CONCISE REPORT

Heterogeneity of Cell Lineages in L3 Leukemias

By Benjamin Koziner, Roland Mertelsmann, Michael Andreeff, Zalmen Arlin, Herbert Hansen, Etienne De Harven, Susan McKenzie, Timothy Gee, Robert A. Good, and Bayard Clarkson

Five cases of adult leukemia with L3 morphology in bone marrow were studied for the presence of immunologic, metabolic, and enzymatic markers. Among the five patients, there were four males and one female. Median age was 66 with a range of 16–80 yr. Median survival was only 5 mo. Serum lactate dehydrogenase (LDH) levels, 3H-thymidine labeling indices, and DNA/RNA content of the 13 lymphoblasts were markedly elevated. B-cell markers were found in three cases. Two of 16–80 yr. Median survival was only 5 mo. Serum lactate dehydrogenase (LDH) levels, 3H-thymidine labeling indices, and enzymatic markers. Among the five patients, there were four males and one female. Median age was 66 with a range of 16–80 yr. Median survival was only 5 mo. Serum lactate dehydrogenase (LDH) levels, 3H-thymidine labeling indices, and DNA/RNA content of the 13 lymphoblasts were markedly elevated. B-cell markers were found in three cases. Two of

A TTEMP TS to classify the leukemic processes on the basis of the in vitro growth, biochemical, enzymatic, and immunologic properties of the neoplastic cell populations are currently underway.1,2 However, at the present, taxonomic schemes based on the morphological features of the leukemic cells under light microscopy are needed to facilitate the communication of therapeutic results and allow correlation with the findings of a rapidly expanding methodology.

In recent years, the new scheme proposed by the French-American-British Cooperative Group3 has been applied to the morphological classification of cases with leukemia seen at the Hematology/Lymphoma Service of Memorial Sloan-Kettering Cancer Center. Among 88 cases of adult acute lymphatic leukemia seen over a 2-yr period, 5 cases met requirements for classification in the L3 category. Immunologic characterization of the L3 lymphoblasts in these 5 patients revealed a heterogeneity of cell lineages despite cell kinetic measurements indicating similar rapidly proliferating cell populations.

MATERIALS AND METHODS

Clinical characterization of the cases under study is described in Table 1. All patients had bone marrow involvement on presentation, but only 2 (cases 2 and 3) had circulating leukemic cells. Review of the available histologic material in nodal and extranodal tissues in patients 1–4 revealed an “undifferentiated” or Burkitt lymphoma according to the taxonomic scheme of Rappaport.4 Patient 5 had a histologic diagnosis of large cell or diffuse histiocyctic lymphoma based on a specimen of involved skin. Despite intensive chemotherapy with different protocols used at our center (including the administration of vinca alkaloids, corticosteroids, alkylating agents, anthracyclines, and antimetabolites), survival from the time of diagnosis was short with a median of 5 mo for the 5 patients.

Inclusion in the L3 category required that the cells were large and characteristically homogeneous; they had a dense but finely stippled nuclear chromatin; the nucleolus was oval to round and regular with or one more prominent vesicular nucleoli; the cytoplasm was moderately abundant, intensely basophilic, and completely surrounded the nucleus, and prominent cytoplasmatic vacuolation was present in a majority of the cells.5

Independent review of the peripheral blood and/or bone marrow material was carried out on tetrachrome-stained smears by four different investigators. Cytochemical characterization of the neoplastic cells included staining for acid phosphatase (AP),5 PAS,6 sudan black (SB), alpha-naphthyl acetate esterase (α-NAE), and naphthol-ASD chloro acetate esterase (N-ASD),7 as previously reported.

Lymphocyte preparation and immunofluorescent staining were performed as previously reported. A polyvalent rabbit antiserum to human immunoglobulin and antisera specific for gamma, mu, alpha, delta, kappa, and lambda light chains were used. Fluorescein conjugated Fab1 fragments of specific antisera to gamma, mu, alpha, and delta heavy chains were purchased from Kallestad Laboratories (Chaska, Minn.). Indirect binding of aggregated IgG was performed as reported by Dickler et al.8 The cells were examined with a Leitz Ortholux microscope equipped with fluorescent illumination. Spontaneous rosette formation with sheep erythrocytes (SRBC-rosette) was performed as described by Bentwich et al.9 SRBC-rosette determination after incubation at 37°C for 1 hr was carried out as reported by Borella and Sen.10 High avidity Fe receptors on mononuclear cells were determined with a human EA (Ripley) rosette technique11 modified from Fröland and Natvig.12 EAC rosettes were prepared as described by Shevach et al.13 Terminal deoxynucleotidyl transferase (TdT) enzymatic activity was determined in cell homogenates as previously reported.14

Cell suspensions from peripheral blood, bone marrow, or solid tissues were incubated in McCoy's 5 A medium containing 1 μCi/ml of tritiated thymidine (3H-TDR, specific activity 6 Ci/mmole) for 1 hr at 37°C. Autoradiographs were taken from...
cytocentrifuge preparations as per previous description. The \( ^{1}H\)-TDR labeling index was determined as percentage of all leukemic cells with more than 5 grains over the nucleus. Simultaneous measurement of DNA and RNA content of single unfixed cells using acridine orange and a computer interfaced flow cytometer was carried out as previously reported. The RNA index (RI) calculated from the \( G_0/G_1 \) RNA content of the sample over the median \( G_0/G_1 \) RNA content of control lymphocytes multiplied by 10, represented the relative RNA value of the sample as compared with the reference.

For transmission electron microscopy (TEM), Ficoll-Hypaque-separated cells were fixed in 2.5% glutaraldehyde in RPMI 1640, postfixed in osmium tetroxide, embedded in epon, thin sectioned, and studied with Siemens 101 electron microscope following routine procedures.

**RESULTS**

In all cases more than 50% of the bone marrow cellularity was represented by blastic cells with the characteristic L3 morphology (Table 1). Cytocentrifuge preparations as per previous description. The \( ^{1}H\)-TDR labeling index was determined as percentage of all leukemic cells with more than 5 grains over the nucleus. Simultaneous measurement of DNA and RNA content of single unfixed cells using acridine orange and a computer interfaced flow cytometer was carried out as previously reported. The RNA index (RI) calculated from the \( G_0/G_1 \) RNA content of the sample over the median \( G_0/G_1 \) RNA content of control lymphocytes multiplied by 10, represented the relative RNA value of the sample as compared with the reference.

For transmission electron microscopy (TEM), Ficoll-Hypaque-separated cells were fixed in 2.5% glutaraldehyde in RPMI 1640, postfixed in osmium tetroxide, embedded in epon, thin sectioned, and studied with Siemens 101 electron microscope following routine procedures.

---

**Table 1. Clinical Characteristics of 5 Patients With L3 Leukemia**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (Cells/10^9)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>Platelets (Cells/10^9)</th>
<th>Bone Marrow Blasts (%)</th>
<th>Lymph Nodes</th>
<th>Bone</th>
<th>Gastro-intestinal</th>
<th>Central Nervous System</th>
<th>Other</th>
<th>Survival From Diagnosis (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 M</td>
<td>18.8</td>
<td>11.7</td>
<td>235</td>
<td>56</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>46 M</td>
<td>45.4</td>
<td>9.5</td>
<td>90</td>
<td>93</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>68 M</td>
<td>11.3</td>
<td>11.9</td>
<td>76</td>
<td>92</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Subcutaneous tissue</td>
<td>+</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>80 F</td>
<td>6.7</td>
<td>10.1</td>
<td>445</td>
<td>90</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>-</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>66 M</td>
<td>11.6</td>
<td>9.8</td>
<td>136</td>
<td>57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Skin</td>
<td>+</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

---

**Fig. 1.** Acid phosphatase staining of L3 lymphoblasts in case 5.

**Fig. 2.** Transmission electron microscopy of L3 lymphoblasts in case 5 (X 7200).
fourth patient, no characteristic surface phenotype could be determined with a predominance of cells failing to display SRBC rosetting or surface immunoglobulin. However, in two separate occasions a significant proportion of the blastic population was Fc-receptor-positive as determined by the binding of heat-aggregated IgG and EA rosetting by the Ripley method. TdT activity was found to be markedly elevated to the levels seen in cases of "non-B-non-T ALL."4 The fifth patient had a predominance of blasts that were capable of forming spontaneous rosettes with SRBC at both 4°C and 37°C. However, no TdT activity was found.

High levels of serum lactate dehydrogenase (LDH) were observed in all patients with a mean value of 4406 U/liter. The 3H-thymidine labeling index was significantly elevated in the 3 patients that were studied, arguing further about the rapid rate of proliferation of the cells involved. Figure 3 illustrates the computer-drawn three-dimensional DNA-RNA bone marrow histogram in patient 5 with a T-cell, TdT-negative phenotype and a diploid DNA content. A similar pattern with regard to DNA-RNA distribution, except for hyperdiploidy was observed in patient 3 with a monoclonal B-cell phenotype (Fig. 4). The RNA index was above 18 in both patients. In a series of 26 patients with acute lymphatic leukemia with either L1 or L2 morphology seen at Memorial Hospital, the mean RNA index was 11.8 ± 2.0.16

**DISCUSSION**

The taxonomic scheme proposed by the French-American British Cooperative Group appears to be of clinical usefulness in classifying the different leukemic types on the basis of their morphological appearance under light microscopy.3 Correlation with cell marker analysis will follow, helping to determine the accuracy of this type of approach. Although an L3 morphology was originally described to represent the leukemic phase of an underlying Burkitt or undifferentiated lymphoma of monoclonal B-cell type,3,17 we have found it related to cell lineages other than B. Since in all cases in the present series, as well as in the reported series in the literature,18,20 tissue masses were frequently observed at presentation or upon progression of the disease, the processes under discussion could be considered as the leukemic phase of an underlying malignant lymphoma, more frequently of the Burkitt or "undifferentiated" variety. However, in
one of our patients, the histologic diagnosis on the skin was large cell lymphoma or diffuse histiocytic lymphoma according to Rappaport's scheme. This patient had a T-cell, TdT-negative phenotype. It could be postulated that the low TdT level of this rapidly proliferating T-cell malignancy may reflect a further maturation stage along the thymic lineage, since in the overwhelming majority of other cases with "non-B–non-T" and T acute lymphatic leukemia or malignant lymphoma of lymphoblastic type the levels of enzymatic activity of TdT have been found to be markedly elevated.2,14,21

Another patient failed to reveal a predominance of either T or B cells. However, an increase in cells carrying Fc receptors for immunoglobulin IgG was found. Although the presence of only Fc receptors on these cells may have suggested a monocytic or histiocytic lineage,11 the failure to stain with nonspecific esterases as well as the high levels of enzymatic activity of TdT strongly argued in favor of a lymphoid lineage. However, no further specimens could be obtained to perform separation studies on the cell suspension to determine whether only a subfraction of this population carried high levels of TdT activity.

A consistent feature found in all cases of L3 leukemia was the high level of serum LDH. High levels of LDH have been previously described in Burkitt lymphoma22 and in diffuse histiocytic lymphomas,23 representing a bad prognostic parameter. Moreover, the pulse 3H-thymidine labeling index—which is dependent on the growth fraction as well as on the duration of S phase and the generation time15—was markedly increased in the patients studied, with a mean value of over 30%. The mean labeling indices found in other types of non-Hodgkin lymphoma studied were 5.4% for diffuse poorly differentiated lymphocytic and 20% for diffuse histiocytic lymphomas, respectively.24 Mean values for acute lymphatic and acute nonlymphatic leukemias were 10.6% and 8%, respectively.13 This reflects the very rapid rate of proliferation of these leukemias above the level found in other types of lymphoid neoplasia. In addition, this cell population revealed high proliferation and RNA content above the values found in L1 and L2 lymphatic leukemias.16

It is concluded that although most of the L3 leukemias are neoplasias of B lymphocytes, other lineages may also express this morphology. Regardless of the lineage involved, the high serum LDH levels, 3H-thymidine labeling indices, and DNA/RNA cellular content argue in favor of the rapid rate of proliferation of the neoplastic L3 cells.

REFERENCES

20. Brunning RD, McKenna RW, Bloomfield CD, Coccia P,


Heterogeneity of cell lineages in L3 leukemias

B Koziner, R Mertelsmann, M Andreeff, Z Arlin, H Hansen, E De Harven, S McKenzie, T Gee, RA Good and B Clarkson