Acute Leukemia With Burkitt’s Tumor Cells: A Study of Six Cases With Special Reference to Lymphocyte Surface Markers

By G. Flandrin, J. C. Brouet, M. T. Daniel, and J. L. Preud’homme

In six patients with acute leukemia (about 2% of the patients referred for acute lymphoblastic leukemia) the blast cells invading bone marrow and blood showed all the cytologic, cytochemical, and electron microscopy features of Burkitt’s tumor cells. The presence of monoclonal surface immunoglobulins (their synthesis being proved by in vitro culture experiments), the binding of IgG aggregates, and the absence of rosette formation with sheep red cells documented the monoclonal B-cell origin of these blast cells which is in sharp contrast to the findings in common acute lymphoblastic leukemia. The course of the disease was usually rapidly fatal without chemotherapy-induced remission.

The clinicopathologic and epidemiologic features of Burkitt’s lymphoma first attracted attention on this peculiar variety of lymphoma in African children. It soon became clear that similar cases could be found in various parts of the world, including North America and Europe, and that the clinical presentation may differ from one place to another. The cytologic and pathologic criteria allowing the diagnosis of Burkitt’s lymphoma cell have been therefore precisely defined. Since apparently only one case with a true leukemic presentation has been observed in Burkitt’s lymphoma, we wish to report six patients with acute leukemia and characteristic cytologic and immunologic features of Burkitt’s tumor cells.

Materials and Methods

Patients

The main clinical and hematologic data in the six patients (five males and one female) are summarized in Table 1. In all patients the onset of the disease was abrupt. They were all acutely ill with marked thrombocytopenia (10,000-30,000 platelets/cu mm), anemia (Hb 5-10 g/100 ml), massive bone marrow infiltration, circulating blast cells with leukoerythroblastic blood picture. Death due to intracranial bleeding occurred in two patients before any treatment. A remission was not obtained in the four other patients with a combined chemotherapy (daunomycin, vincristine, prednisone).

Cytologic and Ultrastructural Studies

Cytologic studies were performed on May-Grunwald-Giemsa (MGG)-stained bone marrow and peripheral blood smears. The following cytochemical reactions were used: periodic acid Schiff (PAS), methyl green pyronine, oil red O, peroxidase, acid phosphatase. The blast cells from...
one patient were studied by electron microscopy: the tissue specimens were fixed in glutaeraldehyde and postfixed in osmium tetroxide. Sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

Lymphocyte Surface Markers

Membrane-bound immunoglobulins (S.Ig) were studied on blast cells obtained from bone marrow aspirates and defibrinated blood by direct immunofluorescence using monospecific sera to μ, γ, α, ν, and λ-chains as previously described.9,10 A conjugate specific for λ-chain was used also in two cases.

To investigate if the S.Ig were or were not actually synthesized by the cells which carried them, the cells from two patients were treated with trypsin and incubated in a culture medium for 6-7 hr before staining, with appropriate controls.11

Heat-aggregated human IgG coupled to rhodamine were used at a concentration of 1-2 mg/ml in a direct immunofluorescent test.11

Spontaneous sheep erythrocytes rosette formation was performed according to Jondal et al.12

RESULTS

Morphologic Studies

Bone marrow. In each case, bone marrow aspiration showed a massive infiltration (>90%) by the tumor cells. The blast cells varied slightly in size (20–25 μ in diameter) but were otherwise identical. The cytoplasms were uniformly and intensely deeply basophilic without granules. Most cells contained empty cytoplasmic vacuoles (1–2 μ in diameter) on the MGG smears. The nuclei were round and large with a finely clumped chromatin. Many mitotic figures were observed (Fig. 1). The blast cells showed a marked cytoplasmic pyroninophilia. They were usually devoid of PAS-positive material. Coarse lipid droplets were demonstrated in the cytoplasmic vacuoles by oil red O staining. The acid phosphatase reaction showed a small amount of granular positivity. The peroxidase reaction was negative. Some macrophages with light cytoplasmic matrix and cell debris were observed. Acid phosphatase activity was very strong in these macrophages.

Electron microscopic study showed large regular blast cells. The cytoplasmic matrix contained lipid vacuoles slightly stained by osmium. The most characteristic feature of the cytoplasm was the large number of ribosomes. Mitochondria were large and had a tendency to polarize. The nuclei were round or

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>White Blood Cells per cu mm</th>
<th>Circulating Tumor Cells (%)</th>
<th>Bone Marrow Tumor Cells (%)</th>
<th>Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>28,000</td>
<td>48</td>
<td>100</td>
<td>Hepatosplenomegaly; lytic bone area (humerus)</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>10,300</td>
<td>50</td>
<td>100</td>
<td>Hepatosplenomegaly; bone tumors (fibias)</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>29,000</td>
<td>4</td>
<td>98</td>
<td>Lymphadenopathies; pelvic tumor; pleural and meningeal involvement; priapism</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>6,900</td>
<td>1</td>
<td>100</td>
<td>Hepatic and neurologic involvement</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>10,000</td>
<td>3</td>
<td>90</td>
<td>Splenomegaly</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>15,700</td>
<td>27</td>
<td>100</td>
<td>Splenomegaly; secondary occurrence of a jaw tumor</td>
</tr>
</tbody>
</table>
Fig. 1. (left). Bone marrow smear, May-Grünewald-Giemsa stain. x 1200.
Fig. 2. (right). Tumor cells in the peripheral blood. x 1200.

oval with a relatively high nucleocytoplasmic ratio. The chromatin was abundant and clumped at the nuclear envelope and around the nucleoli. The interchromatinic substance was relatively clear. Nucleoli were quite large. In some cells a strong margination of the chromatin was observed.

Peripheral Blood

Circulating tumor cells were found in all the cases. The blast cells in the blood were very similar to the marrow cells. However, they varied more in size than on bone marrow smears (Fig. 2).

The number of circulating blast cells greatly varied from patient to patient at first examination, accounting for 30%–50% of the white blood cells in three patients. In the last three patients, less than 5% blast cells were found in the blood, and the leukoerythroblastic picture (observed in all cases) was the most striking feature.

Lymphocyte Surface Markers

In all six cases, virtually 100% of the blast cells bore a monoclonal S.Ig belonging to the IgM class in five cases and to the IgG class in one instance. The light chain type of the monoclonal S.Ig was λ in four cases and κ in the two others. In the two cases where in vitro studies were performed, the monoclonal S.Ig was shown to be synthesized by the cells left in culture after trypsinization. Delta chains were associated to the μ-chains in the two cases studied with the specific anti-δ-chain antiserum. In four cases the spotty fluorescence was very bright, suggesting a high density of S.Ig; in the two other cases the staining was faint.

Virtually all the tumor cells were able to bind heat-aggregated IgG. They did not form spontaneous rosettes with sheep erythrocytes.

DISCUSSION

The blast cells from six patients (about 2% of the patients referred during the last 3 yr to our department of hematology for acute lymphoblastic leukemia
(ALL)) were indistinguishable by cytologic, cytochemical, and immunologic criteria from the tumor cells characteristic of Burkitt’s lymphoma. In these six patients the course of the disease was acute, and anemia, thrombocytopenia, and fever developed rapidly. At first examination, a massive bone marrow infiltration and a peripheral blood involvement by blast cells were found. Neither the clinical findings nor the past history could distinguish the disease from common ALL. It is worth noting that the mean age of these six patients was somewhat higher than that of children with common ALL. However, the number of patients in this series is too small to draw any conclusion. The combined chemotherapy regimen used in ALL did not result in any remission; four patients died in the first month of the illness, and the two others are currently under treatment 2 mo after the onset of the disease.

The blast cells from these six patients fulfilled the current histopathologic and cytochemical criteria defining Burkitt’s tumor cells, and their electron-microscopic pattern was identical to that previously described in Burkitt’s lymphoma. Moreover, as in Burkitt’s lymphoma, these blast cells were shown to be of B-cell origin. Indeed, they bore S.Ig, which are detected only on B-cells by rather insensitive methods such as immunofluorescence. They were able to bind aggregated IgG, another reliable B-cell marker, and they were devoid of the most commonly used human T-cell marker, i.e., rosette formation with sheep erythrocytes. It was of utmost importance to prove the in vitro synthesis of the S.Ig by the tumor cells, since antibodies directed towards surface determinants have been found on blast cells in acute leukemias and Burkitt’s lymphoma. The monoclonal nature of the B-cell proliferation in our patients is established by the finding of a monoclonal S.Ig on the cells (IgM in four cases, IgMv and IgGv in one case each). The simultaneous presence of ζ- and δ-chains in the two cases where studies with anti-δ-chain sera were performed does not argue against this interpretation, since normal or leukemic lymphocytes bearing ζ-chains commonly synthesized also δ-chains. The finding of a monoclonal B-cell proliferation further delineates these patients from those with common ALL. In this latter condition the blast cells are usually devoid of B- or T-cell markers or could be tentatively related to the T-cell line.

A true acute leukemic presentation is probably a very rare event in Burkitt’s lymphoma, since only one such case has been previously published. Blood or diffuse bone marrow involvement was not mentioned in early reports. However, recent studies have documented the relative frequency of bone marrow infiltration during the course of the disease in African (16%) as well as in American (more than 50%) patients. In these cases, a leukemic picture was not apparent except as a terminal event. The occurrence of leukemia at a late stage of the disease has been reported also in several other cases of Burkitt’s lymphoma.

The possibility of a true leukemic presentation further enlarges the clinical spectrum of Burkitt’s lymphoma. Although rare, such cases deserve special emphasis, since Burkitt’s lymphoma cells have been reported to be highly resistant to some drugs usually effective in common ALL, an observation confirmed in our patients. The identification of such cases on cytologic and immunologic criteria might therefore lead to other therapeutic approaches.
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