Possible Specific Chromosome Change in Prolymphocytic Leukemia

By Naoki Sadamori, Tin Han, Jun Minowada, Marvin L. Bloom, Edward S. Henderson, and Avery A. Sandberg

The chromosomes of unstimulated and stimulated blood lymphocytes from 5 cases with B-cell prolymphocytic leukemia (PLL) were examined following the use of polyclonal B-cell activators (PBA). Banding techniques revealed a common and specific chromosome abnormality to be present in each of the cases, which was due to a translocation between chromosomes 6 and 12 (t(6;12)(q15;p13)). The fact that this specific chromosome change has not been reported in other lymphoproliferative disorders may indicate that PLL is a distinct clinical entity and different from other lymphoproliferative disorders, whether it occurs de novo or complicates chronic lymphocytic leukemia (CLL).

Galton et al.1,2 proposed the term, prolymphocytic leukemia (PLL), in 1963, and in 1974, reported a series of 15 cases with PLL in which more precise morphological criteria for the disease were defined. According to the reported cases,3-4 PLL is a distinct clinicopathologic entity characterized by splenomegaly, minimal or absent lymph node enlargement, a pronounced leukocytosis with large numbers of circulating prolymphocytes, and is of monoclonal B- or T-cell origin. Galton et al.2 and Enno et al.5 asserted that these clinicopathologic entities are distinct and different from classical chronic lymphocytic leukemia (CLL), prolymphocytic transformation in CLL, lymphosarcoma cell leukemia, and acute lymphoblastic leukemia (ALL). On the other hand, Kjeldsberg et al.6 recently suggested that the entities of PLL, prolymphocytic transformation of CLL and Richter’s syndrome, are less distinct than had been thought previously. Thus, some argument about PLL as a separate entity exists. This article presents the chromosome characteristics of five cases with PLL, and the results are compared with the karyotypic findings in other lymphoproliferative disorders reported in the literature.

Case Reports

The diagnosis of PLL in the cases studied were based on accepted clinical and laboratory criteria, as set forth in the report by Galton et al.2 Clinical, hematologic, and immunologic data at the time of clinical and laboratory criteria, as set forth in the report by Galton et al.2 were examined following the use of polyclonal B-cell activators (PBA). Banding techniques revealed a common and specific chromosome abnormality to be present in each of the cases, which was due to a translocation between chromosomes 6 and 12 (t(6;12)(q15;p13)). The fact that this specific chromosome change has not been reported in other lymphoproliferative disorders may indicate that PLL is a distinct clinical entity and different from other lymphoproliferative disorders, whether it occurs de novo or complicates chronic lymphocytic leukemia (CLL).

Case 1

R.M., a 66-yr-old white male retired physician was found to have an elevated white blood cell count, splenomegaly, and lymphadenopathy in August 1981. He was admitted to the Medical Oncology Service at Roswell Park Memorial Institute (RPMI) on September 23, 1981. Upon physical examination, there was a 1 x 1 cm lymph node in the right axilla. The spleen was markedly enlarged, measuring 13 cm below the left costal margin. The white blood count was 90,400/μl, with over 90% small to medium sized lymphocytes and rare prolymphocytes. Bone marrow aspiration showed a hypercellular specimen infiltrated predominantly with medium-sized lymphocytes (70%). About 20% of the lymphoid cells appeared to be prolymphocytes or lymphoblasts. The diagnosis of possible PLL was made, and he was treated with intermittent high doses of chlorambucil (20-30 mg/sq m every 2 wk) and prednisone (150 mg daily for 5 days, every 4 wk) from September through December 1981. The response was minimal. Peripheral blood smear in December 1981 showed an increase in the prolymphocyte count to approximately 20%. In January 1982, he was started on cyclophosphamide (1 g/sq m i.v. on days 1 and 8, every 4 wk), vincristine (1 mg/sq m i.v. on days 1 and 8, every 4 wk), and prednisone (150 mg p.o. daily for 5 days, every 4 wk). In February 1982, the prolymphocyte count further increased to 80%-90%.

Case 2

J.Ca., a 78-yr-old, white male was diagnosed as having CLL in the summer of 1979 when he presented with lower abdominal tenderness and was found to have an enlarged spleen, as well as a high white blood cell count (26,000/μl). In July 1979, he was referred to RPMI for a second opinion and treatment. His blood findings, when he first presented at RPMI, were hemoglobin 14.2 g/dl, platelets 137,000/μl, and white blood cell count 26,000/μl with 83% mature lymphocytes. Bone marrow aspiration showed 38% lymphocytes and 12% prolymphocytes. In December 1981, it was apparent that his disease had progressed (splenomegaly of 11 cm, white blood cell count 156,000/μl, hemoglobin 11.9 g/dl, platelets 114,000/μl). Bone marrow aspiration at that time showed 46% lymphocytes and 36% prolymphocytes. He was started on intermittent high doses of chlorambucil and prednisone. He had some response to this treatment. In May 1982, he was admitted because of complaints of fatigue, weakness, and night sweats. On physical examination, he was noted to have hepatosplenomegaly (8 cm and 11 cm, respectively, below the costal margin). Hematologic examination showed hemoglobin 9.1 g/dl and WBC 85,000/μl with 82% immature lymphocytes. Bone marrow aspiration showed 10% lymphocytes and 65% prolymphocytes.

Case 3

J.Co., a 69-yr-old, white male was found to have CLL in September 1981; the disease was accidentally found when he was admitted to the hospital for a left inguinal hernia repair. On physical examination, he was noted to have generalized but minimal lymphadenopathy. His laboratory workup showed a white blood cell count of 88,000/μl, mainly lymphocytes. He also had massive splenomegaly.
ly. Node biopsy done at that time was consistent with non-Hodgkin's lymphoma. This was felt to be a tissue manifestation of CLL. In November 1981, his hemoglobin was 9.7 g/dl, platelets 118,000/µl, and WBC of 133,700/µl. Since then, he has been treated with intermittent chlorambucil, which was later changed to cyclophosphamide and prednisone; however, the disease progressed despite this treatment. The patient returned in May 1982 with huge splenomegaly (18 cm), a white blood count of more than 200,000/µl, anemia, and thrombocytopenia. Bone marrow aspiration showed 23% lymphocytes and 70% prolymphocytes. The patient underwent splenectomy on May 13, 1982. The pathologic findings of the spleen were consistent with CLL. After splenectomy, the patient's count gradually improved, with a thrombocyte count of around 60,000/µl. The patient was started on a cyclophosphamide, vincristine, and prednisone regimen on the basis of review of slides, which showed a predominant population of prolymphocytes.

Case 4

M.A., a 72-yr-old, white female presented in May 1982 complaining of general weakness, perspiration, and weight loss of about 4 kg over the past month. She was found to have CLL and was referred to RPMI for evaluation of the disease. On physical examination, she was noted to have splenomegaly (5 fingerbreadths below the left costal margin), but no lymphadenopathy or hepatomegaly. Her hematologic findings were hemoglobin 8.7 g/dl, platelets 120,000/µl, and white blood cell count 246,000/µl with 94% prolymphocytes (Fig. 1). Bone marrow aspiration showed 90% prolymphocytes and

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Table 1. Clinical, Hematologic, and Immunologic Data at the Time of Cytogenetic Examination

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex</th>
<th>Date</th>
<th>Hb (g/dl)</th>
<th>Platelets (x 10³/µl)</th>
<th>WBC (x 10³/µl)</th>
<th>Prolymphocytes (%)</th>
<th>Lymphocytes (%)</th>
<th>Treatment Before Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) R.M.</td>
<td>66/M</td>
<td>9/24/81</td>
<td>10.2</td>
<td>135</td>
<td>90.4</td>
<td>(Proly. + Ly. = 93)</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>(2) J.Ca.</td>
<td>77/M</td>
<td>4/2/81</td>
<td>14.1</td>
<td>133</td>
<td>36.0</td>
<td>76</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>(3) J.Co.</td>
<td>69/M</td>
<td>5/13/82</td>
<td>9.1</td>
<td>109</td>
<td>63.5</td>
<td>50</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>(4) M.A.</td>
<td>72/F</td>
<td>7/19/82</td>
<td>8.7</td>
<td>120</td>
<td>246.4</td>
<td>70</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>(5) E.P.</td>
<td>87/F</td>
<td>8/19/82</td>
<td>13.3</td>
<td>210</td>
<td>35.0</td>
<td>39</td>
<td>54</td>
<td>1</td>
</tr>
</tbody>
</table>

*Not done.

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Fig. 1. Blood smear showing prolymphocytes with the indented and cleft nuclei characteristic of PLL and a chromatin structure that is intermediate between that of lymphoblasts and mature lymphocytes.
only 2% lymphocytes. The patient had not received any therapy at the time of the cytogenetic examination.

Case 5

E.P., an 87-yr-old, white female was seen on August 19, 1982. She had been referred because of profound progressive weakness, coldness of the extremities, and intermittent dyspnea. On physical examination, she was noted to have hepatosplenomegaly (5 cm and 5 cm, respectively), but no lymphadenopathy. Hematologic examination showed hemoglobin 13.3 g/dl, platelets 210,000/µl, and white blood cell count 35,000/µl with 39% prolymphocytes. The bone marrow sample was hypercellular, mainly due to dense lymphocytic proliferation. Tartrate-resistant acid phosphatase reactivity of the lymphoid cells was negative. Electron microscopy did not show hairy cells. The patient had not received any therapy at the time of cytogenetic examination.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date</th>
<th>Source</th>
<th>Total Number of Metaphases Analyzed</th>
<th>Abnormal Metaphases in Abnormal Clones</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>9/24/81</td>
<td>Blood</td>
<td>20</td>
<td>19</td>
<td>48,XY,+t(6;12)(q15;p13),+12,−13,+mar,t(1:?)(p36;?),t(16;6;14;16;q21;q32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cowan I</td>
<td>9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PWM</td>
<td>29</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EBV</td>
<td>40</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LPS</td>
<td>40</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td>4/2/81</td>
<td>Blood</td>
<td>5</td>
<td>0</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PWM</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EBV</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>5/13/82</td>
<td>Spleen</td>
<td>10</td>
<td>10</td>
<td>44,XY,−2,−17,t(6;12)(q15;p13)</td>
</tr>
<tr>
<td></td>
<td>5/13/82</td>
<td>Lymphnode</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/27/82</td>
<td>Blood</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>(4)</td>
<td>7/19/82</td>
<td>Marrow</td>
<td>4</td>
<td>3</td>
<td>43, X, −X, +t(6;12)(q15;p13), −17, −18, −22, +mar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cowan I</td>
<td>47</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PWM</td>
<td>15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EBV</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LPS</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>(5)</td>
<td>8/19/82</td>
<td>Blood</td>
<td>3</td>
<td>1</td>
<td>45,XX, −6, −17, +r(?),t(5;16)(q22;p13),t(6;12)(q15;p13),t(16;19)(p11;p13)</td>
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<tr>
<td></td>
<td></td>
<td>Cowan I</td>
<td>31</td>
<td>7.2*</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>PPP</td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EBV</td>
<td>33</td>
<td>25.3*</td>
<td></td>
</tr>
</tbody>
</table>

In each case, the asterisks indicate the number of cells with a particular karyotype.

NA, no activator; Cowan I, Staphylococcus bacteria strain Cowan I; PWM, pokeweed mitogen; EBV, Epstein-Barr virus; LPS, lipopolysaccharide W from E. coli.
bacteria strain Cowan I protein (Cowan I, 100 µg/ml) (Calbiochem-Behring Corp., La Jolla, CA), pokeweed mitogen (PWM; Grand Island Biochemical Co., Grand Island, NY) at a dilution of 1:100, Epstein-Barr virus (EBV; supernatant from an EBV-producing permanent cell line B-95-8 at 1:9 v/v of culture) after filtration through 0.22-µ Millipore filters and lipopolysaccharide W from E. coli 055:B5 (LPS, 40 µg/ml; Difco Laboratories, Detroit, MI) as the so-called polyclonal B-cell activators (PBA), as described by Gahrton and Robert,\textsuperscript{9} at 37°C in an atmosphere of 5% CO\textsubscript{2}. Usually, 5 separate 10-ml cultures (4 cultures with each PBA and 1 without any activator) were set up for each sample. The cells were harvested after 3 days in culture. Colcemid was added 2 hr before harvest. Karyotype analyses were performed with G- and Q-bandning techniques.\textsuperscript{19}

**CYTOGENETIC RESULTS**

Table 2 presents the cytogenetic data only from samples that contained metaphases. The number of adequate metaphases varied, depending on the case, sample, and PBA. In only a few samples were metaphases seen in the absence of an activator. On the other hand, PBA readily stimulated the cells in PLL.

All five cases showed complex chromosome abnormalities, although at the first examination of case 2, a normal karyotype was found. Four of the five cases were hypodiploid. Chromosomes Y, 8, 17, 21, and 22 in case 2, chromosomes 2 and 17 in case 3, chromosomes X, 8, 17, 18, and 22 in case 4, and chromosomes 6 and 17 in case 5 were missing. In particular, monosomy-17 was seen in four of the five cases. As reported previously,\textsuperscript{7} and shown in Fig. 2, case 1 was hyperdiploid with trisomy-12, 6q−, 1q+q, t(16;12), −13 and a marker chromosome. Case 5 showed complex structural abnormalities, such as t(5;16), t(6;19) and a ring chromosome besides t(6;12), as shown in Fig. 3.

It is interesting to note that all five cases in the present study were associated with a unique chromosome that was likely caused by t(6;12), as shown in Figs. 2 and 3. Though it was difficult to determine rigorously the breakpoints of these chromosomes in each case, the karyotypic change most likely was due to t(6;12) (q15;p13). Even though we originally thought, on the basis of the material in cases 1–4, that the marker chromosome was a +1p−, the chromosomal material in case 2 afforded us the opportunity to examine the bands of the abnormal chromosome at a higher resolution than in the above-mentioned cases and led us to believe that t(6;12) was the most likely origin of this marker. In cases 3 and 4, terminal deletions of the long arm of the involved chromosome 12, with the material being translocated to a chromosome 6, may have occurred secondarily.

**DISCUSSION**

Prolymphocytic leukemia (PLL) is a distinct clinicopathologic entity based on a distinctive morphology of the cells termed, “prolymphocytes,” first described by Galton et al.\textsuperscript{1,2} Peripheral blood or bone marrow smears usually reveal a uniform population of large lymphoid cells with round nuclei, single prominent nuclei that are large and vesicular, abundant cytoplasm, and nuclear chromatin coarser than that of lymphoblasts but finer than that of lymphocytes. These prolymphocytes can be easily differentiated from L1, L2, and L3 lymphoblasts of ALL, described by the FAB classification.\textsuperscript{11} Lymphoblasts of L1 type from patients with ALL are small, the amount of cytoplasm is scanty, and the nucleoli are usually small and inconspicuous; those of the L2 type of ALL are large but heterogeneous in size, with nuclei that are usually irregular with clefting and indentation. Lymphoblasts of the L3 type from patients with Burkitt-type leukemia or lymphoma are uniformly large with finely stippled nuclear chromatin, very deeply basophilic cytoplasm, and cytoplasmic vacuolation.

The presence of consistent cytogenetic changes has contributed much to the establishment of entities within blood diseases, such as t(9;22) in chronic myelocytic leukemia,\textsuperscript{10} t(8;21) in acute myeloblastic leukemia,\textsuperscript{12} t(15;17) in acute promyelocytic leukemia,\textsuperscript{13} and +12 in CLL.\textsuperscript{9}

As far as we know, no cytogenetic reports on B-cell PLL have appeared. We think that this may be primarily due to its rarity and very low spontaneous mitotic index, as shown in Table 2; in addition, the clinicopathologic entity of PLL remains unclear, as discussed before.

From the present studies it appears that PBA can readily stimulate the leukemic cells of PLL, thus affording an opportunity to examine the cells cytogenetically. In case 2, the first examination before the disease progressed showed a normal karyotype, whereas the second examination after progression revealed an abnormal clone. It is possible that in the first study, PBA stimulated only normal B cells, normal T cells, and/or leukemic cells with a normal karyotype. Interestingly, the immunoglobulin isotype changed from κμ to κγ (Table 1). These findings indicate a possibility that a small group of cells with κγ and an abnormal karyotype existed at the first examination but were not in division and, hence, chromosome abnormalities could not be detected; however, these cells became the major group at the second study.

As shown in Figs. 2 and 3, all five cases showed a unique marker chromosome. Even though the pathogenesis of this marker chromosomes could not be determined exactly, they were probably caused by t(6;12). In fact, we originally interpreted the marker chromosome as being a +1p−, but further studies and
Fig. 3. Karyotype of case 5: arrows point to numerical and structural abnormalities (see Table 1 for details). The structural changes include the t(6;12) (q15;p13) observed in all 5 cases of PLL reported in this article. Other morphological abnormalities were: t(5;16) (q22;p13), t(6;19) (p11;q13), and a ring chromosome. The numerical changes consisted of −6 and −17.
optimal chromosome material in an additional patient (case 5) led us to believe that the translocation described by us is the most likely explanation for this cytogenetic change in PLL and may constitute another specific change in leukemia, joining the list of other leukemias. As far as we know, no cases of a lymphoproliferative disorder with t(6;12) (q15;p13) have been reported.

Even though hyperdiploidy has been observed in most cases of lymphoproliferative disorders with chromosome abnormalities, four of the five patients (cases 2, 3, 4, and 5) in this study showed hypodiploidy caused by missing chromosomes of variable nature, i.e., X, Y, 2, 6, 8, 17, 18, 21, and 22. Among the missing chromosomes, chromosome 17 was involved in 4 cases. Monosomy 17 is seen in malignant lymphoma, ALL, and CLL. This may indicate that the abnormalities of chromosome 17 cannot distinguish PLL from other lymphoproliferative disorders, even though a chromosome 17 anomaly might be seen often in PLL.

Identified chromosome abnormalities (besides the missing ones), i.e., trisomy-12, 6q−, and 14q+ in case 1, 13q+ in case 2, and t(5;16) and t(6;19) in case 5, were seen. Trisomy-12 is most often seen in CLL, 6q− and 14q+ in the various lymphoproliferative disorders and 13q+ in malignant lymphoma and in ALL. Strictly speaking, these abnormal chromosomes cannot distinguish PLL from other lymphoproliferative disorders. The translocations (5;16) and (6;19) in case 5 were not seen in the other four cases of this study. Of interest is case 1, who showed hyperdiploidy (48 chromosomes) with trisomy-12 in addition to the t(6;9), raising the possibility that this patient had CLL with prolymphocytic transformation, inasmuch as +12 is the most common karyotypic change in CLL.

Thus, t(6;12) (present in all five patients studied by us) and possibly hypodiploidy (present in four of the five cases presented) in PLL suggest that this leukemia may be a distinct entity, whether it occurs "de novo" or complicating CLL, with the karyotypic change t(6;12) characterizing leukemic cells of prolymphocytic nature. However, more cases of PLL (and CLL) will have to be studied before a final conclusion can be reached.

NOTE ADDED IN PROOF

In a recent paper (Pittman S, Catovsky D: Chromosome abnormalities in B-cell prolymphocytic leukemia: A study of nine cases. Cancer Genet Cytogenet 9:355-365, 1983), which appeared while our report was in press, are presented the cytogenetic findings in 9 cases of B-cell CLL. The most common abnormality involved chromosome 14 (14q+ in 7 cases), followed by chromosome 6 (6 cases with 6q−) and chromosome 1 (6 cases). No consistent or specific karyotypic change, including t(6;12), was described.

ACKNOWLEDGMENT

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REFERENCES

17. Kaneko Y, Hayashi Y, Sakurai M: Chromosomal findings


Possible specific chromosome change in prolymphocytic leukemia

N Sadamori, T Han, J Minowada, ML Bloom, ES Henderson and AA Sandberg