B Cell Acute Lymphoblastic Leukemia (ALL) With a 14q+ Chromosome Abnormality

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An adult patient with acute lymphoblastic leukemia associated with a 14q+ marker chromosome is presented. The abnormality resulted from a translocation of material from the long arm of chromosome 11. The leukemic cells were found to be B cells on the basis of surface immunoglobulins, lack of receptors for sheep erythrocytes, and a characteristically low level of adenosine deaminase activity. In other patients with ALL studied by us or reported by others in whom chromosome banding was done, a 14q+ chromosome was present in only one instance, also a case of B cell ALL. These two cases are the only examples of B cell ALL associated with a 14q+ marker chromosome studied with chromosome banding reported to date. The frequent occurrence of a 14q+ chromosome in other malignant lymphoproliferative diseases of B cell origin suggests that a general association may exist between the 14q+ abnormality and B cell neoplasms. Cytogenetic analysis may therefore be useful in defining subtypes of ALL and in relating specific chromosomal abnormalities to lymphoproliferative disorders.

CYTOGENETIC ANALYSIS with banding techniques has revealed patterns of chromosomal abnormalities that are distinctive for certain types of nonlymphocytic leukemia. In addition to the 9;22 translocation of the Philadelphia chromosome in chronic granulocytic leukemia, a translocation involving chromosomes 15 and 17 has been identified in patients with acute promyelocytic leukemia. In a series of ten patients who developed acute leukemia after therapy for lymphoma, deletion of a B group chromosome was found in nine; this abnormality is uncommon in acute nonlymphocytic leukemia arising de novo.

In acute lymphoblastic leukemia (ALL), no similar correlations of cytogenetic findings with specific clinical or morphologic variants have been identified. Recent studies have shown, however, that subtypes of ALL can be defined on the basis of cell surface properties and of certain enzymatic activities.

We propose, in agreement with Minowada et al., that ALL of B cell origin may be associated with an abnormality of chromosome 14 (14q+), which has previously been described as the most common chromosome abnormality in malignant lymphoma. In the following case report, the 14q+ marker chromosome was present in lymphoblasts from a patient with ALL. The circulating leukemic cells had surface immunoglobulin as well as a low level of adenosine deaminase activity, characteristic of B cells. In the only other reported case of B cell ALL examined...
with chromosome banding techniques, the 14q+ abnormality was also found. A preliminary report of this work has been presented.9

CASE REPORT

A 33-yr-old white male was admitted to the University of Chicago Hospitals in August 1977 with symptoms of fever, night sweats, bleeding gums, and hemoptysis for the previous 2 wk. Retinal hemorrhages and petechiae of the lower extremities were present on physical examination. Multiple 1–2-cm lymph nodes were palpable in cervical, axillary, and inguinal sites. The liver span was 15 cm, and the spleen was palpable 2 cm below the left costal margin.

The hemoglobin level on admission was 9.7 g/dl, hematocrit 27%, platelets 34,000/μl, white cell count 20,400, with 42% lymphoblasts, 29% lymphocytes, 9% neutrophils, 7% bands, 5% myelocytes, 1% promyelocytes, 5% monocytes, 1% eosinophils, 1% basophils, and 2 nucleated red cells/100 WBC. Histochemical stains of a buffy coat smear for acid phosphatase and with Sudan black were negative. Cerebrospinal fluid examination was normal. Chest x-ray findings were also normal, with no evidence of a mediastinal mass.

Bone marrow aspiration was a dry tap, and a core biopsy confirmed the diagnosis of ALL (Fig. 1). In the bone core biopsy specimen, the marrow was diffusely replaced by large lymphoid cells with a prominent nuclear membrane. The majority of cells had a coarse chromatin pattern. Occasional cells showed a prominent pink nucleolus. Some of the neoplastic cells had an irregular and somewhat convoluted nucleus. Mitoses were frequently seen. In addition to replacement by blasts, increased reticulin was present, which was thought to account for the leukoerythroblastic changes in the blood. Peroxidase, PAS, and α-naphthylbutyrate esterase stains of the marrow blasts were negative.

When chemotherapy with vincristine and prednisone was begun, there was a prompt decrease in the white cell count, and the lymphadenopathy and hepatosplenomegaly disappeared. Lymphoblasts and the
leukoerythroblastic changes were no longer present in peripheral blood smears, but bone marrow samples showed persistence of leukemia during 10 wk of vinristine-prednisone therapy.

In October 1977 involvement of the central nervous system by leukemia occurred. Intrathecal methotrexate was begun, and daunorubicin was added to the systemic chemotherapy. A complete remission of the bone marrow and CNS was documented 3 wk later.

Consolidation therapy with daunorubicin and cytosine arabinoside (ara-c) was instituted, but leukemia recurred rapidly in the CNS and was quickly followed by bone marrow relapse. Cranial radiotherapy provided control of neurologic symptoms, but leukemia persisted in the marrow despite chemotherapy with three courses of 6-mercaptopurine, ara-c, and high-dose methotrexate with folic acid rescue and later with cyclophosphamide, vincristine, ara-c, and prednisone (COAP). In March 1978 diffuse interstitial pneumonia developed. Bronchial brushing demonstrated Pneumocystis carinii and a fungal organism that could not be further identified. Blood cultures were positive for Candida species. Despite treatment with appropriate antimicrobial drugs, the patient died with the clinical findings of septic shock.

MATERIALS AND METHODS

Chromosome studies. Cytogenetic analyses were performed on peripheral blood leukocytes obtained prior to therapy (8/2/77), on bone marrow samples during remission (11/1/77) and during the early stages of relapse (12/1/77), and on bone core biopsies after relapse (12/28/77 and 1/6/78). Peripheral blood was cultured for 24 hr without phytohemagglutinin (PHA) and for 72 hr with PHA. Bone marrow was studied directly, after 24-hr culture, or both. Bone core samples were minced in RPMI-1640 medium and cultured for 24 hr without PHA in the same manner as bone marrow. Analyzable metaphases were photographed after Giemsa staining. After destaining, the slides were restained with quinacrine mustard, and the same metaphases were rephotographed with fluorescence. Karyotypes were analyzed by comparison of Giemsa-stained and Q-banded photographs of the same cell.

Some cells from the initial sample were also studied with C banding after routine Giemsa staining both with and without intervening Q banding. Other cells were studied after Q banding with R banding using acridine orange.

Cell surface markers. Leukemic cells were purified on Ficoll-Hypaque gradients and tested for surface immunoglobulins and E-rosette formation. Fluorescein-conjugated polyvalent antiserum (Meloy Laboratories, Springfield, Va.) was diluted 1:4 with phosphate-buffered saline (PBS) and filtered to remove aggregates. An aliquot of $5 \times 10^6$ cells was suspended in 0.1 ml diluted antiserum and incubated at room temperature for 30 min. The cells were rinsed three times in Hanks' balanced salt solution and examined with an American Optical fluorescent microscope, and the percentage of fluorescing cells was calculated.

A second aliquot of $5 \times 10^6$ cells was suspended in 0.4 ml absorbed fetal calf serum and 0.4 ml of a 0.5% solution of washed sheep red blood cells in PBS was added. The sample was incubated at 37°C for 30 min, centrifuged at 200 g for 5 min, and placed on ice for 2 hr. The cells were gently resuspended and placed in a hemocytometer. One hundred cells were counted; those with three or more adhering erythrocytes were counted as E rosettes.

Adenosine deaminase (ADA) assay. Leukemic cells purified from peripheral blood on Ficoll-Hypaque gradients were used for the enzymatic assay. Cell pellets were frozen at −80°C, and extracts were prepared just prior to assay. Cells were disrupted by rapid thawing at a final concentration of $10^8$ cells/ml in 50 mM potassium phosphate pH 7.6, 2.5 mM mercaptoethanol, 0.1 mM EDTA, 6 mM magnesium chloride, and 0.3 mM ATP. The extract was stirred at 4°C for 30 min, and insoluble material was removed by centrifugation at 12,000 g for 10 min.

The assay measures adenosine-dependent production of ammonia by a colorimetric method. Samples contained 1 µmole adenosine (PL Biochemicals, Milwaukee, Wisc.) and 2.5 µmole barbital buffer pH 7.5 in a final volume of 0.4 ml. After incubation at 37°C for 20 min, the reaction was stopped by adding 0.85 ml 1 mM sodium nitroprusside in 5% (w/v) phenol. Color development with bleaching reagent (12.4 ml 4-6% sodium hypochlorite per 100 ml 0.6 N NaOH) was carried out at 55°C for 50 min.

The ammonia concentration was determined by measuring the optical density at 625 nm in a Zeiss spectrophotometer, using ammonium chloride as the standard. Assays were routinely performed with
Table 1. Cytogenetic Studies of Samples During the Course of the Disease in a Patient With B Cell ALL

<table>
<thead>
<tr>
<th>Date</th>
<th>Clinical Status</th>
<th>Source</th>
<th>No. of Cells</th>
<th>Chromosome Number§</th>
<th>Karyotype</th>
<th>Percentage Abnormal%</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/02/77</td>
<td>AC</td>
<td>PB</td>
<td>24</td>
<td>Q R C</td>
<td>≤ 45 46 47 48 49 50 ≥ 51</td>
<td>49,X,Y,+7,+12,+13, &lt;p,+&lt;p,der(13)t(1;13), t(6;18)t(11;14)/50, XY.same,+20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>1(11) 6(6**)</td>
<td>5(4) 9(4) 10(1)</td>
<td>46,X,Y/49,X,Y, as above/50,X,Y, as above</td>
</tr>
<tr>
<td>11/01/77</td>
<td>CR</td>
<td>BM</td>
<td>d24</td>
<td>12 23</td>
<td>7(3** 15(8**) 1(1)</td>
<td>46,X,Y/49,X,Y, as above, but with second der(13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/01/77</td>
<td>RL</td>
<td>BM</td>
<td>d</td>
<td>16 25</td>
<td>22(15**) 1(1)</td>
<td>46,X,Y/49,X,Y, as above, but with +20</td>
</tr>
<tr>
<td>1/06/78</td>
<td>RL</td>
<td>BC</td>
<td>24</td>
<td>9 11</td>
<td>6(5) 3(3) 2(1)</td>
<td>50,X,Y, as above†</td>
</tr>
</tbody>
</table>

* AC, active disease (initial episode). CR, complete remission. RL, relapse.
† PB, peripheral blood. BM, bone marrow. BC, bone core.
‡ 24, 24-hr culture without PHA. 72, 72-hr culture with PHA. d, direct processing.
§ Number in parentheses, number of banded cells.
∥ Banded cells only.
† One cell normal, or broken normal.
** All cells normal.
†† Owing to poor quality of this material, no cell was completely analyzable, however, all scorable cells displayed various of the rearrangements seen in previous samples.
duplicate samples of three different levels of extract. Duplicate blank samples for each extract level were also included and were prepared by adding the phenol/nitroprusside reagent prior to the extract.

Protein was measured by the Lowry method, with bovine albumin as the standard.

RESULTS

The results of the cytogenetic analyses of four samples are shown in Table 1. The fifth sample, that of 12/28/77, provided no analyzable metaphases and is excluded from the table. Thirty-nine cells from the pretreatment sample (8/2/77) were analyzed with banding techniques (Fig. 2). Only two cells had a normal karyotype; for the others, a bimodal chromosome number of 49 and 50 was obtained. The karyotype of the 49-chromosome cell line was: 49,XY,+7,+12,-13, +9p+,+der(13),t(1;13)(q12.2;p13),t(6;18)(p25;q21),t(11;14)(q23;q32). The 50-chromosome cell line evolved from this line by the addition of one No. 20 (Fig. 2). Further clonal evolution involved additions of one No. 22 and of a second der(13) chromosome.

The identification of the material translocated to the No. 14 to form the 14q+ marker chromosome was initially determined with Q banding and subsequently verified with R banding. The partial karyotype (Fig. 3) of normal and rearranged

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**Fig. 2.** Q-banded chromosomes of a cell from the pretreatment sample of the patient (8/2/77), from a 24-hr peripheral blood culture without PHA. This cell represents the second stage of clonal evolution, with the addition of a third No. 20 to the initial abnormal cell line of 49 chromosomes. One No. 21 is missing due to random loss. Arrows indicate the translocation between a No. 11 and a No. 14 to produce the 14q+ marker. Karyotype of this cell is 49,XY,+7,+12,-13,-20,-21,+9p+,+der(13), t(1;13)(q12.2;p13),t(6;18)(p25;q21),t(11;14)(q23;q32). Second No. 9 shows unusual banding morphology, which was observed only in this cell. Number 9 chromosomes, including the additional 9p+, from another cell are shown in the inset.
Nos. 11 and 14 from the initial sample showed a narrow, darkly staining R-banded region on No. 11q. Whether this is 14q32 or a portion of 11q23 cannot be determined.

The 72-hr PHA-stimulated sample showed the same abnormalities as the unstimulated sample, but at a reduced frequency (47%) (Table I). Of 15 Q-banded cells, 7 were of normal karyotype.

A bone marrow sample obtained during complete remission (11/1/77) showed only 1 abnormal cell among 12 that were Q banded; this cell had the previously noted abnormalities plus a second translocation chromosome, t(1;13). During the early stage of relapse (12/1/77), prior to the second sample obtained for cell surface marker and enzyme studies, a similar low frequency of abnormal cells was observed in the bone marrow; later in relapse (1/6/77), shortly after the cell surface marker and enzyme studies, a bone core sample showed 100% abnormal cells (Table I). None of the cells from this last sample could be fully analyzed, but one or more rearrangements of the type noticed in previous samples were seen in these cells.

The results of cell surface marker studies and ADA assays are presented in Table 2. In the initial sample obtained at the time of diagnosis, 40% of the peripheral blood cells had surface immunoglobulins (SIg) as measured by a polyvalent antiserum. The proportion of T cells was reduced to 15%.

The specific activity of ADA in the patient’s leukemic cells at the time of diagnosis is the lowest value among the eight patients with ALL studied in our laboratory to date. Two of the eight patients were classified as having T cell ALL.

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**Table 2. Cell Surface Markers and Adenosine Deaminase (ADA) Activity**

<table>
<thead>
<tr>
<th>Source</th>
<th>No of Pts</th>
<th>Date</th>
<th>WBC</th>
<th>Blasts (%)</th>
<th>B cells (%)</th>
<th>T cells (%)</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other ALL patients</td>
<td>7</td>
<td>32(20-429)</td>
<td>68(20-98)</td>
<td>10(0-24)</td>
<td>40(0-79)</td>
<td>314(103-1321)</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>8/77</td>
<td></td>
<td></td>
<td>18(16-24)</td>
<td>65(59-77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12/77</td>
<td></td>
<td></td>
<td>3(1-78)</td>
<td>75(75)</td>
<td>2(2)</td>
<td>26.7</td>
</tr>
</tbody>
</table>

Median values are given for peripheral blood cells with the range of values in parentheses. B and T cells were determined as described in Materials and Methods. Normal controls were healthy laboratory personnel. Other ALL cases included two T cell and five null cell types. ADA activity is expressed as nmoles/min/mg as described in Materials and Methods.
and five the non-B, non-T type. The subject of the present report is the only one with B cell ALL.

After the patient's relapse in December 1977 there was an increase in the percentage of blast cells in the peripheral blood and a corresponding increase in the proportion of SIg-positive cells to 75%. The ADA activity in the later sample decreased further compared to the initial value, as shown in Table 2.

DISCUSSION

The morphology of the bone marrow from this patient was that of ALL and was not consistent with lymphoma of the lymphocytic or Burkitt type. The B cell characteristics of the patient's lymphoblasts are reflected by the low ADA activity as well as the presence of SIg. Normal B lymphocytes are reported to have lower ADA activity than normal T cells. Relatively low levels of this enzyme are also characteristic of B cell neoplasms. The lymphocytes of chronic lymphocytic leukemia have even lower levels of ADA than normal B cells. In a study of ALL in children, substantially lower ADA activity was found in 3 cases with B cell properties compared to 24 others of T cell or non-B, non-T types. In another study, cultured cell lines derived from B cells, including ALL, multiple myeloma, and Burkitt lymphoma, had significantly lower ADA activity than lines established from T cell ALL.

The finding of the 14q+ chromosome in this case is of particular interest because this marker has been reported in only one other case of ALL, which was also a B cell type. These are the only two cases of B cell ALL known in which karyotype studies were done with banding techniques, and both had the 14q+ abnormality. The karyotype in the previous case, although less complex, had other features in common with the present case: an additional No. 7, a loss of No. 13, and a 6p+ rearrangement. In our patient, the extra chromosomal material of the 14q+ marker was translocated from the distal one-fourth of the long arm of chromosome 11: t(11;14)(q23;q32). The origin of the additional material on the 14q in the previous case was not determined.

Of further interest is the fact that a 14q+ chromosome was the most common cytogenetic defect detected in a recent series of patients with various forms of malignant lymphoma. It was most common in poorly differentiated lymphocytic lymphoma (PDL), also a B cell neoplasm, where it was demonstrated in each of 8 patients. At least 3 of 16 patients with PDL had translocations reported to involve chromosome 11: t(11;14)(q13.1;q32). In two patients with diffuse histiocytic lymphoma (DHL) the translocation involved the No. 11 at q13.

Five of twelve abnormal karyotypes from patients with Hodgkin disease in the same series also displayed the 14q+ marker chromosome. Patients with African Burkitt lymphoma and cell lines derived from African and American cases have a 14q+ chromosome. The same translocation has been reported in a single case of chronic lymphocytic leukemia (CLL). Two other patients with T cell CLL had a 14q+ marker; in one of these the donor chromosome was No. 11 at q13.1. The 14q+ marker has also been reported in multiple myeloma (four cases) and in plasma cell leukemia (two cases). Both of the latter patients had a translocation between Nos. 11 and 14, with one patient showing the same breakpoints as in the present case: t(11;14)(q23;q32).
The 14q+ marker chromosome therefore appears to be a common occurrence in certain lymphoproliferative disorders, particularly those of B cell origin. Similar conclusions were reached by Minowada et al.6 The infrequent occurrence of this abnormality in ALL may only reflect the low incidence of the B cell subtype of ALL.

The low ADA activity and the 14q+ abnormality appear to be independent characteristics of B cell neoplasms. The gene for ADA in man has been localized to chromosome 20 by cell hybridization studies.25 Furthermore, in CLL low ADA activity is almost always present, whereas the 14q+ chromosome is rare. It is possible that abnormalities of chromosome 14 in undifferentiated B cell neoplasms influence ADA activity in an indirect manner, but there are no data in support of such an effect.

The finding of the 14q+ chromosome in the only two cases of B cell ALL investigated sufficiently to detect it may represent another example of the value of cytogenetic studies in identifying discrete variants of hematologic disease. As more patients are studied with the current techniques, additional associations of specific cytogenetic abnormalities with particular syndromes are likely to be found.

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B cell acute lymphoblastic leukemia (ALL) with a 14q+ chromosome abnormality

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