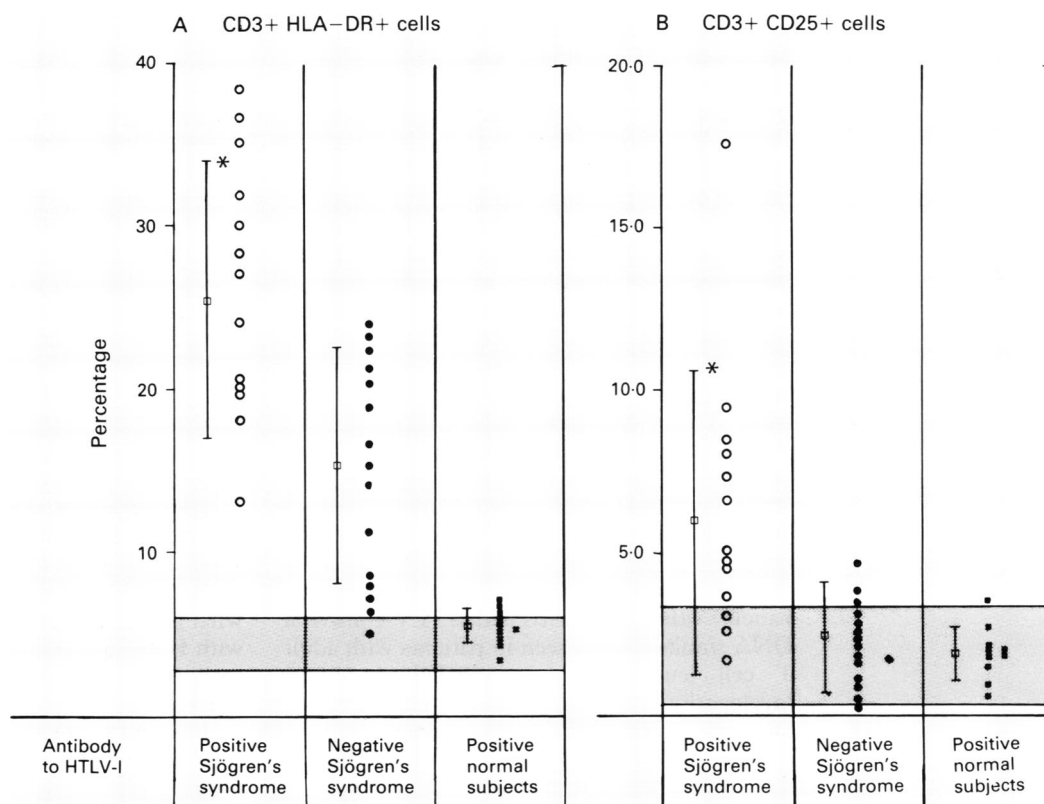


Figure 3 Activated T cells in peripheral blood mononuclear cells from patients with Sjögren's syndrome and normal subjects with or without antibodies to HTLV-I. (A) Percentage of CD3+HLA-DR+ cells among CD3+ cells in patients with Sjögren's syndrome with or without antibodies to HTLV-I and normal subjects. (B) Percentage of CD3+CD25+ cells among CD3+ cells. (—□—) Mean (SD). Tinted area gives the normal (mean (SD)) range from normal subjects without antibodies to HTLV-I. (\*)  $p < 0.05$  versus patients without antibodies to HTLV-I.

Table 5 Lymphocyte subpopulations in peripheral blood mononuclear cells from patients with Sjögren's syndrome and normal subjects with or without antibodies to HTLV-I. Results are mean (SD) %

Lymphocyte subpopulation	Patients with Sjögren's syndrome		Normal subjects	
	Patients with antibodies to HTLV-I (n=13)	Patients without antibodies to HTLV-I (n=15)	Subjects with antibodies to HTLV-I (n=10)	Subjects without antibodies to HTLV-I (n=10)
CD3+ cells	76.5 (8.0)	71.7 (6.9)	75.1 (5.8)	70.4 (4.4)
HLA-DR+CD3+/CD3+	25.0 (8.8)*,†	15.3 (7.3)†	5.5 (1.1)	4.3 (1.5)
CD25+CD3+/CD3+	5.9 (4.7)*,†	2.4 (1.7)	1.9 (0.8)	1.8 (1.5)
CD26+CD3+/CD3+	54.7 (15.5)†	55.8 (12.0)†	15.8 (4.0)	12.3 (3.2)
CD4+ cells	28.7 (10.0)*,†	37.4 (8.3)	47.7 (7.2)	41.2 (8.7)
CD29+CD4+/CD4+	68.5 (10.8)*,†	51.9 (4.1)†	49.5 (7.1)	43.4 (8.7)
CD45RA+CD4+/CD4+	31.5 (10.8)*,†	48.1 (4.1)†	50.5 (7.1)	54.3 (8.7)
CD8+ cells	40.0 (12.4)	45.1 (23.3)	40.0 (8.2)	43.5 (8.8)

\* $p < 0.05$  versus patients without antibodies to HTLV-I.

† $p < 0.05$  versus normal subjects without antibodies to HTLV-I.

and that in a third area which excludes Nagasaki is less than 5%. Furthermore, 15/240 (6%) serum samples from outpatients in our hospital were positive for antibodies to HTLV-I. Thirteen of the 36 patients had serum samples which were positive by ELISA and the particle agglutination assay for antibodies to HTLV-I and were confirmed to be positive by western blotting. We found antibodies to HTLV-I in none of seven patients with primary Sjögren's syndrome who were born in the first area, 13/26 (50%) patients who were born in the second area, and none of three patients who were born in the third area.

A comparison of the clinical features of our seropositive and seronegative patients showed that extraglandular manifestations including uveitis, myopathy, and recurrent high fever were higher in seropositive than in seronegative patients. Patients with HTLV-I associated myelopathy/tropical spastic paraparesis are known to have rheumatic syndromes such as T lymphocyte alveolitis,<sup>12 23-25</sup> polyarthritis,<sup>26</sup> polymyositis,<sup>27</sup> and uveitis.<sup>28</sup> It is important that such rheumatic syndromes may be associated with HTLV-I infection. Vernant *et al* reported that all of five patients with HTLV-I associated tropical spastic paraparesis had lymphocytic alveolitis and Sjögren's syndrome was confirmed histologically.<sup>12</sup> Only one of the five patients had xerophthalmia and xerostomia. It has been reported that HTLV-I provirus DNA was detected in the alveolar lymphocytes of patients with tropical spastic paraparesis.<sup>25</sup> Patients with adult T cell leukaemia who presented with proliferative synovitis have also been described.<sup>29</sup> We reported previously that patients with rheumatoid arthritis had a high prevalence of antibodies to HTLV-I,<sup>30</sup> and an HTLV-I infected T cell line established from mononuclear cells taken from the synovial fluid of a patient with polyarthritis had HTLV-I proviral DNA similar to that seen in patients with adult T cell leukaemia and HTLV-I associated myelopathy.<sup>31</sup> Clinical features suggest that Sjögren's syndrome complications in seropositive patients with rheumatoid arthritis were higher than in seronegative patients.<sup>30</sup> In this study, the prevalence of interstitial pulmonary fibrosis and polyarthritis was slightly increased in patients with antibodies to HTLV-I compared with patients without these antibodies. An increase of the serum creatine kinase level was observed in eight of 13 patients who had antibodies to HTLV-I but in only one of 23 patients who did not. All of the patients except one man had chronic or relapsing increases of their serum creatine kinase level (less than 250 IU/l) and could not be diagnosed as having polymyositis by needle electromyography or muscle biopsy, or both. One man had diffuse weakness of the proximal muscles of his hands and feet and was found to have polymyositis by taking a muscle biopsy sample. A case of polymyositis in a patient infected with HTLV-I and the human immunodeficiency virus (HIV) has been reported.<sup>32</sup> In this patient, HTLV-I nucleic acids and antigens were localised specifically to atrophic muscle fibres in regions of inflammation. There were myopathic changes

and the HTLV-I tat protein had accumulated in the muscle, making it resemble the muscle of a transgenic mouse carrying the HTLV-I tax gene.<sup>32</sup> The striking degree of inflammation, however, was not seen in the transgenic mouse model. These results support strongly the belief that myopathy, with or without inflammation, is associated with HTLV-I infection.

Patients with uveitis are known to have a high prevalence of antibodies to HTLV-I.<sup>28</sup> Uveitis has been found in one patient with HTLV-I polymyositis.<sup>32</sup> In our clinical study, uveitis was found in four of 13 seropositive patients but in none of 23 seronegative patients. As uveitis is thought to be rare in patients with primary Sjögren's syndrome,<sup>8 9</sup> uveitis may be a distinct clinical feature which is observed in HTLV-I associated Sjögren's syndrome. Among patients with primary Sjögren's syndrome, those who were positive for antibodies to HTLV-I could not be distinguished clinically from those who were negative. It is possible, however, that the extraglandular manifestations which include uveitis, myopathy, and recurrent high fever may typify seropositive patients with Sjögren's syndrome.

The laboratory data showed that there was no significant difference in the amounts of serum  $\gamma$  globulins, immunoglobulins, and complement between seropositive and seronegative patients. Patients with antibodies to HTLV-I had a lower prevalence of antinuclear antibodies and antibodies to SS-B (La) than did patients without antibodies to HTLV-I. This difference, however, was not significant. Talal *et al* reported that a subset of patients with Sjögren's syndrome had serum antibodies to the HIV-I p24 gag protein and were remarkable for their paucity of antibodies to SS-A (Ro) and SS-B (La).<sup>10</sup> Furthermore, the absence of antibodies to SS-A (Ro) and SS-B (La) is strikingly reminiscent of what has been seen in patients who are infected with HIV-I and who present with parotid gland swelling, xerostomia, or other features of Sjögren's syndrome including positivity for rheumatoid factor and antinuclear antibodies.

We found that the spontaneous proliferation of peripheral blood lymphocytes is increased in seropositive patients compared with that in seronegative patients with Sjögren's syndrome. Furthermore, normal subjects with antibodies to HTLV-I had an increased spontaneous proliferation of mononuclear cells. It has been shown that the spontaneous proliferation of peripheral blood lymphocytes is increased markedly in patients with HTLV-I associated myelopathy<sup>33 34</sup> as well as in healthy HTLV-I carriers.<sup>35</sup> Furthermore, HTLV-I carriers with or without HTLV-I associated myelopathy had increased levels of interleukin 2 activity and soluble interleukin 2 receptors in the culture supernatants of their peripheral blood lymphocytes.<sup>36</sup> In this study, seropositive patients with Sjögren's syndrome had increased percentages of HLA-DR+ T cells, CD25+ (interleukin 2 receptor  $\alpha$  positive) T cells and CD29+CD4+ cells compared with seronegative patients with Sjögren's syndrome. The increased expression on the T cells of patients with Sjögren's syndrome of activated antigens such as HLA-

DR, interleukin 2 receptors, T cell lineage specific antigen 1, and late activation antigen on the T cells of patients with Sjögren's syndrome has been reported.<sup>37 38</sup> In this study, we also showed that patients with seronegative Sjögren's syndrome had higher percentages of CD26+ T cells, and CD29+CD4+ cells among CD4+ cells than did normal subjects. Furthermore, patients with Sjögren's syndrome with antibodies to HTLV-I had increased percentages of HLA-DR+ T cells, CD25+ T cells and CD29+CD4+ cells among CD4+ cells compared with seronegative patients with Sjögren's syndrome. On the other hand, the percentages of CD4+ cells and CD45RA+CD4+ cells among CD4+ cells in patients with antibodies to HTLV-I were less than those in patients without those antibodies. It has been shown that patients with HTLV-I associated myelopathy have unusually high proportions of HLA-DR+ T cells and interleukin 2 receptor positive cells than normal subjects.<sup>39 40</sup> It is apparent that HTLV-I has a preferential tropism for CD4+ cells in vivo and has the capacity to induce interleukin 2 production and CD25 expression and to trigger T cell proliferation.<sup>41 42</sup> In particular, HTLV-I infected T cells synthesise the CD25 chain, which is probably induced by the tat-I gene product of the virus. Given these findings, it seems likely that the increase in the percentage of CD25+ T cells found in seropositive patients with Sjögren's syndrome may be induced not by Sjögren's syndrome but by HTLV-I infection.

The pathogenesis of HTLV-I associated Sjögren's syndrome is unknown. It is possible that a disease triggering virus infection results from HTLV-I induced immunosuppression.<sup>43 44</sup> Other mechanisms may be direct slow virus infection of the salivary glands, a cytotoxic immune reaction against HTLV-I infected cells in the salivary glands, or a humoral autoimmune disorder in which HTLV-I infected salivary gland cells share common antigenic determinants. On the basis of the clinical features and laboratory data of our patients, we suggest that HTLV-I plays a part in the pathogenesis of a subset of cases of primary Sjögren's syndrome.

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