Chromosome Aberrations and Clinical Features of Adult T Cell Leukemia-Lymphoma Not Associated With Human T Cell Leukemia Virus Type I

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Chromosome aberrations and clinical features of three patients with adult T cell leukemia-lymphoma (ATL) not associated with human T cell leukemia virus type I (HTLV-I) are described. From their clinical features, two patients were diagnosed as acute type and one patient was diagnosed as chronic type, which later converted to acute crisis. Clonal and many chromosomal abnormalities were observed before therapy in the two acute type cases and at relapse in the chronic type case. Karyotype aberrations, including trisomy 3, trisomy 7, trisomy 21, del(6)(q21), del(10)(p13), 14q11 translocation, and loss of X chromosome, all of which are frequently found in HTLV-I-associated ATL, were also seen in these cases of HTLV-I-negative ATL.

We showed in 1983 that some patients with ATL have typical clinicohematologic, morphologic, and immunophenotypic features, but test negative for antibody to human T cell leukemia virus type I (HTLV-I). By Southern blot analysis, we recently proved that HTLV-I was not associated in five patients with anti-HTLV-I antibody-negative ATL. These findings suggested that HTLV-I may not be involved in leukemogenesis of some ATL patients. To examine leukemogenesis and the exacerbation process of such ATL patients, we conducted karyotype analysis of the leukemia cells. We present evidence that chromosome abnormalities similar to those found in typical HTLV-I-associated ATL were also found in HTLV-I-negative ATL.

MATERIALS AND METHODS

Patients. Previously, we confirmed that any nucleotide sequences of the provirus genome of HTLV-I were not integrated into DNA of leukemia cells from all five patients with anti-HTLV-I antibody-negative ATL. In these patients, the leukemia cells had flower cell morphology as well as the inducer/helper phenotype, both of which are characteristics of ATL. Karyotype analysis could be made in the leukemia cells from three of the five patients with HTLV-I-negative ATL. The patients were classified according to clinical subtypes of ATL, as described elsewhere.

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Cytogenetics. Cytogenetic studies were done on specimens of peripheral blood and pericardial effusion which were obtained before therapy and at relapse. Mononuclear cells prepared from these sources by the usual Ficoll-Hypaque gradient method were examined according to conventional techniques after culturing for 18 to 48 hours without mitogens such as phytohemagglutinin or T cell growth factor. After hypotonic treatment with KCl (0.075 mol/L) for 20 minutes, the cells were fixed in methanol acetic acid (3:1), dropped onto glass slides, and air dried. Conventional Q-banding techniques were applied to the slides. Metaphase and prometaphase cells were photographed and then arranged according to the International System for Human Cytogenetic Nomenclature (ISCN). The criteria adopted at the First International Workshop on Chromosomes in Leukemia were used to identify abnormal clones. Karyotypes of cases 1 and 2 were determined after complete reviews by expert members of ATL Karyotype Review Committee—1985.

RESULTS

Clinical features. The clinical features of the three patients with HTLV-I-negative ATL are summarized in Table 1. The age at onset of the disease ranged from 43 to 71 years. There were two men and one woman. Two patients (cases 1 and 2) became acutely ill, and their symptoms at onset were subfever, lymph node swelling, skin rash, and hepatosplenomegaly. Leukemic manifestation and high serum LDH levels were also observed. Pulmonary infiltration of leukemia cells developed later. The patients died of the disease 3 and 11 months, respectively, after the diagnosis (Table 1). In another patient (case 3), leukocytosis due to abnormal inducer/helper T lymphocytosis of flower cell morphology was accidentally found when the patient complained to his physician of dizziness due to iron-deficiency anemia. His T lymphocytosis remained unchanged for ~2 years without specific therapy and then converted to acute phase. Skin lesions, lymph node swelling, hepatosplenomegaly, pulmonary infiltration, pleural effusion, and pericardial effusion developed. Hypercalcemia developed later, and the patient died of cardiac insufficiency 34 months after the diagnosis and 3 months after the acute transformation (Table 1).

Hypercalcemia was observed in two patients. Immunoglobulin levels were found to be within the normal range at onset of the disease in all three patients. Combination chemotherapies, including vincristine, cyclophosphamide, prednisolone, and dexamethasone (VEPA or CHOP therapy), were effective. Two patients were induced into remission, but the effect was transient. From the clinical features,
CHROMOSOME ABERRATIONS OF HTLV-I-NEGATIVE ATL

Table 1. Clinical Features

<table>
<thead>
<tr>
<th>Factor</th>
<th>Case No.</th>
<th>Case No.</th>
<th>Case No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset, sex</td>
<td>71, M</td>
<td>43, F</td>
<td>47, M</td>
</tr>
<tr>
<td>Complaints at presentation</td>
<td>LN swelling, anorexia, subfever</td>
<td>LN swelling, erythrodema, subfever</td>
<td>Dizziness, back pain</td>
</tr>
<tr>
<td>Organs involved</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>- → +</td>
<td>- → +</td>
<td>- → +</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>- → +</td>
<td>-</td>
<td>- → +</td>
</tr>
<tr>
<td>Laboratory examination</td>
<td>WBC (per µL)</td>
<td>Abnormal</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>56,800</td>
<td>74,600</td>
<td>17,300</td>
</tr>
<tr>
<td>Treatment and response</td>
<td>VEPA</td>
<td>CHOP, MTX</td>
<td>CHOP, MTX</td>
</tr>
<tr>
<td>Survival after diagnosis (m)</td>
<td>3</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>ATL, acute type</td>
<td>ATL, acute type</td>
<td>ATL, chronic type</td>
</tr>
</tbody>
</table>

LN, lymph node; WBC, white blood cell; L, lymphocyte; BM, bone marrow; LDH, lactic acid dehydrogenase; N, normal; NR, no remission; PR, partial remission; CR, complete remission; ATL, adult T cell leukemia-lymphoma.

In one patient (case 1), chromosome constitution was extremely complicated: Among abnormalities, +der(2)(2;?)(?:p16q31;?), -6p-, and breaks at the proximal portion of chromosome 14 [14p11 and 14q11---+der(14)(14;?)(p11;q1) and +der(1)(1;14)(p11;q1)] were noted (Fig 1). In case 2, partial trisomy 3 and

Table 2. Cytogenetic Findings

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Source of Cells</th>
<th>Modal Chromosome Constitution</th>
<th>No. of Cells Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>b: 4n + of a.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24-h and 48-h PB</td>
<td>c: 48,X.,-X,-5,-12,+der(3)(3;?)q12;?,+der(5)(5;12)(q22; q14),+mar1,+mar2,+min.</td>
<td>23/69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d: 47,-X,-5,(X;6)(q12;p22),+der(3)(3;?)q12;?,+der(5)(5;12) (q22;q14),del(12)(q14),+min.</td>
<td>19/27</td>
</tr>
<tr>
<td>3</td>
<td>24-h PB*</td>
<td>f: 46,XY.</td>
<td>18/18</td>
</tr>
</tbody>
</table>

PB, peripheral blood; PE, pericardial effusion.

* Cytogenetic study was performed at relapse.

Cases 1 and 2 were diagnosed as acute type and case 3 was diagnosed as chronic type, according to the classification of subtypes of ATL* (Table 1).

Cytogenetic findings. Karyotype analysis was made before therapy in all three patients and again at relapse in two (cases 2 and 3). The results are summarized in Table 2.
t(5;12)(q22;ql4) were found in addition to three marker chromosomes (Fig 2A). Chromosome constitution obtained at relapse showed almost the same as that obtained before therapy. But, a translocation involving an X chromosome-t(X;6)(ql2;p22)—was newly found, suggesting clonal evolution (Fig 2B). On the other hand, in case 3, the karyotype remained apparently normal, and no gross abnormality was found before therapy. At relapse, however, cells in the pericardial effusion also showed very complicated karyotypes (Fig 3A and B). Two abnormal clones were determined, one of which had a missing Y and partial trisomy 7 (Fig 3B). Many rearranged chromosomes, including del(3)(p12), del(6)(q21q23), del(10)(p13), +der(11)(t(11;?)(p15;?), +del(21)(t(17;21)(q11;p13)) were common to each other, however, suggesting that both cell lines originated from the same progenitor cell. Thus, all three patients had complicated chromosome constitutions, although their modal numbers were near-diploid range. Abnormalities common to three patients were not detected. Abnormalities similar to those found in HTLV-I-associated ATL were also found in these three cases of HTLV-I-negative ATL, however.

DISCUSSION

We present the chromosome aberrations and clinical features of three patients with ATL not associated with HTLV-1. These findings and the hematologic, pathologic, and immunophenotypic features of these cases as reported elsewhere are identical to those of HTLV-I-associated ATL. The patients in this report are the same as the patients in the previous report, in which we proved that the patients showed neither integration of the HTLV-I proviral genome into chromosomal DNA of leukemia cells nor anti-HTLV-I antibody in serum. In addition, no anti-HTLV-I antibody was detectable in any family members. These facts indicate that HTLV-I was not associated with leukemogenesis in any of these cases of ATL, but we could not exclude the possible presence of a small amount of anti-HTLV-I antibody and of a very small fragment of the HTLV-I provirus that could not be detected with current techniques. Diversity in the clinical course of HTLV-I-associated ATL has been reported. Like HTLV-I-associated ATL, there was also diversity in the clinical course of HTLV-I-negative ATL, ie, acute type and chronic type were observed in HTLV-I-negative ATL in this series.

Clonal and many chromosomal abnormalities are documented for HTLV-I-associated ATL by karyotype analysis. Rowley and colleagues reported that trisomy for chromosomes 3 and 7 or 7q, and monosomy for X chromosome, 14q+, and 6q— were noted as the most common abnormalities in ATL, but 14q+ abnormality was not identified in any of the Caribbean ATL patients. Recently, karyotype analysis by the ATL Karyotype Review Committee—1985 of 78 ATL patients from 13 institutions in Japan revealed that +3, +7, -X rearrangements involving 6q15, 6q21, 10p11–13, 14q11, and 14q32 were the most common abnormalities in ATL. In the early phases of ATL, however, such as in smoldering ATL, or in chronic ATL, minimal chromosomal abnormalities have been reported.
As presented here, in acute HTLV-I-negative ATL, clonal and many chromosomal abnormalities were observed. In chronic HTLV-I-negative ATL (case 3), no chromosomal abnormalities were found at chronic phase, but clonal and many abnormalities, including del(6)(q21q23), +der(7)(7;?)(q22;?), and del(10)(p13), were found at relapse. In case 1, abnormalities involving the proximal portion of chromosome 14, +der(1)(1;14)(q13;q11) were found. Recently, abnormalities involving 14q11-q13 have been increasingly reported in various T cell malignancies, including ATL. In case 2, trisomy for chromosome 3, +der(3)(3;?)(q12;?), and monosomy for X chromosome were observed. From these cytogenetic findings, it is speculated that HTLV-I-negative ATL cells also have chromo-
somal abnormalities similar to those reported in HTLV-I-positive ATL or other T cell malignancies.

The number of HTLV-I-negative ATL cases reported is very small, indicating a need for close scrutiny of this disease. Results indicate no detectable differences between the clinicohematologic, cytopathologic, immunologic, and karyotype features of HTLV-I-positive and HTLV-I-negative ATLs. The findings of HTLV-I-negative ATL suggest that there is a similar underlying mechanism(s) in leukemogenesis as well as in the malignant process of both HTLV-I-related and HTLV-I-unrelated ATL. The presence of HTLV-I-negative ATL suggests the existence of unknown cellular oncogenes closely related to the transformation of inducer/helper T cell. We consider the role of HTLV-I in the etiology of ATL to be similar to that of Epstein-Barr virus in Burkitt’s lymphoma. The causative agent of ATL may not always be
HTLV-1; hence, the disease entity of ATL may not always be defined as an HTLV-1-induced T cell malignancy.

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Chromosome aberrations and clinical features of adult T cell leukemia-lymphoma not associated with human T cell leukemia virus type I

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