ACUTE LYMPHOBLASTIC LEUKEMIA WITH PRE-B-CELL CHARACTERISTICS

By J. C. Brouet, J. L. Preud’homme, C. Penit, F. Valensi, P. Rouget, and M. Seligmann

Blast cells from 6 of 50 patients with acute lymphoblastic leukemia (ALL) displayed intracytoplasmic μ chains in the absence of detectable light chains and surface immunoglobulins. These cells also expressed λ-like and common ALL antigens. Terminal deoxynucleotidyltransferase was detectable in 2 of 5 cases tested. These blast cells are probably related to early B-cell precursors (pre-B cells). In 4 of 6 cases the disease had a tumoral presentation; the prognostic significance of this new subgroup, which accounts for 20% of patients with non-T non-B ALL, remains to be established.

STUDIES OF LYMPHOID CELLS from fetal liver of rabbit, mouse, and man have strongly suggested that the first identifiable cell in the B-cell lineage is a cell called a pre-B cell that lacks surface receptors and contains intracytoplasmic IgM and that is the progenitor of surface Ig-positive B cells.1 In adults, pre-B cells are found almost exclusively in the bone marrow.2 Vogler et al.3 recently showed that leukemic cells with pre-B-cell characteristics may be detected in a fair percentage of children affected with acute lymphoblastic leukemia (ALL). The aim of this report is to further delineate the immunologic and clinical features of the pre-B-cell leukemias.

MATERIALS AND METHODS

Between January and October, 1978, cells from 50 consecutive patients with ALL were studied with conventional lymphoid markers4 and were also screened for pre-B-cell phenotype, e.g., the presence of intracytoplasmic IgM in the absence of surface Ig. Washed cell suspensions obtained from either bone marrow or peripheral blood and containing 80% or more blast cells were first stained with a polyvalent anti-human-Ig goat antiserum coupled to fluorescein to detect surface immunoglobulins. After fixation in 90% ethanol, intracytoplasmic staining was performed with rhodamine-conjugated F(ab')2, fragments of rabbit IgG specific for human μ chains at optimal concentration. The cells with features suggestive of pre-B cells were further studied with a panel of membrane markers: surface and cytoplasmic Ig with monospecific antisera to μ, γ, α, δ, ε, and λ chains.5 E-rosette formation, common ALL antigen,6 p23.30 Ia-like antigen.7 The leukemic cells were also studied in immunofluorescence tests with antisera to T cells8 and with an antiserum obtained by immunization of rabbits with B-derived chronic lymphocytic leukemia cells. The latter reagent reacted with B cells and most non-T non-B ALL cells.9 The presence of terminal deoxynucleotidyl transferase (TdT) was determined either by an enzymatic quantitative assay8 or by indirect immunofluorescence and immunoperoxidase procedures.9 In these procedures we used anti-calf-TdT antibodies provided by P.C. Kung and anti-chicken-TdT antibodies purified by us.10
Table 1. Cellular Ig in Pre-B-Cell Leukemia as Percentage of Positive Cells

<table>
<thead>
<tr>
<th>Case</th>
<th>Intracytoplasmic Ig</th>
<th>Surface Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μ</td>
<td>γ</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

* Very dim staining.

RESULTS

Leukemic cells with intracytoplasmic IgM in the absence of surface Ig were found in 6 patients with ALL (5 of 40 children and 1 of 10 adults). The results of the study of surface and cytoplasmic Ig in these cases are shown in Table 1. Cytoplasmic staining for IgM was usually very dim but was detectable in 50%–90% of the cells. The cells displayed a perinuclear rim of fluorescence and/or a granular and reticular pattern. Pre-B-cell cases were confirmed by two independent observers. Since the fluorescence was faint, despite optimal optical conditions and a very low background, the results were checked with another batch of antibodies to μ chains obtained from Dr. M.D. Cooper; identical results were obtained, and in these cases where only 50% of the cells stained for μ with one conjugate, double-labeling experiments showed that the same cells were recognized by the two different antisera.

In 2 cases very small percentages of cells also stained with anti-γ and/or anti-α sera. Light-chain determinants were not found in the cytoplasm of the positive blast cells except in 2 cases (patients 5 and 6), where the antiserum to κ chains stained (with a characteristic pre-B-cell pattern) a low percentage of the blast cells.

In 2 cases very faint fluorescence was detectable on the surfaces of 10% and 30% of the blast cells with the anti-μ antisera without simultaneous positivity with the antisera to light chains. In cases 2 and 4, 1%–2% of the circulating cells had surface IgM with a single type of light chain. The surface-Ig-positive cells were usually smaller than the blasts and appeared as small lymphoid cells on phase-contrast examination. Although the very low percentage of positive cells precluded definite conclusions as to the existence of a monoclonal lymphocytic population, it is of

Table 2. Non-Ig Markers in Pre-B-Cell Leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>ALL</th>
<th>p23.30</th>
<th>Anti-CLL Serum</th>
<th>Anti-T Serum</th>
<th>TdT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>NT*</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>(+)†</td>
<td>–</td>
<td>(+)</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>+ (10%)</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+ + ‡</td>
<td>+</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

* NT, not tested.
† (+) = very faint staining.
‡ ++ = strong staining.
### Table 3. Pre-B-Cell Leukemia: Clinical and Hematologic Data at Presentation

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>WBC (× 10⁹/L)</th>
<th>Blood (%)</th>
<th>Bone Marrow (%)</th>
<th>Hb (g/dL)</th>
<th>Platelets (× 10¹⁰/L)</th>
<th>Lymph Nodes</th>
<th>Spleen</th>
<th>Liver</th>
<th>Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>12</td>
<td>100</td>
<td>95</td>
<td>95</td>
<td>8</td>
<td>50</td>
<td>+ + *</td>
<td>+</td>
<td></td>
<td>Bone marrow relapse 3 mo after complete remission (CR); meningeal relapse 2 mo after second remission</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>4</td>
<td>276</td>
<td>98</td>
<td>100</td>
<td>8</td>
<td>40</td>
<td>+ + +</td>
<td>++</td>
<td>++</td>
<td>In CR after 7 mo</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>4.5</td>
<td>10</td>
<td>45</td>
<td>100</td>
<td>7.5</td>
<td>230</td>
<td>+</td>
<td>+</td>
<td></td>
<td>In CR after 6 mo</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>1</td>
<td>120</td>
<td>99</td>
<td>100</td>
<td>5.8</td>
<td>5</td>
<td>+ + +</td>
<td>+</td>
<td>+</td>
<td>Death from sepsis at day 20 after induction therapy</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>10</td>
<td>20</td>
<td>75</td>
<td>100</td>
<td>11</td>
<td>150</td>
<td>+ +</td>
<td>--</td>
<td>--</td>
<td>In CR after 2 mo</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>23</td>
<td>200</td>
<td>99</td>
<td>99</td>
<td>7</td>
<td>75</td>
<td>+ + +</td>
<td>+</td>
<td>+</td>
<td>In CR after 2 mo</td>
</tr>
</tbody>
</table>

* + = slight enlargement; ++ = moderate enlargement; +++ = massive enlargement.

interest that the staining for the opposite light-chain type was negative as well for IgD. In the other cases, polyclonal B cells were found.

The study of non-Ig markers (Table 2) showed that the usual phenotype of the leukemic cells was ALL-antigen-positive, p 23,30-antigen-positive, CLL-antigen-positive, anti-T-serum-negative, E-rosette-negative.

No TdT could be detected in the leukemic cells from 3 patients. A very low amount of TdT (0.3 U/10⁶ cells) was found in case 1 and a substantial quantity (4 U/10⁶ cells) in case 2.

The main clinical and hematologic data are listed in Table 3. In 4 cases the leukemia had a tumoral presentation with several high-risk factors. In all cases superficial lymph nodes were enlarged, with a striking parotid involvement in patients 1 and 4. Four patients were classified in the L₁ cytologic subclass of ALL, according to Fab group,¹¹ and 2 patients were in the L₂ subgroup. Patients 3 and 5 entered into remission on vincristine and prednisone therapy and are presently in complete remission under maintenance therapy. The other patients who presented with known high-risk factors were treated with an intensive chemotherapy regimen including prednisone, vincristine, daunorubicin, and cyclophosphamide. Patient 4 died from sepsis, and patient 1 experienced an early relapse despite vigorous maintenance therapy using the same drugs as during the induction phase and central nervous system prophylactic irradiation.

**DISCUSSION**

Studies of the leukemic cells with immunologic membrane markers¹² disclosed an unexpected heterogeneity in the cellular origin of ALL, since three main groups were found: T-derived ALL, B-derived ALL, and non-T non-B ALL. This third group includes more than 70% of the cases and is defined by the absence on the blast cells of the usual markers and antigenic determinants of peripheral B and T cells. It should be stressed that this non-T non-B subgroup is itself clearly heterogeneous with respect to some phenotypic features and that the delineation between T ALL and non-T non-B ALL is not always clear.¹² Several findings suggest that in many patients non-T non-B ALL cells may represent T-cell progenitors.¹² On the other hand, Vogler et al.¹³ recently showed that some other cases should be related to the B-cell series. Indeed, the cells from 4 of 22 patients displayed features thought to be characteristic of pre-B cells, e.g., the presence of...
intracytoplasmic IgM in the absence of surface Ig. We found similar characteristics of the blast cells in 6 of our series of 50 cases (including 12 cases with T-cell features, 3 cases of B-derived ALL with Burkitt’s cells, and 29 cases with the non-T non-B phenotype).

In these pre-B-cell leukemias, small amounts of cytoplasmic material reacting with antisera to \( \mu \) chains were found. An unusual perinuclear and reticular immunofluorescence staining pattern of these leukemic cells was observed that was similar to that found in the study of two cultured cell lines that showed pre-B-cell features. It is worth noting that immunoelectron microscopic study of these lines showed that the intracytoplasmic IgM was not located inside endoplasmic reticulum but at the level of free polyribosomes. Strikingly, no light-chain determinants could be detected by immunofluorescence in these leukemic pre-B cells (with the exception of a small percentage of blasts that were stained, in 2 cases, by the antiseraum to \( \lambda \) chains). This negative finding remains unexplained. It may merely be due to insufficient sensitivity of the immunofluorescence assay, since in the two cultured cell lines with pre-B-cell characteristics mentioned previously, monoclonal light chains could not be detected by immunofluorescence but were found at the ultrastructural level by an immunoperoxidase method. Molecular characterization of the intracytoplasmic immunoglobulin material by radiolabeling and biosynthesis experiments is clearly warranted.

The detection of a small amount of surface IgM on blast cells from 2 of our patients may indicate that the differentiation arrest of the leukemic clone had taken place beyond the pre-B-cell stage. As already pointed out by Vogler et al., \( \gamma \) and \( \alpha \) determinants were detected in a small percentage of the pre-B leukemic cells in 2 of our patients, but the significance of this finding is presently unsettled. In spite of the low percentage of immunoglobulin-bearing cells, we observed an imbalance in heavy- and light-chain distribution at the surface of the blood or marrow B lymphocytes in 2 of our patients (Table 1). This finding suggests that these relatively mature B cells may be involved in the leukemic process, but formal evidence for the monoclonality of pre-B-cell leukemia is still lacking.

The ALL antigen described by Greaves and the Ia-like antigens were found in 70% and 90% of non-T non-B ALL cases, respectively. It is now apparent that pre-B-cell ALL belongs to this ALL-antigen- and Ia-antigen-positive subgroup, as shown by the report of Vogler and by the present study. It is of interest that in the presently reported studies the pre-B leukemic cells were shown to contain TdT in a fair percentage of cases. TdT was initially considered as a characteristic enzyme of thymic and prethymic cells. However, suggestive evidence for the presence of TdT in early murine B-cell precursors has recently been presented. The presence of TdT in early pre-B cells might be related to the possible role of TdT in somatic mutations and in the generation of antibody diversity.

The clinical significance of pre-B-cell leukemias is still uncertain. The patients studied by Vogler et al. had no high-risk factors, and all entered complete remission. In contrast, 4 of our 6 patients had tumoral disease, 1 patient died during the induction phase with blastic bone marrow, and another patient relapsed 3 mo after the onset of remission. These findings show that a sizable proportion of patients with pre-B-cell leukemia do carry high-risk factors, as do patients with T-derived ALL. Future studies involving a larger number of patients are necessary.
in order to delineate the prognosis of this new subgroup of ALL and to work out possible therapeutic implications. In this regard, it should be noted that normal pre-B cells are sensitive to alkylating agents.9

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REFERENCES


*Since this manuscript was submitted, 4 new cases of ALL with such pre-B-cell characteristics have been found in our laboratory (among 18 patients).
Acute lymphoblastic leukemia with pre-B-cell characteristics

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