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 L3 lymphoblasts were markedly elevated. B-cell markers were found in three cases, two exhibiting surface membrane IgM-λ, and one IgG-K. Terminal deoxynucleotidyl transferase (TdT) enzymatic activity was consistently low in this group. In one case, the L3 lymphoblasts displayed only surface Fc receptors demonstrated by the binding of aggregated IgG. TdT activity was found to be significantly increased. In another instance, the lymphoblasts formed spontaneous rosettes with sheep erythrocytes and exhibited paranuclear staining with acid phosphatase. TdT activity was found to be low. Although most of the L3 leukemias are neoplasias of B lymphocytes, other lineages may also express this morphology.

Attempts 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. 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 Group 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. Patient 5 had a histologic diagnosis of large cell or diffuse histiocytic 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 nucleus was oval to round and regular with one or more prominent vesicular nucleoli; the cytoplasm was moderately abundant, intensely basophilic, and completely surrounded the nucleus, and prominent cytoplasmic vacuolation was present in a majority of the cells.

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), PAS, sudan black (SB), alpha-naphthyl acetate esterase (alpha-NAE), and naphthol-ASD chloroacetate esterase (N-ASD), as previously reported.

Lymphocyte preparation and immunofluorescent staining were performed as previously reported. A polyclonal rabbit antisera to human immunoglobulin and antisera specific for gamma, mu, alpha, delta, kappa, and lambda light chains were used. Fluorescein conjugated Fab' 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. 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. SRBC-rosette determination after incubation at 37°C for 1 hr was carried out as reported by Borella and Sen. High avidity Fc receptors on mononuclear cells were determined with a human EA (Ripley) rosette technique modified from Fröland and Natvig. EAC rosettes were prepared as described by Shevach et al.

Terminal deoxynucleotidyl transferase (TdT) enzymatic activity was determined in cell homogenates as previously reported. Cell suspensions from peripheral blood, bone marrow, or solid tissues were incubated in McCoy's 5 A medium containing 1 uCi/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. A 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 G0/G1 RNA content of the sample over the median G0/G1 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). Cytological characterization of the neoplastic cells was essentially negative after testing with SB, α-NAE, N-ASD, and PAS, except for case 3 in which the lymphoblasts were positive for PAS (digested with diastase). Patient 1 showed a mild and diffuse staining with AP and patient 5 a strong polar staining, suggesting increased enzymatic activity of AP in the paranuclear Golgi zone. Again, in patient 5, on TEM the cell surface displayed pseudopods but practically no microvilli. The cytoplasm had little rough endoplasmic reticulum and a large number of free ribosomes. Many large intracytoplasmic lipid vacuoles were observed in most cells. The nuclei showed a mottled appearance of condensed heterochromatin and 2 or 3 nucleoli. The nuclear envelope occasionally showed a deep cleft, more discernible in ultrathin sections than under the light microscope (Fig. 2).

Three of the patients under study had a B-cell monoclonal phenotype with two patients carrying predominately surface membrane IgM of lambda type and one patient IgG of kappa light chain type (Table 2). This immunoglobulin pattern persisted after overnight incubation in serum-free medium and staining with Fab'2 fragments of immunoglobulin. TdT enzymatic activity was undetectable in this group. In the

<table>
<thead>
<tr>
<th>Table 1. Clinical Characteristics of 5 Patients With L3 Leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic Values</strong></td>
</tr>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<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." 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,3-7 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,8-20 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 maturational 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 fraction 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.15 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

1. Koziner B, Filippa D, Mertelsmann R, Gupta S, Clarkson B, Good RA, Siegal FP: Characterization of malignant lymphomas in leukemic phase by multiple differentiation markers of mononuclear cells: Correlation with clinical features and conventional morphol-


10. Borella L, Sen L: E receptors on blasts from untreated acute lymphocytic leukemia (ALL). Comparison of temperature depen-


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