CONCISE REPORT

Acute Lymphoblastic Leukemia With Burkitt Cell Morphology and Cytoplasmic Immunoglobulin

By Dorothy J. Ganick and Jonathan L. Finlay

A case of acute lymphoblastic leukemia with morphological characteristics of Burkitt's leukemia (L3 morphology) is presented. This patient's lymphoblasts were lacking in surface immunoglobulin, but were found to contain cytoplasmic IgM. This is the first report of a morphologically B-cell leukemia showing pre-B-cell characteristics immunologically.

DESPITE ADVANCES in the treatment of acute lymphoblastic leukemia (ALL) in children, many with the disease do not respond favorably to current chemotherapeutic regimens. Immunologic characterization of the leukemic blasts as to their cell of origin has afforded better prognostic information.1-4 Children with the T-cell type ALL, comprising about 20%-25% of all cases of childhood leukemia, do not have prolonged remissions.1-5 Similarly, children with the B-cell type, which comprises only 1%-2% of all cases, respond poorly to current chemotherapy.3 The majority (about 80%) of children with ALL are of the "null" cell type.1,2 The response to chemotherapy within this group is variable. The majority of children have prolonged remissions, but a significant percentage of cases fail to respond to treatment. Controversy exists as to the exact origin of the "null" cell. From immunologic marker studies some investigators feel that the "null" cell is of B-cell origin,6 while others feel it is of T-cell origin.1 Peripheral blood null cells also contain circulating myeloid and erythroid progenitor cells.7 Recently, a new subset of ALL, the pre-B-cell type, has been described.8,9 The blast cells of this type have no distinctive features by light microscopy. However, by using immunofluorescent markers, these blasts display a unique pattern of cytoplasmic IgM in a perinuclear distribution. To date, pre-B-cell ALL has been found to comprise between 12% and 18% of cases in two studies.8,9 Initial reports as to response to induction chemotherapy were favorable for this group, but long-term follow-up is needed to confirm the prognostic significance that can be ascertained.

We wish to report a case of acute lymphoblastic leukemia in a child whose blast cells were of L3 morphology according to the FAB criteria10 and thus resembled Burkitt's lymphoma cells. Burkitt's lymphoma and acute leukemia (of Burkitt's cell morphology) have been shown to possess monoclonal surface immunoglobulins and thus are of B-cell origin.11,12 Immunologic typing of the blast cells in our case revealed them to lack surface immunoglobulins, but to contain cytoplasmic IgM. Thus, this case is unique in that the pre-B-cells have not previously been reported to resemble Burkitt's cells morphologically.

MATERIALS AND METHODS

Leukemic cell marker studies were performed on our patient on three separate occasions: at presentation (prior to any therapy), on day 56 (at the time of relapse), and on day 84. On each occasion, bone marrow was aspirated into heparinized syringes and separated by Ficoll-Hypaque gradient centrifugation to yield a mononuclear cell population. T lymphocytes were enumerated by the spontaneous erythrocyte rosetting assay.13 B lymphocytes were enumerated both by the spontaneous mouse erythrocyte rosetting assay13 and immunofluorescent staining;14 in the latter, fluorescein isothiocyanate (FITC)-labeled goat anti-human light-chain serum, FITC-labeled (Fab')2 goat anti-human mu-chain serum, rhodamine-labeled (Fab')2 goat anti-human gamma-chain serum, and rhodamine-labeled (Fab') goat anti-human alpha-chain serum were used at 1 mg/ml. These class-specific antisera were prepared by papain digestion.14

Cytoplasmic IgM was detected by a modification of the method described by LeBien et al.15 Bone marrow mononuclear cells were washed three times with phosphate-buffered saline (PBS) pH 7.2, incubated at 37°C for 30 min, washed twice with PBS, and the cell pellet resuspended in 0.5 ml PBS with one drop of 30% bovine serum albumin (Dade). This cell suspension was then cytocentrifuged for 5 min at 500 rpm (Shandon Elliott-Cytospin) onto a glass slide, fixed 18 hr in PBS. Finally, after a 5-mm wash in PBS, the slides were read under uv fluorescence at x 1000 magnification (Zeiss microscope).

CASE REPORT

A 10-yr-old young man presented with a 1-wk history of fatigue and easy bruisability. On physical examination he was found to have an enlarged spleen (6 cm below the left costal margin) and liver (93.5 cm below the right costal margin) as well as multiple ecchy-
moses and petechiae over his body. Initial blood count revealed a hemoglobin of 8.5 g/dl; white blood count of 24,900/cu mm with 5% monocytes, 5% metamyelocytes, 17% bands, 8% neutrophils, 6% eosinophils, 35% lymphocytes, and 24% large blast cells, and a platelet count of 33,000/cu mm. Additional laboratory values included a uric acid of 15.9 mg/dl, LDH greater than 1500 IU/liter, and SGOT of 231 IU/liter. Serum immunoglobulins were normal (IgG, 709 mg/dl, IgA, 69 mg/dl, and IgM, 19.5 mg/dl). Bone marrow aspirate revealed a hypercellular specimen containing an almost homogenous population of lymphoblasts. No normal marrow elements were seen. Spinal fluid contained no malignant cells.

The patient was started on induction chemotherapy for acute lymphoblastic leukemia (Childrens Cancer Study Group Protocol 162). He received weekly vincristine, daily prednisone, and L-asparaginase 3 times per week for a total of 9 doses. Central nervous system prophylaxis consisted of cranial irradiation at a dose of 1800 rads and intrathecal methotrexate.

The patient was found to be in bone marrow remission by day 14. Repeat bone marrow aspiration on day 56 revealed a relapse. Despite reinduction therapy with vincristine, prednisone, and cyclophosphamide, the patient did not attain a second remission and died on day 97 from uncontrolled bleeding. No autopsy was obtained.

RESULTS

Leukemic Cell Characterization

Morphological Studies

As shown in Fig. 1, the initial bone marrow aspirate contained a uniform population of large vacuolated blast cells with blue cytoplasm and very fine nuclear chromatin with an occasional nucleolus; frequent mitotic figures were observed. The large blast cells resembled Burkitt's lymphoma cells, and the marrow was classified according to the FAB classification as L3. Cytochemical staining of the blasts with Oil-Red-O, periodic acid Schiff, and Sudan Black, and staining for esterase with Naphthol-AS-D chloroacetate were all negative. Karyotyping of the blast cells revealed cells containing 60 chromosomes. There were 3 marker chromosomes (2 very long metacentrics resembling a giant number 1 and a giant number 2 and a large submetacentric resembling a giant B), as well as extra chromosomes in the C, D, E, F, and G groups.

Immunologic Studies (Table 1)

Mononuclear cell suspensions of the patient's bone marrow cells on days 0, 56, and 84 did not reveal surface immunoglobulins upon immunofluorescent staining with monospecific (Fab')2 fluorescent anti-human heavy-chain sera. Spontaneous sheep erythrocyte rosetting showed less than 1% rosetting lymphocytes in the bone marrow on all three occasions. Mouse erythrocyte rosetting performed on days 56 and 83 also showed less than 1% rosetting lymphocytes in the bone marrow. The presence of cytoplasmic IgM was sought only on day 84; using the method detailed above, 40% of bone marrow mononuclear cells demonstrated a distinctive pattern of cytoplasmic fluorescence with fluorescein-labeled F(ab')2, anti-human mu serum: there was extensive cytoplasmic distribution of fluorescence, predominantly in the perinuclear area.

DISCUSSION

Burkitt's lymphoma presenting as leukemia with blast cells in the peripheral blood is rare. There have been only a few cases reported in the literature. Bone marrow involvement by Burkitt's lymphoma either initially or during the course of the disease has been documented in American cases. Flandrin et al. reported six cases of acute leukemia with Burkitt's

![Fig. 1. Leukemic blast cells at diagnosis.](image-url)
Studies of mixed lymphocyte reactions, surface B cell antigens, and characteristics and potentials, in Golde DW, Cline Mi, Metcalf D, acute lymphoblastic leukemia. Cancer 42:817, 1978. Prognostic factors in acute lymphoblastic leukemia. N Engl J Med 294:245, 1976. Intracytoplasmic immunoglobulins in “null cell” acute lymphocytic leukemia and increased B-cell markers. J Pediatr 89:956, 1976. Characteristics of Burkitt’s lymphoma with leukemic transformation. In the series of Brouet et al. had bulky disease. One patient failed induction and had an early relapse. In our case, although the blasts morphologically resembled Burkitt’s tumor cells, having L3 morphology, they lacked surface immunoglobulins. Also, the blast cells showed marked chromosomal aneuploidy but without the chromosomal translocation [t(8:14)], which has been described in several cases of Burkitt’s lymphoma. The cells contained cytoplasmic IgM, which is characteristic for pre-B-cells, but of a more extensive cytoplasmic distribution with predominance in, but not restriction to, the perinuclear area. Since pre-B-cells probably are the progenitor cells of the marrow B cell, this may indicate that our case was a primary marrow malignancy, rather than of extramedullary origin. The blast cells did not rosette with mouse erythrocytes; the capacity to do so is considered a property only of mature B lymphocytes.

Leukemic blast cells that type immunologically as pre-B-cells have been reported in two series of cases of ALL. This type of blast cell has also been reported in four cases of chronic myelogenous leukemia in blast crisis. Several of the ALL cases of pre-B origin in the series of Brouet et al. lysed bone lesions. These and extensive cytoplasmic distribution with predominance in, but not restriction to, the perinuclear area. Since pre-B-cells probably are the progenitor cells of the marrow B cell, this may indicate that our case was a primary marrow malignancy, rather than of extramedullary origin. The blast cells did not rosette with mouse erythrocytes; the capacity to do so is considered a property only of mature B lymphocytes. 14.

Burkitt cell ALL with cytoplasmic IgM

<table>
<thead>
<tr>
<th>Day</th>
<th>Percent Lymphoblasts in Bone Marrow</th>
<th>Surface Immunoglobulin-Bearing Cells</th>
<th>Sheep Erythrocyte Rosetting Cells</th>
<th>Mouse Erythrocyte Rosetting Cells</th>
<th>Cytoplasmic IgM-Bearing Cells</th>
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<tr>
<td>0</td>
<td>97%</td>
<td>&lt;1% &lt;1% &lt;1% &lt;1% &lt;1%</td>
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<td>56</td>
<td>39%</td>
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<tr>
<td>84</td>
<td>49%</td>
<td>&lt;1% &lt;1% &lt;1% &lt;1% &lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>40%</td>
</tr>
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*Normal bone marrow controls contain less than 1% cytoplasmic IgM-bearing cells.

References

14. Potter MR, Moore M: Characterization and separation of ACKNOWLEDGMENT

We thank Dr. Richard Hong for making available to us facilities for fluorescent analysis. We gratefully acknowledge the technical assistance of Wayne Borcherding. We also wish to thank Dr. L. F. Meisner for the karyotype analysis.


16. Lachman PJ: Purification of specific antibody as F(ab')2 by the pepsin digestion of antigen-antibody precipitates, and its application to immunoglobulin and complement antigens. Immunochromistry 8:91, 1971


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