An Analysis of Clinical and Laboratory Features of Acute Lymphocytic Leukemias With Emphasis on 35 Children With Pre-B Leukemia

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In 35 of 191 patients with acute lymphocytic leukemia (ALL) malignant cells were similar in phenotype to B-lymphocyte precursors. Both these patients' lymphoblasts and normal pre-B-cells contain cytoplasmic immunoglobulin (Ig) µ heavy chains, but have no surface Ig. In patients with pre-B leukemias, lymphoblasts containing cytoplasmic µ chains alone were often accompanied by cells of identical morphology that expressed no Ig and less frequently by lymphoblasts bearing scant amounts of surface µ. This spectrum of cellular Ig expression suggests that "null," pre-B, and intermediate pre-B/B ALLs represent closely related malignancies with complete or partial arrests at different stages of maturation. When pre-B, B, T, and "null" cell categories of ALL were compared for 22 different clinical and laboratory features, including remission rate and short-term remission duration, no statistical differences were observed between the pre-B and "null" groups. These early results suggest that pre-B-cell leukemias represent a relatively good prognostic subclass of ALL, do not require more intensive treatment than that proven to be effective for "null" cell ALL, and should be distinguished from the less common, but more clinically aggressive, B-cell subclass of ALL. Longer follow-up will be required to confirm these preliminary conclusions.

Techniques

Leukemic cells were examined morphologically at member institutions and independently by a panel of four collaborating hematologists and one pathologist and classified according to F(French) A(American) B(British) criteria. In addition, leukemic cells were examined for Sudan black B, periodic acid Schiff, nonspecific esterase, chloroacetate esterase, and acid phosphatase cytochemical reactivities, for surface immunoglobulin, and for receptors of sheep erythrocytes, IgG, and the third component of complement by established and quality controlled techniques at member institutions. Cytoplasmic Ig was examined at the University of Alabama in Birmingham by a previously described method. At member institutions, cells were stained for surface Ig, centrifuged onto glass slides, fixed in cold 95% ethanol-5% acetic acid, washed, and mailed submerged in phosphate-buffered saline. Ia-like, T and "common ALL" antigens were examined at Duke University by microcytotoxicity assays using heteroantisera as described on vials labeled in heparinized RPMI-1640 media. Glucocorticoid receptors were determined by the dexamethasone binding assay described by Lippman. Serum immunoglobulin concentrations, determined at member institutions, were compared to local age-matched normal controls.

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Definitions

All leukemias were classified into pre-B, B, T, or “null” ALL subgroups according to the following criteria: pre-B, if ≥10% of marrow lymphoblasts contained cytoplasmic μ; B, if ≥10% of marrow lymphoblasts had easily detected surface Ig without cytoplasmic Ig; T, if ≥10% of marrow lymphoblasts formed rosettes with sheep erythrocytes at 4°C; and “null,” all ALLs lacking these defining features.

Statistics

The comparability of the features in the subclasses of ALL were tested using the Wilcoxon rank sum test when the data were not grouped. In cases in which the data are grouped in binary fashion, a test of comparability was performed by usual chi square techniques or Fisher’s exact test when appropriate. The survival and remission plots were computed using the Kaplan-Meier procedure.

RESULTS

Of the 191 patients with ALL examined, 35 (18.3%) were classified as pre-B, 127 (65.9%) “null,” 6 (3.1%) B, and 23 (12.0%) T cell in phenotype. The presenting clinical and laboratory features of pre-B-cell leukemia, including morphological, cytochemical, and additional immunologic characteristics of the leukemic cells, are given in Table 1. In no case did pre-B lymphoblasts bind sheep erythrocytes or express T-cell surface antigens. Cells from all 20 patients with pre-B ALL examined for Ia-like surface antigen were positive, and 19 of 20 demonstrated “common ALL” surface antigens. An equivalent proportion of patients with “null” ALL, 60 of 63 (95%), had cells that were “common ALL” positive. Expression of receptors for the Fc portion of IgG and for C3 was variable, but

<table>
<thead>
<tr>
<th>Table 1. Comparative Features of Subclasses of ALL*</th>
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<tbody>
<tr>
<td>Pre-B (n = 35)</td>
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<td>----------------</td>
</tr>
<tr>
<td>Demographic</td>
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<tr>
<td>Age (yr)</td>
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<td>Sex (male:female)</td>
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<td>Race (white:nonwhite)</td>
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<tr>
<td>Corporal</td>
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<tr>
<td>Liver (cm below RCM)</td>
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<tr>
<td>Spleen (cm below LCM)</td>
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<tr>
<td>Moderate to marked</td>
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<tr>
<td>Lymphadenopathy (%)</td>
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<tr>
<td>Mediastinal mass (%)</td>
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<tr>
<td>Extramedullary involvement (%)‡</td>
</tr>
<tr>
<td>Bone involvement (%)§</td>
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<tr>
<td>Hematologic</td>
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<tr>
<td>Hemoglobin (g/dl)</td>
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<tr>
<td>White cells (10⁹/liter)¶</td>
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<tr>
<td>Blood blasts (%)</td>
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<td>Marrow blasts (%)</td>
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<tr>
<td>Platelets (10⁹/liter)</td>
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<tr>
<td>Cell phenotypic</td>
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<tr>
<td>FAB L1 (%)</td>
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<tr>
<td>FAB L2 (%)</td>
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<tr>
<td>FAB L3 (%)</td>
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<tr>
<td>Periodic acid Schiff reactive (%)</td>
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<td>Acid phosphatase reactive (%)</td>
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<tr>
<td>IgG Fc Receptor* (%)</td>
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<tr>
<td>C3 Receptor* (%)</td>
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<tr>
<td>Glucocorticoid receptor (10⁻³ sites/cell)</td>
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<tr>
<td>Therapeutic</td>
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<td>Remission induction (%)**</td>
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</tbody>
</table>

*Expressed as arithmetic means.
†Not significant (p > 0.05).
‡Central nervous system or testicular infiltration or massive hepatosplenomegaly with margins below the umbilicus.
§Radiographic lytic lesions.
¶Median white cell counts for pre-B, “null,” B, and T-ALL groups were 15.9, 8.9, 16.0, and 43.8 x 10⁹/liter, respectively.
||Determined on cells of patients from the University of Alabama in Birmingham and Emory University in Atlanta. Dissociation constants for dexamethasone binding to pre-B, “null,” B, and T leukemic cells were 5.3 ± 0.3, 6.4 ± 0.8, 14.3 ± 5.65, 4.9 ± 2.5 nM, respectively.
**See Fig. 1 for chemotherapeutics administered.
I. REMISSION DURATION OF PATIENTS WITH DIFFERENT FORMS OF ALL

Fig. 1. Curves above were derived from children with ALL enrolled in treatment regimens of the Pediatric Division of the Southwest Oncology Group (SWOG). The maximum follow-up time for any patient is 36 mo. Patients with "null" and E-rosette-negative T-ALL and all but four patients with pre-B ALL were stratified on the basis of age and white counts into different prognostic risk groups. All patients received vincristine and prednisone induction with or without asparaginase, central nervous system prophylaxis consisting of 2400 rads of cranial irradiation, and 5 doses of intrathecal methotrexate given over 2.5 wk or intrathecal methotrexate, arabinosylcytosine and hydrocortisone given as every 2 wk doses for 12 wk and then given every 2 mo during maintenance. Maintenance therapy included daily 6MP, weekly methotrexate, and periodic reinforcement with vincristine and prednisone. Consolidation therapy varied with different treatment groups. Patients with E-rosette-positive T-ALL, B-ALL and the first four patients with pre-B ALL were treated with a more intensive chemotherapeutic protocol as described by Wollner et al. and modified by the SWOG.

Most leukemic pre-B-cells lacked them. None of these leukemic cells were positive for Sudan black B, nonspecific esterase, or chloroacetate esterase reactions, while periodic acid Schiff and acid phosphatase reactivities were variable. Both pre-B and "null" leukemic cells had high levels of specific receptor sites for glucocorticoids.

No striking elevations, depressions, or monoclonal representation of serum immunoglobulins were observed in patients with pre-B ALL. With regard to cellular immunoglobulin expression, however, considerable heterogeneity was observed. The mean percentage of marrow lymphoblasts containing intracytoplasmic Ig μ chains was 71 (normal, 1.0% ± 0.6% of nucleated bone marrow cells), but the range between individual patients was broad (12.0%–99.5%). In most patients, a significant subpopulation of leukemic cells did not express Ig determinants, while μ expression was common to both μ+ and μ− members of the population of lymphoblasts in these individuals. Three patients had both pre-B leukemic cells and an additional subpopulation (24.8%, 49.2%, and 60.7%, respectively) identical in size, morphology, and cytoplasmic μ staining characteristics, which also expressed scant amounts of surface μ. In five cases, more than 10% of the leukemic cells expressed γ heavy chains. When examined for kappa and lambda light chain expression, cells from the majority of patients with pre-B ALL were negative. The variable expression of Ig heavy and light chains in these individuals is the subject of a separate report.

When the features of pre-B-cell leukemia were compared with those of the other immunologically stratified groups of ALL (Table I), no statistically significant differences between the pre-B and "null" cell groups were identified. Furthermore, over a relatively short period of observation (up to 156 wk), a preliminary analysis indicated that the duration of remission and overall survival of patients with pre-B-cell leukemia was parallel to that of patients in the "null" cell group (Figs. 1 and 2). This correlates with the presence of "common" ALL antigen, associated with a favorable prognosis, on cells from the majority of patients in each group. The single patient with...
“common” ALL antigen-negative pre-B leukemia was still in remission at 12 mo. Of three patients with “common” ALL antigen-negative “null” leukemia, one was in remission at 12 mo, one died on the first day of therapy, and one died of infection in remission at 3 mo.

DISCUSSION

In addition to our initial study, lymphoblastic leukemia of pre-B phenotype has been reported in both children and adults by other investigators. The 35 cases of pre-B leukemia presented here provide a better appreciation of the clinical spectrum and cellular heterogeneity of this ALL subgroup.

The incidence of pre-B-cell leukemia among ALLs in this analysis was very similar to that observed originally, 18.3%. When compared with other immunologically defined groups of ALL with regard to multiple clinical and laboratory features, pre-B-cell leukemias were distinguishable from the “null” cell group only by their expression of cytoplasmic immunoglobulin. As previously observed, cells from the majority of patients with pre-B leukemia bore the “common” ALL surface antigen defined by Greaves et al., indicating that pre-B leukemia is largely a subset of the “common” ALL group. Since pre-B leukemia in 1 of 20 patients in our study and 1 of 29 patients reported by Greaves et al. lacked “common” ALL antigen, these ALL subgroups do not entirely overlap. With regards to morphology, it is of interest that 1 of 31 patients with pre-B leukemia had cells of L3 type, while 3 of 4 patients with B-ALL had L3 cells. Acid phosphatase reactivity was higher in T-cell ALL than in other groups, but the difference was not statistically significant. This lack of acid phosphatase discrimination for T-cell ALL in our study was very similar to that observed in previous studies. In this analysis, the genetic mechanisms of isotype switching may be functional at a very early stage in B-cell differentiation. Leukemic cells expressing these additional heavy chains, traditionally associated with more mature stages of development, and those expressing no immunoglobulin determinants were indistinguishable from accompanying lymphoblasts containing μ chains only, on morphological and cytochemical examination.

The observation of subpopulations of leukemic blasts having “null,” pre-B, and intermediate pre-B/B cell features in the same patient suggests that “null” ALL and pre-B ALL may be malignancies of the same cell lineage with variable progression of differentiation. In support of this view is the fact that most “null” ALL cells bear membrane DR antigens and B-lineage-specific differentiation antigens that are also expressed on normal pre-B and B lymphocytes. Moreover, the recent observation of Philadelphia chromosome-positive lymphoblasts with pre-B-cell features in patients with chronic myelogenous leukemia in blast crisis indicates that oncogenic events occurring at the level of the hematopoietic stem cell may lead to malignancies involving daughter cells within the B-cell pathway of differentiation.

An important observation from this study is the similarity between the pre-B and “null” leukemic groups in remission induction and duration and in overall survival during the period of study. In this regard, it should be noted that “common” ALL antigen, which has been shown to correlate with a favorable prognosis, was present on both pre-B and “null” leukemic cells in 95% of patients examined. Too few patients with “common” ALL antigen-negative cells were present in either group to provide a meaningful separate analysis or to significantly alter remission and survival curves. In sharp contrast to the pre-B and “null” leukemia groups was the outcome of patients with cells having a mature B-cell phenotype; i.e., expressing readily detectable amounts of surface Ig.
and lacking cytoplasmic Ig. As in other series, high numbers of glucocorticoid receptors in pre-B and "null" leukemic cells, representing early differentiation arrests, and low numbers of specific receptors in T and B leukemic cells suggest that glucocorticoid receptors may be lost during maturation within both the T- and B-cell pathways of differentiation. Lippman et al. have shown a positive correlation between the quantity of glucocorticoid receptors and the duration of complete remission in patients with ALL independent of cell type. Whether or not the number of receptors per cell in the different subgroups of ALL as described here is related to cellular maturation or to clinical prognosis remains controversial.

REFERENCES


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