Chromosome Studies in Adult T-Cell Leukemia in Japan: Significance of Trisomy 7

By Yoshimi Ueshima, Shirou Fukuhara, Toshio Hattori, Takashi Uchiyama, Kiyoshi Takatsuki, and Haruto Uchino

Chromosomes were studied in cells from 15 Japanese patients with adult T-cell leukemia (ATL). Mitoses were obtained from unstimulated peripheral blood in 12 patients and a lymph node in one patient. In two other patients, mitotic cells were obtained solely from peripheral blood stimulated with phytohemagglutinin (PHA). Chromosomally abnormal cells were seen in 14 of the 15 patients. The abnormal cells had a modal number of chromosomes in near diploid range in 12 patients, and in near triploid and tetraploid range in the remaining 2 patients, respectively. Eight of the nine patients analyzed by Q-banding had clonal chromosome abnormalities. The most common abnormality was trisomy no. 7 or 7q, which was seen in 5 cases and has been primarily observed in lymphoid neoplasms. A 14q+ marker chromosome was found in two patients and a Dq+ in one patient; loss of a sex chromosome was found in three patients. Most chromosomes were involved in gains, losses, or structural rearrangements, but abnormalities of no. 11, which have been frequently found in lymphoid malignancies, was not observed in our series. The significance of these chromosome abnormalities is discussed.

MATERIALS AND METHODS
Twenty-four patients with ATL were studied. The diagnosis was based on clinical and hematologic findings (Table 1).

Cytogenetics
Specimens were obtained from peripheral blood, bone marrow, and lymph node. Peripheral blood mononuclear cells were separated by Ficoll-sodium metrizoate gradient centrifugation and examined after a short-term culture (24-120 hr) without phytohemagglutinin (PHA). In 10 patients, 3-4-day cultures with PHA were also prepared. Chromosome preparations were made by a slightly modified method of Moorhead et al.2 Bone marrow was examined without prior culturing. Lymph node biopsy specimen was minced and examined after 1 day of culture. Suitable Giemsa-stained metaphases were photographed in an ordinary microscope and Q-banded metaphases in a Zeiss fluorescence microscope. In some cases (cases 1, 3, and 9), Giemsa-stained slides were restained with quinacrine mustard more than a year after the original staining. Karyotypes were arranged according to the Paris Conference.4

Cell Surface Marker Analysis
Details of the characterization of neoplastic cell membranes were as previously described.2 In brief, surface markers of neoplastic cells in the peripheral blood and lymph node were detected by the test for sheep (E) red blood cell rosette formation and membrane immunofluorescence for surface immunoglobulins.

RESULTS
Clinical and Hematologic Findings
The clinicohematologic findings are shown in Table 1. The birthplaces of 18 patients were in the southwestern districts of Japan, while all the rest, except 2, lived in central Japan. Skin lesions were noted in 13 patients; erythroderma in most of the patients, and cutaneous nodules in one patient (case 10). Hepatomegaly and splenomegaly were detected in 15 and 7 patients, respectively. A mediastinal mass was not detected roentgenologically in any patient. Prior to chromosomal examination, antileukemic therapy was...
prescribed for 15 patients; corticosteroids and/or vinca alkaloids for 10 patients; combination chemotherapy for 4 patients; and immunotherapy for 1 patient. Most of the therapies were ineffective, although some response was noted in a few patients. One patient (case 11) achieved remission with adriamycin therapy later and is still alive, and one patient (case 10) reacted fairly well to immunotherapy OK432 with disappearance of the cutaneous nodules. Two patients (cases 15 and 17) have a mild course and long survival time without antileukemic therapy. Survival time from onset of the disease ranged from 1 mo to over 4 yr, but was usually less than 6 mo. In May-Giemsa-stained smears, leukemic cells showed a pleomorphism in 20 patients and monomorphism in 4 patients. E-rosette-forming cells accounted for more than half the number of peripheral blood lymphocytes, whereas less than 3.6% of the cells had surface immunoglobulins.

Cytogenetic Studies

Cytogenetic findings are summarized in Table 2. Mitotic cells were obtained from 15 patients, 14 of whom had chromosomally abnormal cells. The 15th patient had a normal karyotype (case 15) and was studied successfully only in a PHA culture of peripheral blood. Abnormal karyotypes were noted in cultures of unstimulated peripheral blood from 12 patients and in the lymph node of 1 patient (case 8), and in 1 patient (case 14) without spontaneous mitotic cells, there were abnormal mitotic cells only in PHA-stimulated peripheral blood culture. Bone marrow cells from 2 patients (cases 3 and 6) and PHA-stimulated peripheral blood culture from 3 patients (cases 9, 10, and 11) were fairly normal, whereas they had abnormal chromosomes in the unstimulated culture. Two patients (cases 12 and 13) had a similar percentage of metaphases, with abnormal chromosomes in both types of culture.

The modal chromosome number was hyperdiploid in 7 patients, pseudodiploid in 3 patients, hyperdiploid in 2 patients, and near triploid or tetraploid in 2 patients.

Chromosomes from 10 patients were examined with Q-banding. One of these patients (case 15) had a normal karyotype and another patient (case 3) had too few mitotic cells to be analyzed. The other 8 patients had a clonal chromosome abnormality that involved most of the chromosomes, however, chromosomes no. 11 and 20 were not involved in these 10 cases.

The most common single abnormality was the gain of a no. 7 chromosome, which was noted in four patients (cases 4, 9, 11, and 12), and a gain of 7q in one (case 10) (Figs. 1, 2, 3, and 4). These patients showed no other common chromosome alterations. One patient (case 9) had trisomy 3 and another (case 4) had trisomy 12 in addition to trisomy 7 (Figs. 1 and 2). The extra 7q in case 10 was translocated to the distal short arm of no. 4 (Fig. 4). This patient had the simplest karyotype in this series (45X, -X, 4p+), but the other 2 cells among the 85 cells had 15q+ in addition to the original karyotype, which could be the result of karyotypic evolution. A 14q+ marker chromosome was found in 2 patients (cases 1 and 3). Lymph node cells in another patient (case 8) had a Dq+ marker, which was not examined with banding.
Table 2. Cytogenetic Findings in 15 Patients With Adult T-Cell Leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>Source/Culture</th>
<th>Rearranged Cells/Total Metaphases Analyzed</th>
<th>Modal Chromosome No.</th>
<th>Banding</th>
<th>Modal Karyotype</th>
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<tbody>
<tr>
<td>1 PB/1</td>
<td>25/25</td>
<td>46</td>
<td>Q</td>
<td>46,X, -Y, -1, 1p +, del(9)(q32), 12p +, +14q +, +2 markers</td>
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</tr>
<tr>
<td>2 PB/2,3,4</td>
<td>6/11</td>
<td>48</td>
<td>Q</td>
<td>48,XY, -8, +3 markers</td>
<td></td>
</tr>
<tr>
<td>3 PB/1</td>
<td>4/4</td>
<td>45</td>
<td>Q</td>
<td>45XX, -1,2q +, +14q +</td>
<td></td>
</tr>
<tr>
<td>BM</td>
<td>0/6</td>
<td>46</td>
<td>Q</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>4 PB/1</td>
<td>31/31</td>
<td>48,49</td>
<td>Q</td>
<td>49,XX, +7, +8, +12, 8q +, 8p +, del(10)(q23), 21q +</td>
<td></td>
</tr>
<tr>
<td>5 PB/1,4</td>
<td>3/6</td>
<td>46,47</td>
<td>Q</td>
<td>47,XY, +marker/46,XY</td>
<td></td>
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<tr>
<td>6 PB/1</td>
<td>3/3</td>
<td>91</td>
<td>Q</td>
<td>86 – 91</td>
<td></td>
</tr>
<tr>
<td>BM</td>
<td>0/4</td>
<td>46</td>
<td>Q</td>
<td>46,XY</td>
<td></td>
</tr>
<tr>
<td>7 PB/1,4</td>
<td>6/6</td>
<td>48</td>
<td>Q</td>
<td>48,XX, +2 markers</td>
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<tr>
<td>8 PB/1,3</td>
<td>0/0</td>
<td>48</td>
<td>Q</td>
<td>48,XY, -8, Dq +, +3 markers</td>
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<td>LN/1</td>
<td>7/9</td>
<td>48</td>
<td>Q</td>
<td>48,XX, +3, -6, 7, del(10)(p13&amp;q24), 10q +, + marker</td>
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<tr>
<td>9 PB/1,5</td>
<td>16/18</td>
<td>48</td>
<td>Q</td>
<td>48,XX, +3, -6, +7, del(10)(p13&amp;q24), 10q +, + marker</td>
<td></td>
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<td>PHA/4</td>
<td>0/29</td>
<td>46</td>
<td>Q</td>
<td>46,XX</td>
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<td>10 PB/1,5</td>
<td>77/85</td>
<td>45,46</td>
<td>Q</td>
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<td>PHA/4</td>
<td>1/28</td>
<td>46</td>
<td>Q</td>
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<td>11 PB/1,3</td>
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<td>73</td>
<td>Q</td>
<td>73,XX, -Y, +1, +1, +2, +3, -6, +7, +8, +11, +12, +13, +16, +17, +17, +18, +19, +19, +20, +22, +12p +, +9 markers</td>
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</tr>
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<td>PHA/3</td>
<td>0/6</td>
<td>46</td>
<td>Q</td>
<td>46,XY</td>
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<tr>
<td>12 PB/1,3</td>
<td>51/51</td>
<td>47</td>
<td>Q</td>
<td>47,X, -Y, -4, +7, del(1)(q32), i(18q), 21q +, i(22q), +2 markers</td>
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</tr>
<tr>
<td>PHA/3</td>
<td>28/28</td>
<td>47</td>
<td>Q</td>
<td>47,X, -Y, -4, +7, del(1)(q32), i(18q), 21q +, i(22q), +2 markers</td>
<td></td>
</tr>
<tr>
<td>13 PB/1,3</td>
<td>54/54</td>
<td>46</td>
<td>Q</td>
<td>46,XY, -10, -13,5p +, +t(10;13)(10qter-&gt;10p13::13q32-&gt;13q11) 21p +, + marker</td>
<td></td>
</tr>
<tr>
<td>PHA/3</td>
<td>22/22</td>
<td>46</td>
<td>Q</td>
<td>46,XY, -10, -13,5p +, +t(10;13)(10qter-&gt;10p13::13q32-&gt;13q11) 21p +, + minute</td>
<td></td>
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<tr>
<td>14 PB/2,4</td>
<td>0/0</td>
<td>48</td>
<td>Q</td>
<td>48,XX, +2 markers</td>
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<tr>
<td>PHA/4</td>
<td>17/17</td>
<td>48</td>
<td>Q</td>
<td>48,XX, +2 markers</td>
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</tr>
<tr>
<td>15 PB/1,3</td>
<td>0/0</td>
<td>46</td>
<td>Q</td>
<td>46,XX</td>
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</tbody>
</table>

PB, peripheral blood; BM, bone marrow; LN, lymph node; PHA, PHA-stimulated peripheral blood.

Fig. 1. Q-banding karyotype of a cell from case 4. Abnormalities consist of +7, +8, +12, 8q +, 8p +, 10q +, +21q +. The absence of one no. 19 and a normal no. 8 in this figure are due to a broken metaphase. Inset: partial karyotype of no. 7 and no. 8 from another metaphase showing +del(7)(p13). Two normal no. 7, 8q +, 8p +, and normal no. 8.

Fig. 2. Q-banding karyotype of a cell from case 9. 48,XX, +3, -6, +7, del(10)(p13 & q24), 10q +, + marker. Inset shows trisomy 7 from another cell.
SIGNIFICANCE OF TRISOMY 7

The donor chromosome involved in the 14q+ markers was not determined because of the complex karyotypes, but each of the markers showed a different shape and fluorescent pattern. Loss of a sex chromosome was found in 3 patients: loss of Y in 2 males (cases 11 and 12) and loss of X in one female (case 10) (Figs. 3 and 4). Another patient (case 1) had an F-size chromosome similar to the Y, but the fluorescent band was narrow. Abnormalities of no. 1 were found in 3 patients: in case 1, one no. 1 was absent and the other was 1p+; case 3 lacked one no. 1; and case 12 had a 1q−[del (1) (q32)] (Fig. 3). Chromosome no. 10 was involved in structural rearrangements in 3 patients: one (case 4) had del (10) (q23); one (case 13) had t (10; 13) (13q11→13q32::10p13→10qter); and case 9 had two abnormal no. 10s and no normal no. 10 (Figs. 1, 2, and 5). Abnormalities of no. 12 were found in 3 patients: a gain in one patient (case 4) (Fig. 1) and 12p+ in two patients (cases 1 and 11). Chromosome no. 21 was rearranged in 3 patients: 21q+ in two patients (cases 4 and 12) and 21p+ in one patient (case 13) (Figs. 1, 3, and 5). Undetermined markers were found in nearly all patients except cases 4 and 10.

Clinical findings of patients with trisomy 7 or 14q+ varied, yet were much the same as in patients without the abnormalities.

DISCUSSION

Our results showed the high frequency of patients with chromosomal anomalies in Japanese ATL. In acute lymphocytic leukemia (ALL) or acute myelocytic leukemia (AML), about 50% of all patients do not show karyotypic changes even upon close banding analysis.5 In contrast, in our study, all the patients with dividing leukemic cells showed karyotypic changes, which seemed to be one of the characteristics of ATL, as well as of chronic T-cell leukemia (T-CLL)6 or Sézary syndrome.7

Although the karyotypic changes appeared to be variable, some recurring changes were noted in our series (Table 2). Trisomy 7, or at least 7q, which was found in 5 of 9 banded cases, was the most frequent abnormality in our series. Trisomy 7 has been occasionally reported in cases of lymphoid malignancies,5,10 22 including malignancies with B- or null-cell characteristics and a case of Sézary syndrome,b but has not been reported in nonlymphoid malignancies, except for four cases of Ph"-positive AML and CML.23 26 It was suggested25 that three23 25 of these four patients had a lymphoid type of the blastic stage of CML. In addition, these three patients had trisomy 12, which is rare in nonlymphoid malignancies. The
remaining case of AML\textsuperscript{26} with trisomy 7 and a Ph\textsuperscript{1} also had a 14q+ marker, which has been observed exclusively in lymphoid malignancies. This may be an atypical case of AML. In lymphoid malignancies, trisomy 7 was first noted by Zech et al.,\textsuperscript{10} who reported that trisomy 7 might belong to the unstable group of autosomes. Including other reported findings,\textsuperscript{5,10,22,27-29} trisomy 7 or 7q was observed in about 13% of banded cases of lymphoid malignancies: 5 of 76 cases of ALL,\textsuperscript{5,18} 30 of 151 cases of lymphomas,\textsuperscript{10-17} and 4 of 28 cases of multiple myeloma.\textsuperscript{21,22} Thus, trisomy 7 or 7q may represent important karyotypic changes in lymphoid neoplasms, although the frequency is not as high as that of the 14q+ marker.\textsuperscript{27,28} The histology of these lymphomas with trisomy 7 or 7q varies and includes one case with Hodgkin’s disease,\textsuperscript{10} six with lymphosarcoma,\textsuperscript{10,13,14} and three with nonendemic Burkitt’s lymphoma.\textsuperscript{15,29} Immunologic markers were described in five cases: one patient with null type poorly differentiated lymphoma,\textsuperscript{13} two with B-ALL,\textsuperscript{19,25} and two with null-ALL.\textsuperscript{18} Chromosomes from over 20 patients with T-cell malignancies have been analyzed with banding,\textsuperscript{6,8,28,30,33} and only one\textsuperscript{6} was found to have trisomy 7. It is possible that trisomy 7 is common only in a subset of T-cell disorders, as for example, the ATL seen in Japan.

A 14q+ marker chromosome, perhaps the most significant marker in lymphoid malignancies,\textsuperscript{27,28,34,35} was found in two patients (cases 1 and 3). In two cases of ATL including one cell line,\textsuperscript{36} a 14q+ was reported to be the result of 14q32 translocation with 12q and Yq. In our study, the fluorescent pattern of two 14q+ markers showed them to have different sizes, suggesting that they did not originate from these translocations, however, the details could not be determined because of the complex karyotypes. Thus, various chromosomes may participate in the formation of a 14q+ marker in ATL, as well as in diffuse histiocytic lymphoma.\textsuperscript{37} The 14q+ marker occurred in about 14% of ATL, which is lower than in other T-cell disorders (50%) or in B-cell neoplasms (90%).\textsuperscript{27,28}

Leukemic T cells obtained from the patients with ATL were generally not stimulated with PHA, and sometimes only normal residual T cells were stimulated with it. However, in one patient (case 14), dividing cells with an abnormal karyotype were observed only in the PHA-stimulated culture. The dividing cells in this case may be PHA-associated leukemic T cells. Normal chromosomes in the bone marrow of two patients may relate to the slight infiltration of leukemic cells into the bone marrow in these patients.

Isochromosome 17, which was reported in three cases of Sézary syndrome with “small cell” type variant,\textsuperscript{8,31} was not found in our study.

Fewer T-cell neoplasms have been analyzed karyotypically than have B-cell malignant diseases. Our data suggest that the chromosome pattern in ATL differs somewhat from that of other T-cell malignant disorders. However, only further study will determine whether this difference is consistent and whether it is determined by genetic factors in this particular Japanese population or by special functional characteristics of the affected T-cell subgroup.

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REFERENCES

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