The 14q+ Chromosome in Pre-B-ALL

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A child who had acute lymphoblastic leukemia (ALL) associated with an 8;14 chromosome translocation and with a pre-B phenotype is described. The leukemic cells were determined to be pre-B-cells on the basis of intracytoplasmic μ-chain immunoglobulin (clgM+) and the common-ALL antigen, lack of receptors for sheep erythrocytes, and lack of surface immunoglobulin. The 8;14 translocation is frequently found in patients with Burkitt’s lymphoma and in most patients with B-cell ALL and is known to carry a poor prognosis. Thus far, no karyotypes have been reported for patients with pre-B-ALL. The present case indicates that a 14q+ abnormality may provide a proliferative advantage not only to cells with a B-cell phenotype, but also to pre-B-cells. The short survival of our patient also suggests that the 14q+ abnormality and the pre-B phenotype may signal a poor prognosis.

The use of cell surface markers has led to the identification of various subsets of acute lymphoblastic leukemia (ALL), the most clearly defined of which are T-cell, B-cell, and non-T, non-B ALL. The last group, consisting of common and null-cell ALL, is the largest (85% of patients); it is composed of patients whose leukemic cells do not form rosettes with sheep red blood cells (E+) and do not have surface immunoglobulin (slg+). It has recently been recognized that some of these cells are, in fact, B-lymphocyte precursors or pre-B-cells. Pre-B-cells are positive for intracytoplasmic μ-chains (ClgM+) and for the common-ALL antigen (cALL+) and are negative for E rosettes and surface Ig. Vogler et al. first described a series of 22 children with ALL, 4 of whom had the pre-B phenotype; these observations were confirmed by Brouet et al., who found that 6 of 50 ALL patients were pre-B. Neither of these reports, however, described the karyotype of the pre-B leukemic cells. The karyotype is of interest because we6 and others5,8 have reported that an abnormality of chromosome no. 14, which consists of an extra band at the end of the long arm (14q+), is present in the great majority of patients with B-cell ALL. In most patients with an accompanying Burkitt-like solid tumor,6 the extra band is the result of a translocation from no. 8 (l[(8;14) (q24;q32)], the translocation most frequently seen in Burkitt’s lymphoma. Patients who do not have evidence of a solid tumor, the extra material on no. 14 may come from no. 8 or from other chromosome.4

We recently studied the karyotype of a patient whose leukemic cells were of the pre-B type.

CASE REPORT

A 10-yr-old white boy was admitted to a hospital in July 1979, with a 1 mo history of skin rash, malaise, low-grade fever, left knee pain, and weight loss. The lower edge of the spleen was palpated below the left costal margin, but no hepatomegaly, abdominal tumor, or systemic lymphadenopathy was found. His hemoglobin was 11.6 g/dl, white blood cell (WBC) count, 7400/μl, and platelet count, 237,000/μl. The blood smear contained a few immature granulocytes and nucleated red blood cells, but no leukemic cells. His bone marrow aspirate was slightly hypocellular, with the marrow elements completely replaced by lymphoblasts. The lymphoblasts were not very large; they were homogeneous, with somewhat clumpy chromatin and inconspicuous nucleoli (Fig. 1A). The scanty cytoplasm showed vacuolization in some of the cells. The leukemic cells were compatible with the L1 type according to the FAB classification. The lactate dehydrogenase (LDH) value was 2100 mU/ml (normal range, 100-225). Antibody titers against Epstein-Barr virus capsid antigen were negative.

The patient was treated briefly with vincristine and prednisone. Later, he was transferred to the University of Chicago Hospitals and was treated with a regimen consisting of systemic vincristine (VCR), prednisone, and L-asparaginase. A complete remission was easily achieved; cranial irradiation and intrathecal methotrexate (MTX) injections were administered for CNS prophylaxis. After 30 days of maintenance therapy, consisting of daily oral 6-mercaptopurine and weekly oral MTX, he appeared on 11-16-79 to be in relapse with leg pain, fever, and an enlarged spleen. The hemoglobin was 8.3 g/dl, and the WBC count was 7900/μl with 16% blasts. A bone marrow aspirate revealed many large cells containing stippled chromatin and conspicuous nucleoli. The cytoplasm in some cells was moderately abundant and basophilic and contained many vacuoles. These findings supported the designation as L3 (or Burkitt type), although some features, such as heterogeneity of the cell size, were compatible with a diagnosis of L2 (Fig. 1B). The leukemic cells were negative for periodic acid Schiff stain, acid phosphatase, and peroxidase. The patient was treated with a reinduction regimen consisting of VCR, prednisone, and L-asparaginase. He developed pancytopenia and died of sepsis 4 mos after the initial admission.

MATERIALS AND METHODS

Chromosome and Immunologic Studies

Chromosome and immunologic marker studies on the leukemic cells were performed 1 wk after the beginning of induction therapy and at relapse. Chromosomes were studied initially from bone marrow and from peripheral blood cells cultured for 24 hr without
Fig. 1.  (A) Bone marrow aspirate at the time of diagnosis of ALL, showing L1-type morphological features. (B) The bone marrow in relapse contained large cells with stippled chromatin and abundant cytoplasm with vacuoles (Wright-Giemsa).

The cells were analyzed with regular Giemsa stain and with Q-, G-, and R-banding methods. Seven of 12 cells from bone marrow and 15 of 19 from peripheral blood had an abnormal karyotype, 46,XY, -8,del(3)(q12q25),t(8;14)(q24;q32),+der(8)t(1q;8q) (cen;cen) (Fig. 2). The remaining cells had a normal male karyotype. The peripheral blood cells at relapse were cultured for 24 hr without PHA and were analyzed with regular Giemsa stain and Q-banding methods. Of 16 cells analyzed with banding, 5 cells had the chromosome abnormalities noted in the initial sample. In addition, 2 of the 5 cells had an extra segment of unknown origin at the end of the long arm of one no. 13, and this marker was described as der(13)(t(13;?)(q34;?)). The 13q+ chromosome was duplicated in a third cell. These 3 abnormal cells provided evidence of karyotypic evolution.

Leukocytes were prepared for immunologic studies from the EDTA-anticoagulated specimens by Ficoll-Hypaque density gradient centrifugation. Cryopreserved cells served as positive or negative controls and were assayed in parallel with the patient's cells, as we have described previously. The cells were examined for conventional surface markers (E rosettes, EAC rosettes), and aliquots of each rosette-forming cell suspension were also cytocentrifuged and stained with Wright-Giemsa for morphological evaluation. Cells positive for cALL antigen were detected by immunofluorescence with reagents kindly provided by Dr. M. F. Greaves, London.

Fig. 2.  Q-banded chromosomes of a cell from peripheral blood of the patient 1 wk after the beginning of chemotherapy. Arrows show abnormal chromosomes; interstitial deletion of no. 3, reciprocal translocation between second no. 8 and no. 14, and a derivative chromosome consisting of 1q and 8q. Karyotype is indicated as 46,XY, -8,del(3)(q12q25),t(8;14)(q24;q32),+der(8)t(1q;8q) (cen;cen). R- and G-banded partial karyotypes are shown in the inset; the R-band-positive region on the second 8q, 8q24, is translocated to 14q32. The same chromosomes were analyzed with both Q- and R-banding methods.
Table 1. Results of Immunologic Analysis of Cells From Patient
and Controls

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<th>Date</th>
<th>Source</th>
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BM, bone marrow; PB, peripheral blood.
NE, not evaluable; ND, not done.
*Percent of malignant cells in the peripheral blood sample that were positive for the marker.
†Patient with ALL (E−, EAC−, Slg−, cALL−).
‡Human lymphoblastoid cell line (Slg+, clgM+).
§Human lymphoblastoid cell line (Slg−, clgM−).

Slg-positive cells were detected by reaction with a polyvalent anti-human Ig goat antiserum coupled to peroxidase (Cappel Laboratories, Inc., Cochranville, Pa.) and staining of the cytocentrifuged smears for peroxidase.13 For cytoplasmic IgM, cell smears were fixed in cold ethanol-acetic acid, reacted with peroxidase-conjugated goat serum specific for human μ-chains (Cappel), and stained for peroxidase.13 The leukemic cells were identified on the basis of their size and the ratio of nuclear to cytoplasmic area.

The initial blood specimen (8/30/79) contained 13% lymphoblasts, of which fewer than 5% formed E or EAC rosettes and none were Slg-positive. At that time, cALL and clgM were not evaluated. A blood specimen taken at relapse (11/19/79) contained 30% lymphoblasts, of which less than 3% formed E rosettes, none were positive for EAC rosettes or Slg, 63% contained clgM, and 35% were positive for cALL (Table 1). These results were consistent with the pre-B phenotype (E+, EAC−, Slg−, cALL−, clgM+).

DISCUSSION

The combined use of immunologic and cytogenetic techniques in this case has provided new evidence of the close correlation of particular chromosome changes and malignant lymphocytes with specific functional characteristics. At present, with few exceptions,14 patients reported as having B-cell ALL have had a 14q+ marker chromosome.14 The 8;14 translocation was regularly found in patients who also had a solid tumor phase and who would reasonably be considered to have the leukemic phase of Burkitt's lymphoma. In other patients with B-cell ALL in whom the translocation has been defined, various chromosomes have been involved with no. 14, usually no. 8, but also no. 11. This patient is similar to patients with B-cell ALL in several other respects. Of the 10 reported patients, 9 were male. The survival was described in 8 patients, who had a median survival of 4 mo (range, 1–9 mo). Where the information was provided, all had an IgM phenotype and were EBV-negative. Chromosome changes in addition to the 14q+ also showed recurring abnormalities; including the present case, 4 patients have had rearrangements of no. 1 leading to trisomy for the long arm and 4 patients have had a 13q+ chromosome, 2 of them during evolution of the karyotype.

The observation of the 8;14 translocation in the present patient provides additional support for the importance of this particular chromosome rearrangement in providing B cells with a proliferative advantage. This arrangement also appears to provide a proliferative advantage to B cells that are in an earlier stage of maturation, that is, those with a pre-B phenotype. It is generally agreed that the first identifiable cell in the B-cell lineage is the pre-B cell, which lacks surface markers and contains intracytoplasmic IgM.

The present patient would have been classed as having non-B, non-T ALL, which comprises more than 80% of childhood ALL; almost 90% of this group are also cALL+.1 In general, this group, i.e., non-B, non-T, cALL+, is considered to have the best prognosis.1 The prognostic implications of the pre-B phenotype, which may account for up to 20% of childhood non-B, non-T ALL, are unclear. The clinical course of the four patients of Vogler et al.2 appeared to be favorable. On the other hand, two of the six patients described by Brouet et al.3 had a rapid course, and none of them was followed in CR for more than 7 mo; in four of the six patients, the morphology of the blasts was of the L1 type. Thus, although the patient described here was thought to have average-risk ALL because of a low WBC (<10,000/μl), but unfavorable age (10 yr old), his clinical response was of short duration.

At present, few data are available that would help to relate the chromosome pattern studied with banding to survival in ALL. In two series,5,15 patients with an abnormal karyotype had a much shorter median survival (about 10 mo) than did those with a normal karyotype. This survival time is similar to that observed in patients with Ph+ + ALL.16 It is well known that patients with B-cell ALL have a poorer prognosis than those with any other type of ALL. Although the karyotypes of only a few patients with B-cell ALL have been examined with banding, a 14q+ chromosome is usually present. The short survival of our patient suggests that the 14q+ abnormality and the pre-B phenotype may also signal a poor prognosis. A similarly poor prognosis has been reported in most patients with T-cell ALL whose cells have a 14q+ marker. Additional studies combining cytogenetic and immunologic analyses will clarify the significance of these various factors.
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REFERENCES

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