A HTLV-I Carrier Who Showed Various Symptoms and Antibodies of Autoimmune Diseases

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We report a 37-year-old female HTLV-I carrier with complicating primary biliary cirrhosis (PBC) and mixed connective tissue disease (MCTD). Serum anti-HTLV-I antibody titer was ×256. Flower cells (4.5%) were found in the peripheral blood. Southern blot analysis showed no clonal integration in peripheral blood lymphocyte (PBL) DNA. Polymerase chain reaction showed the HTLV-I genome in PBL DNA. As cholestatic liver dysfunction and serum titer of anti-mitochondrial antibody were found, a clinical diagnosis of PBC was made. This patient later developed MCTD. These diseases responded well to prednisone. The pathogenetic relationship of HTLV-I infection with various autoimmune diseases is discussed.

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Key words: primary biliary cirrhosis, mixed connective tissue disease, anti-mitochondrial antibody, anti-RNP antibody, steroid

Introduction

Retroviruses appear to be among the causative agents of autoimmune disease. A xenotropic virus was isolated from New Zealand Black mice, a model SLE animal, and immune complexes of viral gp70, gp30 and reactive antibodies were detected in this animal (1, 2). Human T-cell lymphotropic virus type I (HTLV-I), a human retrovirus, has been identified as the etiological agent of adult T cell leukemia/lymphoma (3–5). Myelopathy characterized by spastic paraplegia has recently been found to be associated with HTLV-I; this condition has been designated HTLV-I associated myelopathy (HAM) (6–8). This disease is characterized, in part, as an immune system dysfunction in the central nervous system (9, 10). Other diseases characterized as immune system dysfunction, such as polyarthritis, polymyositis, bronchopneumopathy and Sjögren’s syndrome has also been reported to be associated with HTLV-I seropositivity (11–17). It is speculated, therefore, that HTLV-I infection is one of the causes of autoimmune diseases. Here, we report a HTLV-I carrier who showed the symptoms of various autoimmune diseases. Examination of the serum titer for autoantigens revealed autoantibodies specific for various autoimmune diseases. To our knowledge, complications of PBC with HTLV-I have not previously been reported.

Case Report

A 37-year-old-female came to our hospital presenting with neck lymphadenopathy and liver dysfunction in October 1988. She was born in southwestern Japan (Amakusa in Kumamoto prefecture), an area in which HTLV-I is endemic. Her mother died of multiple myeloma. Physical examination showed neck lymphadenopathy and no hepatosplenomegaly. The relevant results of laboratory tests were: erythrocyte sedimentation rate 46 mm/h and leukocyte count 5.5×10⁹/l with 4.5% atypical lobulated lymphocytes (flower cells) as shown in Fig. 1. Serum total protein was 8.0 g/dl and γ globulin was 25.6%, but there was no M peak. Serum lactate dehydrogenase was 217 WU. Serum alkaline phosphatase was 21.8 KAU and γ glutamyl transferase was 169 U/l. C reactive protein and rheumatoid factor were negative. Direct Coombs’ test was positive. Antibody serum titers were: anticytoplasmic antibody, >1×2,560;
Fig. 1. May-Grunwald-Giemsa staining of flower cells in the peripheral blood of the patient. The leukocyte count was 5.5×10⁹/l, of which 4.5% was atypical lobulated lymphocytes (flower cells). (×1,000).

anti-mitochondrial antibody, 1×2,560; anti-M2 antibody, 1×1,600; anti-toxoplasma antibody, 1×1,024; IgM anti-toxoplasma antibody, normal (1×16); anti-HTLV-I antibody, 1×256 (by particle agglutination and determined by Western blotting); and anti-HIV antibody, negative. Surface marker analysis of peripheral blood had the following results: 63.6% of PBL were reactive with anti-CD3, 48.0% with anti-CD4, 27.5% with anti-CD8, 22.4% with CD25, and 16.7% with both anti-CD3 and anti-HLA-DR. Lymph node biopsy revealed a reactive lymph node as shown in Fig. 2.

The clinical course of the patient is illustrated in Fig. 3. Because the serum titer of anti-toxoplasma antibody was high, we speculated that her lymphadenopathy was due to toxoplasma infection. She was treated with sulfamethoxazole/trimethoprim, but as the drug induced an allergic reaction, acetylsalicylamycin was then used. Despite this treatment, the lymph node swelling was not reduced. Meanwhile, cholestatic liver dysfunction deteriorated. As the titers of anti-mitochondrial Ab and anti-M2 Ab were significantly high, a clinical diagnosis of primary biliary cirrhosis was made. The criteria used here were those set out by the committee of the Ministry of Welfare in Japan (18). In July 1989, morning stiffness and arthritis of the arm and interpharyngeal joints was manifested. The patient was treated with azathioprine, which had no effect. In January 1990, she suffered from fever, diarrhea and weight loss. We believed that these symptoms including PBC and arthritis were due to HTLV-I related immune dysfunction such as HAM. As prednisone is the most effective drug for HAM, we used it for this patient, and all the above symptoms improved rapidly.

From February 1990 to August 1991, however, thrombocytopenia, kidney dysfunction characterized by proteinuria and granular casts, Raynaud’s phenomenon, sclerodactyly and lung fibrosis developed as complications. Laboratory data with regard to autoantibodies were: anti-RNP Ab ×256, anti-Sm Ab×16, anti-dsDNA Ab ×7.2, anti-Sc170 Ab(−), anticientromere Ab(−), LE cell (+), anti-thyroglobulin Ab ×1,600, anti-microsome Ab ×6,400. There were symptoms of SLE; arthritis, thrombocytopenia, and kidney dysfunction. LE cells were positive. There were also symptoms of PSS; Raynaud’s phenomenon, sclerodactyly and lung fibrosis. Anti-RNP Ab was positive, and a clinical diagnosis of mixed connective tissue disease (MCTD) was made according to the criteria by the committee of the Ministry of Welfare in Japan (19). In December 1991, after a transient fever, she developed sphincter disturbance and weakness of the bilateral lower extremities. Neurological examination revealed severe symmetric weakness of lower extremities, and weak to absent patellar and achilles tendon reflexes. There was mild weakness of both upper extremities. There were no pathological reflexes and no cortical, cranial nerve, cerebellar, or sensory deficits. Cerebrospinal fluid (CSF) had 158 mg/dl protein, 48 mg/dl glucose, 130.6 mEq/1 chloride and 13/3 cells/µl, that is, acellular rise of total protein. CSF was negative for antibody to HTLV-I, as determined by particle agglutination. Serum creatine phosphokinase was normal. Accordingly, a clinical diagnosis of Guillain-Barré syndrome (GBS) was made and the patient was again treated with prednisone. The rectal disturbance improved within a week and bladder disturbance and muscle weakness gradually ameliorated, eventually showing normal
Materials and Methods

Southern blot hybridization analysis

High molecular weight DNA were extracted from PBL of the patient. After digestion with Pst I and Sac I, 10 µg DNA samples were analyzed by Southern blot hybridization with HTLV-I full length genomic probes (20).

Polymerase chain reaction (PCR)

The viral genome was detected by polymerase-chain-reaction amplification with HTLV-I-specific primer pairs to gag and pX genes, using the method of Fujii et al (21).

Serology assays

Sera from 5 patients with primary biliary cirrhosis who lived in areas nonendemic for HTLV-I in Japan were investigated at this time. To detect HTLV-I antibody in sera, the particle agglutination test was applied.

HLA typing

HLA typing for A, B, C, DR and DQ antigens was performed with the standard National Institutes of Health microcytotoxicity test using selected sera standardized according to the criteria put forward at the Tenth International Histocompatibility Workshop (22). DNA typing to determine HLA DR and DQ loci, was done by PCR and dot blot hybridization, according to the methods outlined at the Eleventh International Histocompatibility Workshop (23).

Results

HTLV-I integration

By Southern blot hybridization, there was no detectable band which hybridized with HTLV-I genome (Fig. 4). However, the HTLV-I genome was positively amplified from the peripheral blood lymphocyte of the patient with primer pairs specific for HTLV-I gag and pX genes, as shown in Fig. 5.

Serological tests for HTLV-I

Sera from all 5 patients with PBC were found to be HTLV-I negative.

HLA typing

Serological HLA typing of this patient was: A26.3, B35, 44,
Fig. 4. Southern blot analysis of HTLV-I proviral DNA. DNA samples derived from peripheral blood were digested with Sac-I and Pst-I and analyzed by Southern blot hybridization with a $^{32}$P labeled HTLV-I full length probe. A positive control was obtained from a patient with acute ATLL and the negative control was from a normal individual. On digestion with Sac-I, the positive control showed a 19.6 Kb band. The patient's sample showed no detectable band. On digestion with Pst-I, the positive control showed an 11.7 Kb band as well as three internal fragments of 2.5 Kb, 1.9 Kb and 1.4 Kb. The patient's sample, again, showed no detectable band.

Fig. 5. HTLV-I pX and gag gene amplification analysis (PCR) of DNA from patient's PBL. The positive and negative controls are as described in Fig. 4. The arrow showed the amplified products, which were hybridized with the respective alkaline-phosphatase-conjugated probe.

Cw9, −, DRw15, −, DQw5, w6.

Abnormal extra reactivity was detected by polyclonal antibodies reacting at loci other than the above; A31, B21, DR13.

By DNA typing of HLA antigens, the DR and DQ loci were determined to be DRw15, w16 and DQw5, w6, respectively.

Discussion

We have reported here what we believed to be the first case of an HTLV-I carrier with complicating multiple autoimmune diseases, including PBC. The patient's serum was positive for anti-HTLV-I antibody and the HTLV-I genome was detected in her peripheral blood by PCR, thus confirming HTLV-I infection. As there were pathological flower cells in her peripheral blood, a provisional clinical diagnosis of smouldering ATL was made (24). According to the revised criteria of Yamaguchi et al (20), by which clinical stage is determined simply according to the integration state of HTLV-I in the peripheral blood by Southern blot analysis, this patient could be reclassified as a healthy carrier of HTLV-I. In the criteria of Yamaguchi et al (20), there is no mention of healthy carriers with pathological flower cells. If sufficient numbers of such cases were to accumulate in the future, such healthy carriers with pathological flower cells could be classified as a new subgroup of ATL.

It has been reported that HTLV-I transgenic mice develop Sjögren's syndrome-like exocrinopathy (25), degeneration of oxidative muscle (26), and arthropathy (27), which, respectively, correspond to human HTLV-I-associated Sjögren's syndrome, HTLV-I-associated myopathy and HTLV-I-associated arthropathy. Although disease models of the transgenic mouse are different from human HTLV-I-associated Sjögren's syndrome or polymyositis in that there are few lymphocyte infiltrations in the affected site in the early stage of these diseases (25, 26), this model offers supportive evidence that HTLV-I could be a causative agent of autoimmune-like diseases in human. In addition to ATL, various other diseases have been found to be associated with HTLV-I infected patients, HAM being the second such HTLV-I associated disease to have become widely accepted (6-9). A number of diseases originally classified as autoimmune diseases have since been reported to be associated with HTLV-I (Table 1) (12, 14-16, 27-33).

HTLV-I-associated diseases have been found as complications in patients with HAM, as shown in Table 1. Thus, HTLV-I-associated diseases sometimes overlap. For example, HTLV-I seropositive patients with Sjögren's syndrome showed a high incidence of complicating extraglandular manifestations such as uveitis, myopathy and recurrent high fever, compared with HTLV-I seronegative patients (17). In the present patient, PBC coincided with MCTD. This complication of multiple autoimmune diseases may be regarded to be characteristic of HTLV-I-associated diseases.

With some exceptions (34), it has been reported that ATLL and HAM seldom co-exist in a patient, one reason being that the HLA type in patients with ATLL differs from that in patients with HAM (35). In our patient, A26 was the only common ATLL-related HLA haplotype reported. There were no other HLA haplotypes associated with ATLL or HAM in this patient; this might therefore explain why this patient did not develop ATLL or HAM. Mann et al (36) and Sonoda et al (37) reported...
Table 1. HTLV-I-Associated Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference(s)</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>1. HTLV-I associated myelopathy (HAM/TSP)</td>
<td>Osame et al (28)</td>
<td>1/1,464 carriers (Kyushu district in Japan)</td>
</tr>
<tr>
<td>2. HTLV-I associated bronchopneumopathy (HAB)</td>
<td>Sugimoto et al (14)</td>
<td>6/6 HAM (abnormal BAL*)</td>
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<tr>
<td></td>
<td>Courdier et al (15)</td>
<td>18/21 HAM (abnormal BAL)</td>
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<tr>
<td>3. HTLV-I associated arthropathy (HAAP)</td>
<td>Kitajima et al (12)</td>
<td>13/40 HAM</td>
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<tr>
<td>4. Sjögren’s syndrome</td>
<td>Vernant et al (16)</td>
<td>5/5 HAM</td>
</tr>
<tr>
<td>5. Myositis</td>
<td>Yoshimine et al (29)</td>
<td>30/99 HAM (abnormal CPK*)</td>
</tr>
<tr>
<td>6. PSS</td>
<td>Vernant et al (30)</td>
<td>1 case (with Sjögren’s syndrome and subsequently ATL)</td>
</tr>
<tr>
<td>7. Uveitis</td>
<td>Mochizuki et al (31)</td>
<td>62 HTLV-I seropositive/145 uveitis</td>
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<tr>
<td></td>
<td>Hino et al (32)</td>
<td>2/5 HAM</td>
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<tr>
<td>8. SLE</td>
<td>Vernant et al (30)</td>
<td>1 case</td>
</tr>
<tr>
<td></td>
<td>Eguchi et al (33)</td>
<td>2 cases (with HAM)</td>
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<tr>
<td></td>
<td>Vernant et al (30)</td>
<td>3 cases</td>
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<tr>
<td>9. Necrotizing vasculitis</td>
<td>Present case</td>
<td>1 HTLV-I seropositive/6 PBC</td>
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*Broncho-alveolar lavage, *Creatine phosphokinase

that T cells infected with HTLV-I induced ‘alien’ HLA antigen on their surfaces (gain of HLA). Sonoda et al (37) also reported that peripheral blood lymphocyte from patients with ATL shows an absence of inherent HLA antigens, deduced from familial haplotype analysis (loss of HLA). The PBL of the present patient showed extra reactivity to HLA test sera in loci other than her inherited loci. It is thus, clear that the PBL of this patient had gained alien HLA antigens. As HTLV-I can infect with B lymphocytes, fibroblasts, endothelial cells and synovial cells in addition to T lymphocytes (38–41), it is likely that HTLV-I infection in multiple organs resulted in the expression of alien HLA-like antigens in all these organs in this patient. The immune response to these antigens may thus have resulted in the multiple autoimmune diseases seen in our patient.

However, it is conceivable that the concomitant existence of PBC and HTLV-I infection in this patient was accidental. We examined titers of anti-HTLV-I in patients with PBC who resided in the Kinki district of Japan, where HTLV-I is not endemic. We found no anti-HTLV-I antibody-positive individual among these patients with PBC. Further investigation of HTLV-I antibody in PBC patients from HTLV-I endemic areas, would contribute to clarification of this problem.

With regard to the therapy used for PBC and MCTD in this patient, we selected prednisone as the first choice rather than ursodeoxycholic acid (UDCA), which is generally regarded as the first choice for the treatment of PBC. We chose this course of action as the patient had multiple autoimmune diseases, including PBC. We suspected that these disease were induced by the HTLV-I-infected state, as in HAM. We therefore used corticosteroid therapy, which proved to be very effective.

References