Four Cases of t(4; 11) Acute Leukemia and Its Myelomonocytic Nature in Infants

By Miharu Nagasaka, Sakan Maeda, Hitoshi Maeda, Hui-Ling Chen, Kenkichi Kita, Osamu Mabuchi, Hideo Misu, Tamotsu Matsuo, and Taketoshi Sugiyama

Four cases of acute infantile leukemia with translocation (4; 11) (q21; q23) are reported. Although leukemia with this chromosomal abnormality has been classified as L2 acute lymphoblastic leukemia by the FAB classification, two of our cases appeared to be of myelomonocyte origin as demonstrated by cytochemical, immunologic, and electron microscopic studies and differentiation induction by 12-tetradecanoyl-phorbol-13-acetate and methylformamide. This chromosomal change is associated with poor prognosis.

The presence of translocation (t)(4; 11) was first reported by Oshimura et al. in null cell leukemia in a 27-yr-old man. Thereafter, Van den Berghe et al. reported four cases, of which two were congenital leukemia and one was found in a 7-yr-old baby. Thereafter, Prigogina reported four cases, two of which occurred in infants under 1 yr; and recently, seven cases of acute lymphoblastic leukemia (ALL) with this abnormality were reported by Bloomfield et al. Parkin et al. demonstrated that t(4; 11) acute leukemia had the cytodifferentiation, cytochemical, and electron microscopic appearance of myeloid cells. Although several neurologic and enzymologic attempts to classify acute undifferentiated leukemia have been reported, precise phenotypic analysis of t(4; 11) leukemia has not been done. We report four cases of t(4; 11) leukemia in infants and present evidence of its myelomonocytic nature.

Materials and Methods

Patients

Four patients under 1 yr of age were hospitalized in the Department of Hematology, Hyogo Prefectural Children's Hospital and the Department of Pediatrics, Kobe University School of Medicine, because of weakness, pallor, and abdominal distension. Leukemia cells from peripheral blood and bone marrow in all patients were classified into L2 ALL according to the FAB classification based on cell size, nucleoli, amount of cytoplasm, and cytochemical staining.

The duration of complete remission was very short, and two patients died of septic shock and two of systemic infiltrations of leukemia cells.

Mononuclear Cell Preparation

Mononuclear cells from peripheral blood and bone marrow were isolated by Ficoll-Hypaque density gradient centrifugation and were washed three times with a phosphate-buffered saline solution.

Cytochemical Staining

Peroxidase, Sudan black B, naphthol AS-D chloroacetate esterase, α-naphthyl esterase, α-naphthyl butyrate esterase, acid phosphatase, and periodic acid Schiff (PAS) stainings were applied to the leukemia cells.

Cytogenetic Analysis

Peripheral blood samples obtained at the time of diagnosis and relapse in case 1, at diagnosis in case 3, and at relapse in case 4 were used for cytogenetic analysis. In case 2, bone marrow cells obtained at diagnosis and relapse were used. Cells were cultured for 12–24 hr and, after exposure to 0.01 μg/ml Colcemid for 2 hr, they were treated with 75mM KCl at 37°C for 40 min and fixed with acetic acid:methanol (1:3). The slides for chromosome studies were made by the conventional air-drying method. About 30 metaphases were analyzed for modal chromosome number, and several selected metaphases were further analyzed by the Giemsa banding technique.

Surface Markers and Immunocytochemical Analysis

Spontaneous rosette formation with sheep erythrocytes (ERFC) was used as a T-cell marker. The receptor for the Fc portion of IgG and IgM (Fcγ, Fcδ) was determined by the method described by Moretta et al. The receptor for complement was detected by the formation of zymosan beads, as described by Huber and Wigzell. Cell surface immunoglobulin (sIg) was tested by direct immunofluorescence with fluorescein-conjugated rabbit anti-human Ig. Human Ia-like antigens (HLA-DR) were examined by indirect immunofluorescence with the anti-HLA-DR monoclonal antibody PTF29.12 (kindly supplied by G. Damiani, Università di Genova, Italy), and fluorescein-conjugated goat anti-rabbit γ-globulin was used as the second reagent. OKM1α (Ortho Pharmaceutical Corporation, Raritan, NJ), T1015 (Antibodies Incorporated, Davis, CA), and common ALL antigen (J5)16 (Coulter Electronics Inc., Hialeah, FL) were used as markers for monocyte, T lymphocyte, and common ALL cells, respectively, for indirect immunofluorescence.

Cytoplasmic Ig (cIg) was tested by direct immunofluorescence using fluorescein-conjugated rabbit anti-human Ig. Cytoplasmic lactoferrin (cLf) and lysozyme (cLy) were tested by indirect immunofluorescence, and rabbit anti-human lactoferrin and lysozyme serum (DAKO, Copenhagen, Denmark) were used as the first reagents, respectively. Terminal deoxynucleotidyl transferase (TdT) was examined by indirect immunofluorescence as described, using...
t(4;11) IN INFANTILE LEUKEMIA

Table 1. Hematologic and Clinical Findings in Infantile Leukemias With t(4;11)

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (mo)</th>
<th>Sex</th>
<th>Hemoglobin (g/dl)</th>
<th>White Blood Cells (10³/Liter)</th>
<th>Platelets (10³/Liter)</th>
<th>Peripheral Blood</th>
<th>Bone Marrow</th>
<th>Hepatosplenomegaly</th>
<th>Lymphadenopathy</th>
<th>CNS Involvement</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>F</td>
<td>4.8</td>
<td>25.0</td>
<td>7.7</td>
<td>92.0</td>
<td>88.2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>F</td>
<td>3.6</td>
<td>27.2</td>
<td>2.5</td>
<td>97.0</td>
<td>87.4</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>M</td>
<td>8.8</td>
<td>3.4</td>
<td>6.5</td>
<td>61.0</td>
<td>82.4</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>M</td>
<td>4.8</td>
<td>2.1</td>
<td>5.9</td>
<td>85.0</td>
<td>ND*</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Not determined.

Electron Microscopy

An aliquot of 2 x 10⁶ cells centrifuged at 900 g was immediately fixed in a mixture of glutaraldehyde, then in 2% buffered osmium. After dehydration in alcohol, they were embedded in an epoxy resin. Ultrathin sections were examined by a Hitachi HS-9 electron microscope after uranyl and lead citrate staining.

Induction of Differentiation

Leukemia cells from the peripheral blood of case 1 at relapse (total nucleated cell number was 14.4 x 10⁹/liter with 81% leukemia cells) and bone marrow aspirate of case 2 at relapse (total nucleated cell number was 49.8 x 10⁹/liter with 90.2% leukemia cells) were used. Cells (6 x 10⁶) were cultured in 2 ml medium for 2 hr and then treated with 10⁻⁸ and 10⁻⁷ M tetradecanoyl-phorbol-13-acetate (TPA) or 100 mM methylformamide (MFA) in 35-mm Petri dishes. Two days after TPA treatment, cells were washed twice in medium, then seeded again in the same dishes. The number of cells that adhered to the bottom of the tissue culture dishes was counted on the second and fourth days of seeding. Phagocytosis was determined after May-Grünwald-Giemsa staining of the control and treated cells 4 days after TPA treatment and after a 1-hr incubation in 0.5% India ink. Differential count was made under a light microscope on 200–400 cells from at least 2 independent glass slides on the fourth day.

Fig. 1. May-Grünwald-Giemsa staining of bone marrow smears from case 1(A), case 2(B), case 3(C), and case 4(D) (x 1,000).
Fig. 2. Electron microscopic features of leukemic cells. (A) Blasts in blood of case 1 at diagnosis. Nuclei are large and generally round except one with an indented nucleus (×4,000). (B) A blast cell in blood from case 2 at relapse. Note azurophil granules, an irregular nucleus with peripherally condensed chromatin, and paranuclear fibrillar arrays. (C) Fibrillar arrays (arrows) in the same cells as (B) at higher magnification. Uranyl acetate and lead citrate. ×10,000.

RESULTS

Hematology and Clinical Course

The clinical data are shown in Table I. Two patients were males and two females, and the age at onset ranged from 3 to 11 mo. The patients showed marked (cases 1 and 2) and moderate (cases 3 and 4) leukocytosis. The percentage of leukemia cells in peripheral blood and bone marrow was high (61%–97%), and they were classified as L2 lymphoblasts (Fig. 1), according to the modified FAB classification.8

Electron microscopic examination of cells in cases 1 and 2 revealed some projections on the cell surface, indented nuclei, and fibrillar arrays, suggesting that the cells were differentiated (Fig. 2). Low hemoglobin and platelet levels and hepatosplenomegaly were present in all cases. Lymphadenopathy was observed in cases 1, 2, and 3, and cerebral involvement in case 1.

Table 2. Treatment and Clinical Course of Four Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Treatment*</th>
<th>Complete Remission</th>
<th>Length of Remission (mo)</th>
<th>Total Course (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VP + MTX i.t.</td>
<td>+</td>
<td>1.5</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>DVP + l-Asp + MTX i.t. + cranial irradiation</td>
<td>+</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>VP → DVP + MTX i.t.</td>
<td>+</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>VP</td>
<td>+</td>
<td>3.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*VP, vincristine + prednisolone; MTX i.t., intrathecal methotrexate; DVP, daunorubicin + vincristine + prednisolone; l-Asp, l-Asparaginase.
Complete remission lasting for only 1.5–7 mo was obtained in all cases (Table 2). The course of leukemias after diagnosis ranged from 4 to 8 mo. Therefore, as already reported,21 t(4;11)-positive leukemia was considered to have a poor prognosis.

**Cytochemical Findings**

Leukemia cells in all four cases showed a negative peroxidase staining (Table 3). Case 2 showed positive staining with Sudan black B (Fig. 3), and cases 1, 2, and 3 gave a slightly positive nonspecific esterase reaction. PAS staining was positive in case 3 and slightly positive in cases 1 and 2. Therefore, from these cytochemical reactions, the leukemia in case 2 was considered to be nonlymphoid.

**Cytogenetic Analysis**

Chromosome analyses at different clinical stages are shown in Table 4. All cases had t(4;11) in 66.7%–90.0% of metaphases. In case 1, chromosome analyses were performed at diagnosis and relapse. The modal number of chromosomes was 46 and its karyotype was 46,XX,t(4;11),t(1;3) (Fig. 4); normal diploid cells were also found. The modal karyotype at relapse revealed another submetacentric marker chromosome of unidentified origin in 4 of 30 metaphases. In case 2,
the modal cells from the bone marrow at diagnosis and relapse revealed 46,XX,t(4;11). Case 3 showed modal cells with 45,XY,t(4;11),-14 at diagnosis. The number of chromosomes in case 4 was between 45 and 52 at relapse, and its modal karyotype was 48,XY,t(4;11) plus 2 marker chromosomes.

Although chromosome abnormality was confined to t(4;11) in case 2, the other 3 cases had additional chromosome abnormalities. In cases 1 and 2, new chromosome changes were noticed at relapse. At remission, a normal karyotype was only found in case 2; however, only case 2 was analyzed at remission.

Surface Markers and Immunocytochemical Staining

Because of negative ERFC and slg, the leukemia cells were considered to be non-T, non-B cells. Precise surface marker studies were made in cases 1 and 2 at diagnosis and relapse (Table 5). Fcy of cases 1 and 2 at relapse, and HLA-DR in these two cases at diagnosis and relapse, showed positive results: OKM1 reacted with 63.3% of the cells of case 1 at relapse. Fcy in both cases at diagnosis, and cLy in case 2 at relapse, were slightly positive. T101 and J5 did not react with blasts at diagnosis and relapse in both cases. These findings indicate that leukemia cells in cases 1 and 2 were cells of a myeloid lineage.

Induction of Differentiation

Leukemia cells from cases 1 and 2 at relapse, separated by a Ficoll-Hypaque gradient, were used for

Table 5. Surface Markers and Cytoimmunologic Staining

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnosis</td>
<td>Relapse</td>
</tr>
<tr>
<td>E</td>
<td>5.6% (-)</td>
<td>6.2% (-)</td>
</tr>
<tr>
<td>Fcy</td>
<td>13.4 (+)</td>
<td>34.0 (++)</td>
</tr>
<tr>
<td>Fcat</td>
<td>1.5 (-)</td>
<td>1.0 (-)</td>
</tr>
<tr>
<td>C3b</td>
<td>2.1 (-)</td>
<td>6.5 (-)</td>
</tr>
<tr>
<td>slg</td>
<td>2.3 (-)</td>
<td>1.0 (-)</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>83.3 (+)</td>
<td>86.1 (+)</td>
</tr>
<tr>
<td>OKM1</td>
<td>21.1 (+)</td>
<td>63.3 (+)</td>
</tr>
<tr>
<td>T101</td>
<td>5.2 (-)</td>
<td>1.8 (-)</td>
</tr>
<tr>
<td>J5</td>
<td>1.8 (-)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>cLy</td>
<td>2.3 (-)</td>
<td>1.2 (-)</td>
</tr>
<tr>
<td>cLy</td>
<td>1.1 (-)</td>
<td>1.1 (-)</td>
</tr>
<tr>
<td>TdT*</td>
<td>0.7 (-)</td>
<td>0 (-)</td>
</tr>
</tbody>
</table>

*Except for case 2 at relapse, used TdT Assay Kit:BRL.
differentiation induction studies. Most cells were leukemic blast cells. Table 6 shows the differential count, adherence of cells to the bottom of Petri dishes, and phagocytic activity following TPA treatment. Almost 80% of the cells were induced to undergo differentiation to intermediate and mature cells in both cases. Mature cells showed a monocyte-like large cytoplasm (Fig. 5A). On the fourth day of TPA treatment, 20.1% and 30.4% of cells adhered to the bottom of Petri dishes in cases 1 and 2, respectively, and the adhering cells aggregated (Fig. 5B). On the fourth day after TPA treatment, phagocytosis was found in 71% and 67% of the cells in cases 1 and 2, respectively. Cytochemical studies also revealed a weak to strong nonspecific esterase reaction. Electron microscopically, after TPA treatment, cell surface projections were increased and nuclei became smaller and more indented; cytoplasm contained azurophil granules, microfilaments, and phagolysosomes (Fig. 6). Differential counts after MFA treatment for 4 days also showed an increase in the number of mature granulocytes. MFA treatment did not cause adherence of cells to dishes, nor did it induce phagocytosis.

The findings revealed by TPA and MFA suggest that t(4;11)-positive leukemia cells are progenitor cells capable of being induced to myelomonocytes.

**DISCUSSION**

According to the report of the Third International Workshop, 19 cases of t(4;11)-positive leukemias have been reported; 10 cases in adults and 9 in children. The break points in our cases were 4q21 and 11q23, as

<table>
<thead>
<tr>
<th>Differential count*</th>
<th>Case 1 Control (%)</th>
<th>TPA (%)</th>
<th>Case 2 Control (%)</th>
<th>TPA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blast cells</td>
<td>92.0</td>
<td>20.0</td>
<td>92.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Intermediate cells</td>
<td>5.0</td>
<td>52.0</td>
<td>6.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Mature cells</td>
<td>3.0</td>
<td>28.0</td>
<td>2.0</td>
<td>33.0</td>
</tr>
<tr>
<td>Cell adherence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second day</td>
<td>5.1</td>
<td>25.6</td>
<td>0.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Fourth day</td>
<td>3.1</td>
<td>20.1</td>
<td>1.6</td>
<td>30.4</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth day</td>
<td>42.0</td>
<td>71.0</td>
<td>34.0</td>
<td>67.0</td>
</tr>
</tbody>
</table>

*Most cells separated by centrifugation were identified as blasts.
in most reported cases. Although case 2 did not reveal other chromosomal changes besides t(4;11) at diagnosis, the cells at relapse revealed additional chromosomal changes such as trisomy of chromosomes nos. 3, 8, and 10. The other 3 cases had complex chromosomal changes, such as t(1;3) in case 1, absence of one chromosome no. 14 in case 3, and 2 unidentified marker chromosomes in case 4 at diagnosis as well as at relapse.

By reviewing other reported cases, t(4;11)-positive leukemia can be classified into two groups: one an infantile type in subjects under 16 mo of age, and the other an adult type in subjects over 11 yr of age. No cases were reported between the ages of 3 and 8 yr, when common ALL with relatively good prognosis occurs frequently. The leukemia with t(4;11) is characterized by leukocytosis, poor prognosis, and blastic leukemia cells of immature nature. Parkin et al. reported that t(4;11)-positive leukemia consisted of myeloid progenitor cells and classified them into three groups: (1) monomorphic and predominantly lymphoid-appearing blasts, (2) predominantly monocytoid, and (3) heterogeneous cell population with monocytoid and large and small lymphoid blasts. Although our cases fall into group 1, in which leukemia cells showed a lymphoblastic appearance, cells in cases 1 and 2 were strongly suggestive of myelomonoblastic leukemia rather than lymphoblastic leukemia for the following reasons. First, α-naphthyl butyrate esterase was weakly positive in cases 1, 2, and 3, and Sudan black B was positive in case 2. Second, lymphocyte markers, such as ERFC, slg, clg, and cALL antigen, were all negative. Although the negative markers and the positive Ia are also traits of undifferentiated ALL cells, these cells are usually negative for Fcγ and OKM1. The last two are positive in acute monocytic leukemia (AMoL) and in undifferentiated AMoL. Our studies showed positive reactions for Fcγ in cases 1 and 2 and for OKM1 in case 1. Third, 30% of TPA-treated cells were found to adhere to the bottom of the culture dishes. These adherent cells are considered to be monocytic cells by positive nonspecific esterase reaction, increased India ink phagocytosis, and electron microscopic findings. Similarly, mature
neutrophils and partially differentiated myeloid cells increased after MFA treatment, just as macrophage-like cells or granulocytes are induced in human promyelocytic leukemia cell line (HL-60) following treatment with TPA, DMSO, and other polar compounds. Fourth, a recent report by Berger et al. showed a specific chromosomal rearrangement involving chromosome no. 11 in 5 of 7 cases of poorly differentiated AMoL. Yunis et al. also described involvement of no. 11 band q23 in one AMoL and two AMMoL cases. Kaneko et al. and Rowley et al. also reported similar results. Thus, t(11q23) involvement is commonly observed in AMoL and AMMoL, although Hage-meijer et al. pointed to a specific translocation in AMoL involving chromosome no. 9 in addition to chromosome no. 11. These results favor the view that t(4;11)-positive leukemia is of myelomonocytic origin. Infantile leukemia has a poorer prognosis than juvenile leukemia, especially in patients with extramedullary involvement. Our cases were all under 1 yr of age and presented with other high-risk factors such as low hemoglobin level, low platelet count, and hepatosplenomegaly. We consider that the presence of t(4;11) indicates cells with a highly undifferentiated nature and is associated with the poor prognosis of this leukemia.

REFERENCES

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