MULTIPARAMETER STUDIES of acute lymphoblastic leukemia (ALL) include the use of clinical, morphological, cytochemical, immunologic, and cytogenetic criteria. Chromosome analysis is important in providing evidence regarding the origin and evolution of leukemic cells. The discovery of the Philadelphia chromosome (Ph') has been a milestone for cytogenetics of hematologic malignancies. Although the Ph' was originally considered relatively specific for chronic myelogenous leukemia (CML), it is now recognized as occurring in various acute leukemias without an antecedent chronic phase. Recently, the Ph' has received much attention, not only because it directs our insights to a stream of stem cell differentiation, but also because its presence has been indicated as a prognostic factor in acute leukemia. On the other hand, an abnormality of chromosome 14, which consists of extra bands at the end of the long arm (14q+), is frequently found in Burkitt's lymphoma and in other types of malignant lymphomas, pre-B ALL, B cell ALL, multiple myeloma, plasma cell leukemia, and adult T cell leukemia in Japan. Therefore, the 14q+ abnormality has been considered to provide lymphocytes, especially those of B-cell lineage, with a malignant proliferative advantage. We have recently studied the karyotype of a patient whose leukemic cells were non-T, non-B type and found both a Ph' and a 14q+ in the same clone.

CASE REPORT
A 13-yr-old Japanese female was admitted on December 5, 1981 with a 2-wk history of general malaise, sore throat, and low-grade fever. Swollen bilateral cervical and inguinal lymph nodes were felt. Examination of her abdomen showed an elastic hard, tender, smooth liver edge descended 2 cm below the right costal margin. No splenomegaly was observed. Her hemoglobin was 11.4 g/dl, white blood cell count 83.5 x 10^9/liter, and platelet count 71.0 x 10^9/liter. The peripheral blood (PB) smears revealed 88% leukemic cells. A sternal bone marrow (BM) aspirate was markedly hypercellular, with 92.8% leukemic cells. Leukemic cells were more than twice as large as small lymphocytes and showed heterogeneity in cell size. The nuclear chromatin was finely dispersed. Nuclear clefting was present, and there were one or more nucleoli. The cytoplasm was basophilic and relatively abundant (Fig. 1). Leukemic cells were positive with periodic acid-Schiff (PAS) reaction. Peroxidase and nonspecific esterase reactions were absent. Therefore, the leukemic cells were compatible with the L2 type, according to the FAB classification. The patient was treated with vincristine (VCR) and prednisolone. A complete remission was achieved on the 26th hospital day; during complete remission, there was no basophilia or eosinophilia, the neutrophil alkaline phosphatase was normal, and serum vitamin B12 was 538.4 pg/ml. After 20 days of maintenance therapy with 6mercaptopurine (6-MP) and methotrexate (MTX), 8.4% leukemic cells were observed in a bone marrow aspirate. Although the patient was treated with a reinduction regimen, consisting of VCR, prednisolone, l-asparaginase, adriamycin, cytosine arabinoside, 6-MP, and MTX, leukemic cells were quite resistant to therapy and it has been difficult to induce complete remission. On June 24th, 1982, she was transferred to another institution to receive an allogeneic bone marrow transplantation.

MATERIALS AND METHODS
Chromosome Studies
Chromosome studies of leukemic cells were performed prior to therapy and throughout the clinical course of this patient. BM cells were cultured for 24 hr at 37°C without phytohemagglutinin (PHA). Metaphase cells were harvested by adding colcemid (GIBCO Diagnostics, Grand Island, NY) 2 hr before fixation. After treatment with hypotonic KCl (0.07 M) for 20 min, the cells were fixed in methanol:acetic acid (3:1), dropped onto glass slides, and flame dried. After incubating at 37°C for 1 wk, the cells were G-banded by the trypsin-Giemsa technique. Metaphases were photographed and then arranged according to the ISCN nomenclature. During complete remission, PB cells were cultured for 72 hr with PHA (HA 15, Wellcome Research Lab., Dartford, U.K.) to examine her constitutional karyotype and treated as described above.

Immunologic Studies
Immunologic studies of cell surface phenotype were performed prior to therapy. The mononuclear cells were separated from a heparinized BM specimen by Ficoll-Hypaque density gradient centrifugation. T cell marker studies included spontaneous rosette formation with neuraminidase-treated sheep erythrocytes as well as examination of OKT3, OKT4, OKT6, OKT8 (Ortho Pharmaceutical Co., Raritan, NJ) reactivity. To detect human Ia-like antigen, OKIa-1 (Ortho) reactivity was examined by indirect immunofluorescence methods, using a fluorescein isothiocyanate (FITC) labeled...
RESULTS

Prior to therapy, cytogenetic analysis was performed on 91 cells. The modal chromosome number was 46. A G-banded karyotype is shown in Fig. 2. The Ph chromosome resulted from a standard translocation, t(9;22)(q34;q11), and the 14q + chromosome resulted from an apparent reciprocal translocation, t(7;14)(p12;q32). There were no metaphase cells with only a Ph or only a 14q + abnormality. No normal cells were observed. On complete remission, all 30 metaphases analyzed revealed a normal karyotype. During complete remission, PB cultured with PHA showed a normal female karyotype (32 cells analyzed). BM specimen taken on the 46th hospital day contained 8.4% leukemic cells. One abnormal metaphase cell with a 47,XX,Ph,14q+,+mar karyotype was found among 47 analyzed metaphases; the remaining cells were normal. An unidentifiable marker chromosome is shown in the inset of Fig. 2. After relapse, cytogenetic examinations performed on the 88th, 129th, and 151st hospital day revealed the coexistence of three cell populations: 46,XX/46,XX,Ph,14q+/47,XX,Ph,14q++,+mar. These results are summarized in Table 1.
Immunologic studies were performed prior to therapy. The specimen obtained from BM contained 92.8% leukemic cells, of which 0.3% formed E rosettes, 0.4% were SIg-positive, 0.4% were ClgM-positive, 52.1% were Ia-positive, and 73.8% were TdT-positive. These results were consistent with a non-T, non-B phenotype. No metaphases, however, could be found that had only a Ph' or a 14q+. The specimen obtained from BM contained 92.8% leukemic cells, of which 0.3% formed E rosettes, 0.4% were SIg-positive, 0.4% were ClgM-positive, 52.1% were Ia-positive, and 73.8% were TdT-positive. These cells were negative with OKT3, OKT4, OKT6, and OKT8. The results were consistent with a non-T, non-B phenotype (E , OKT3 , OKT4 , OKT6 , OKT8 , SIg , ClgM , Ia' , TdT- ).

DISCUSSION

The case presented here has both Ph' and 14q+ abnormalities, and the affected lymphoblasts have a non-T, non-B phenotype. No metaphases, however, could be found that had only a Ph' or a 14q+. The abnormal karyotype disappeared during complete remission. After relapse there has been coexistence of three different karyotypes: 46,XX/46,XX,Ph',14q +/47,XX,Ph',14q+,+mar. Recently, it has become apparent that the Ph', which had originally been considered to be relatively specific for CML, can be found in ALL,17 AML,2 and preleukemia.18 Moreover, leukemic cells during the blast crisis of CML (CML-BC) can have lymphoblastic, erythroleukemic, and monoclonal characteristics. Furthermore, CML can occasionally progress to a basophilic and eosinophilic crisis. In approximately one-third of cases, immunologic studies have revealed a lymphoid origin of leukemic cells of CML-BC.19 In some cases, these leukemic cells have a pre-B20 and a T cell phenotype.21 CML is now considered to be a pluripotent stem cell disease, based on work using glucose-6-phosphate dehydrogenase isoenzyme cellular mosaicism.22 With regard to differences between the blast phase of CML and Ph' -positive acute leukemia without antecedent chronic phase, some investigators explain that, in Ph'-positive acute leukemias, basophilia and splenomegaly could not be observed during the whole clinical course. The Ph' clone usually disappeared or decreased after successful treatment in cases of Ph'-positive acute leukemia, while these phenomena could not be found in CML, except in a few cases.23 However, at present, the relationship between Ph'-positive acute leukemia and CML remains an important unresolved problem.24

In approximately 5% of ALL patients, the origin of the material translocated to 14q was some chromosome other than chromosome 8.25 This case shows involved chromosome 7 as the translocated material to 14q. In patients with ataxia-telangiectasia (AT), specific chromosome rearrangements involving chromosomes 7 and 14 have been frequently reported. A 7;14 chromosome translocation has also been found in lymphocytes from AT patients. These breakpoints are located in 7p14, 7q35, and 14q12 → 14qter.26 The case presented here has no signs of AT, and the 14q+ chromosome abnormality resulted from the translocation, t(7;14)(p12;q32). One AT patient who developed chronic lymphocytic leukemia has been reported.27 Considering an increased incidence of lymphoid malignancies in AT patients, this translocation is quite suggestive of a malignant process in the present case.

The findings of a marker chromosome 14q+ in a case of Ph'-positive CML was recently reported.28 Further cases will be needed to solve the riddle of the presence both of a Ph' and a 14q+ in the same clone.

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Table 1. Serial Cytogenetic Results During the Patient’s Clinical Course

<table>
<thead>
<tr>
<th>Hospital Day</th>
<th>Percent of Leukemic Cells BM</th>
<th>Metaphases Analyzed</th>
<th>Karyotype (BM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>92.8</td>
<td>91</td>
<td>46,XX,Ph',14q +</td>
</tr>
<tr>
<td>26 (CR)*</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>46</td>
<td>8.4</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>88</td>
<td>32.4</td>
<td>33</td>
<td>1</td>
</tr>
<tr>
<td>129</td>
<td>94.8</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>151</td>
<td>94.0</td>
<td>31</td>
<td>3</td>
</tr>
</tbody>
</table>

*CR, complete remission.
†PB cultured with PHA revealed a normal female karyotype (32 cells analyzed).


Ph1-positive acute lymphoblastic leukemia with a 14q+ chromosome abnormality

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