Lactate Dehydrogenase Isoenzymes in Normal and Malignant Human Lymphoid Cells

By Julie Blatt, Robert J. Spiegel, Nick M. Papadopoulos, Spiros A. Lazarou, Ian T. Magrath, and David G. Poplack

Intracellular lactate dehydrogenase (LD) isoenzyme patterns were studied in the malignant cells of patients with a variety of lymphoid malignancies. These were compared with intracellular LD isoenzyme patterns of normal lymphoid cells and were also correlated with immunologic cell surface marker characteristics. Results showed that, in general, the malignant B cells of Burkitt’s lymphoma and the lymphoblasts of T-cell acute lymphoblastic leukemia had isoenzyme patterns similar to those of normal B and T cells, respectively. The isoenzyme patterns of malignant lymphoid cells from patients with non-T, non-B acute lymphoblastic leukemia, cutaneous T-cell lymphoma, and chronic lymphocytic leukemia were more heterogeneous. These data, although based on small numbers of patients, are consistent with the hypothesis that LD isoenzymes may reflect differences in the maturational status of cells within a single diagnostic category.

NORMAL AND MALIGNANT CELLS are now routinely categorized according to immunologic cell surface markers. Recently, a number of biochemical abnormalities have been described in malignant lymphoid cells that are useful for further classifying subsets of leukemia and lymphoma.1

Lactate dehydrogenase (LD), a glycolytic enzyme that converts lactate to pyruvate, is a tetramer formed from two subunits, the varied combination of which results in five isoenzymes, LD-1 through LD-5. Analysis of intracellular LD isoenzymes has been shown to distinguish normal B from T lymphocytes.2,3 Moreover, peripheral blood T cells and thymocytes have unique isoenzyme patterns, and it has been postulated that LD isoenzyme patterns reflect difference in the stage of T-cell differentiation.4 Whether intracellular LD isoenzyme patterns similarly discriminate between subtypes of malignant lymphoid cells is not clear.

In addition, total serum LD is a useful indicator of tumor burden and prognosis in non-Hodgkin’s lymphoma.5,6 Marked elevations of serum LD have also been described in leukemia, particularly acute lymphoblastic leukemia.7,8

In the present study, we compared the LD isoenzyme electrophoretic patterns of malignant cells from patients with a variety of lymphoid malignancies with those from normal donors, and in some instances, with the LD isoenzyme patterns of simultaneously drawn serum samples. Isoenzyme patterns were also examined for possible correlation with the immunologic surface marker characteristics of these cell populations.

MATERIALS AND METHODS

Cells

Leukemic cells were obtained from the peripheral blood or bone marrow of 18 patients with acute lymphoblastic leukemia (ALL), 11 patients with chronic lymphocytic leukemia (CLL), 5 patients with cutaneous T-cell lymphoma (CTCL), and 2 patients with Burkitt’s lymphoma. In addition, malignant cells were obtained from well characterized B-cell lines derived from 7 other patients with Burkitt’s lymphoma.9 Diagnoses were based on clinical presentation and standard histologic criteria.10 In all cases, samples consisted of greater than 80% abnormal cells. Samples were processed and used immediately or cryopreserved as previously described.11 Leukocytes from three normal donors were similarly separated from preservative-free heparinized whole blood and fractionated into T and B cells (99% purity).12 Thymocytes were obtained from three children undergoing surgery for congenital cardiac defects who were otherwise well.

For use in isoenzymes assays, fresh or thawed cell pellets were treated with ammonium chloride in order to remove contaminating erythrocytes, washed twice, and resuspended in 0.45% saline at a concentration of 106 cells/ml. Viability, assessed by trypan blue exclusion, was greater than 90% in all instances. Initial studies had shown that cryopreservation of leukemic lymphoid cells resulted in no alteration of LD isoenzyme patterns.

Cells were lysed by alternately freezing and thawing two times. The resulting suspension was centrifuged, and the supernatant used for LD isoenzyme assays.

Sera

Where possible, serum samples were collected the same day as cell samples from patients whose total serum LD values were greater than twice the normal values. In no instance was there an obvious etiology, other than tumor, for the increase in LD (e.g., serum transaminases were normal).

LD Isoenzyme Assay

LD was separated into its component isoenzymes by agar gel electrophoresis. Isoenzymes were quantitated colorometrically using tetrazolium reduction followed by densitometric scanning as previously described.11

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Cell Surface Markers

Cell samples were examined for the presence of T-cell markers [spontaneous neuraminidase-treated sheep erythrocyte (ER) formation] and B-cell markers [surface immunoglobulin (SIg)] as previously described. Samples containing >20% ER+ abnormal cells were considered to be T-cell populations. Samples containing >10% SIg+ abnormal cells were considered to be B-cell populations. Non-T, non-B samples were those that had fewer than 20% ER+ and 10% SIg+ cells. Non-T, non-B lymphoblasts were further examined for the presence of common acute lymphoblastic leukemia antigen (cALLA). Non-T, non-B lymphoblasts and CTCL samples, where possible, also were examined for reactivity with a panel of T-cell-specific monoclonal antibodies, as previously reported.

RESULTS

Electrophoretic and immunologic characteristics of the samples studied are shown in Table 1. Representative electrophoreses are shown in Fig. 1. Normal peripheral blood T and B lymphocytes could readily be distinguished on the basis of their isoenzyme patterns. In all samples, LD-3 was prominent. However, in B lymphocytes, there was a relative shift to the right (defined arbitrarily as LD-4+5 > LD-1+2), whereas T lymphocytes had a "left shift" (LD-1+2 > LD-4+5). Thymocytes were clearly different from mature T cells, and in each case, despite variability in actual percentages, had large amounts of LD-3 with almost equal amounts of LD-2 and LD-4.

The cells from all the Burkitt's lymphoma samples had LD isoenzyme patterns like those of normal B lymphocytes. Similarly, the lymphoblasts from 7/9 T-ALL patients had LD isoenzyme patterns like those of normal T lymphocytes. The percentage of LD-1 and LD-2 did not appear to correlate with the percentage of ER+ cells (data not shown). Cell samples from the remaining two T-cell ALL patients had LD isoenzyme patterns like those seen in thymocytes.

Among the non-T, non-B ALL samples, LD isoenzyme patterns were variable, although a correlation was seen between LD patterns and cALLA reactivity, as is shown in Table 2. Five of six cALLA+ samples showed a B-cell-like pattern, whereas cALLA- cells showed a pattern similar to that of thymocytes. Of the three cALLA- cell populations, two were reactive with an anti-T-cell antiserum. These differences in LD isoenzyme patterns could not be attributed to differences in sample source, i.e., bone marrow or peripheral blood.

Variability was also seen in the LD patterns of the CTCL samples. Two of the 5 samples (53% and 78% ER+) had LD isoenzyme patterns like those of mature B cells. Two of five (100% and 46% ER+) had patterns like those of thymocytes. The latter samples contained >70% abnormal cells reactive with anti-T-cell antisera. The remaining sample had no ER+ or SIg+ cells, and had an LD isoenzyme pattern more like that seen in B cells; no data are available on the anti-T-cell reactivity of this sample.

In addition, there was considerable heterogeneity among the CLL samples, all of which were B-cell

Table 1. LD Isoenzymes and Immunologic Characteristics of Normal and Malignant Lymphoid Cells

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cell Type</th>
<th>No. Samples</th>
<th>Percent Distribution (Mean ± SDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Peripheral</td>
<td>B</td>
<td>3</td>
<td>LD-1 22 ± 4 LD-2 29 ± 4 LD-3 29 ± 1 LD-4 15 ± 4</td>
</tr>
<tr>
<td>blood lymphocytes</td>
<td>T</td>
<td>3</td>
<td>24 ± 2 36 ± 2 29 ± 0 9 ± 0.7 2 ± 0</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>T</td>
<td>3</td>
<td>10 ± 5 13 ± 6 47 ± 20 14 ± 6 16 ± 8</td>
</tr>
<tr>
<td>Burkitt's lymphoma</td>
<td>B</td>
<td>9</td>
<td>5 ± 2 18 ± 3 31 ± 2 31 ± 3 15 ± 6</td>
</tr>
<tr>
<td>CLL</td>
<td>B</td>
<td>9</td>
<td>8 ± 4 19 ± 4 40 ± 13 22 ± 7 11 ± 10</td>
</tr>
<tr>
<td>ALL</td>
<td>T</td>
<td>9</td>
<td>13 ± 4 31 ± 6 36 ± 8 16 ± 7 4 ± 1</td>
</tr>
<tr>
<td>CTCL</td>
<td>Non-T, non-B</td>
<td>9</td>
<td>6 ± 4 20 ± 8 39 ± 3 28 ± 6 7 ± 2</td>
</tr>
<tr>
<td></td>
<td>T*</td>
<td>4</td>
<td>13 ± 7 24 ± 5 39 ± 11 20 ± 6 4 ± 4</td>
</tr>
<tr>
<td></td>
<td>Non-T, non-B†</td>
<td>1</td>
<td>16 ± 2 20 ± 31 ± 32 ± 11 ± 1</td>
</tr>
</tbody>
</table>

*Rosetting criteria defined in text.
†Reactivity with anti-T-cell antisera not tested.
zyme patterns consistent with that found in normal populations as defined above. Eight of 11 had isoenzyme patterns consistent with that found in normal mature B cells. Three of 11 had T-cell or thymocyte-like LD isoenzyme patterns. As with ALL samples, no correlation was seen between SIg+ or ER+ cell percentages and isoenzyme patterns.

A comparison of LD isoenzyme percent distributions in cells and serum from five patients is shown in Table 3. While there are some similarities, it is clear that the correlation for individual patients is not precise.

DISCUSSION

Normal and malignant lymphoid cells routinely have been classified as T or B cells according to their ability to rosette with sheep erythrocytes and the presence or absence of surface immunoglobulin. More refined criteria, including those defined by monoclonal antibodies and functional assays, have strengthened the biologic basis for this immunologic classification scheme.

Absolute levels and isoenzyme patterns of a number of enzymes have also been found to distinguish immunologic subsets of lymphoid cells. Results of the present study confirm previous reports that unique intracellular isoenzyme patterns of LD are seen in normal B and T lymphocytes. Mature T cells and thymocytes also have patterns that are generally distinguishable.

Moreover, these results indicate that LD isoenzymes in part may also reflect the T- or B-cell origins of malignant cells. Thus, abnormal cells from patients with Burkitt's lymphoma, which are of B-cell origin by a variety of immunologic criteria, including the presence of complement receptors and surface immunoglobulin, demonstrate an isoenzyme pattern identical to that found in normal B cells. In contrast, abnormal cells from patients with T-cell acute lymphoblastic leukemia have isoenzyme patterns (although not necessarily identical percentages of LD isoenzymes) similar to those of normal T lymphocytes or thymocytes.

Interestingly, lymphoblasts from different patients with non-T, non-B ALL that are indistinguishable by routine immunologic techniques are heterogeneous with respect to LD isoenzyme analysis. In one previous report, subsets of normal thymocytes were found to vary in LD-1 content according to their degree of maturation: more mature thymocytes had relatively more LD-1 than did less mature thymocytes. This consideration may explain, in part, the variability within the non-T, non-B leukemia population as well as in the malignant cells of patients with cutaneous T-cell lymphomas. Variability in LD isoenzyme patterns may, in part, also be attributable to contamination by nonmalignant cells. We do not feel, however, that this is likely to be a major source of error, as all samples had at least 80% abnormal lymphoid cells.

While all non-T, non-B lymphoblast samples showed a predominance of LD-3, samples from patients who were cALLA positive had a "right shift" of intracellular LD isoenzymes (LD-4+5 > LD-1+2) similar to that seen in normal B lymphocytes. The reproducibility of this association will require further study. More refined immunologic characterization, such as cytoplasmic immunoglobulin determination, may clarify the relationship of LD isoenzymes within this group.

In this study we did not find a clear correlation between immunologic marker status and LD isoenzyme patterns in CLL. Our data are consistent with a recent report in which the fractionated T and B cells from patients with CLL were shown to have LD isoenzyme patterns distinguishable from those seen in T and B lymphocytes from normal individuals.

The possible prognostic implications of intracellular LD isoenzyme patterns in lymphoid malignancy have not yet been examined. In contrast, total serum LD levels have been correlated with tumor burden and prognosis in non-Hodgkin's lymphoma. In a recent report, it was suggested that elevated serum LD "may relate...to total leukemia cell mass," although values did not correlate either with initial white blood cell or circulating lymphoblast count. This lack of correlation between total serum LD and lymphoblast count is consistent with data from five patients, which showed a poor correlation between serum and intracellular LD isoenzyme patterns. The explanation for this finding is unclear, although one possibility is that a significant

Table 2. Possible Correlation Between Surface Markers and cALLA Reactivity in Non-T, Non-B ALL*

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Diagnosis</th>
<th>Percent Distribution</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ALD-1</td>
<td>ALD-2</td>
</tr>
<tr>
<td>1</td>
<td>Burkitt's lymphoma</td>
<td>11/9</td>
</tr>
<tr>
<td>2</td>
<td>Burkitt's lymphoma</td>
<td>3/7</td>
</tr>
<tr>
<td>3</td>
<td>ALL</td>
<td>3/32</td>
</tr>
<tr>
<td>4</td>
<td>ALL</td>
<td>3/9</td>
</tr>
<tr>
<td>5</td>
<td>ALL</td>
<td>9/32</td>
</tr>
</tbody>
</table>

*See Table 1 for normal lymphocyte LD isoenzymes.

Table 3. LD Isoenzyme Patterns in Malignant Cells and Simultaneously Drawn Serum

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Diagnosis</th>
<th>Percent Distribution</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ALD-1</td>
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<td>ALL</td>
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</tr>
<tr>
<td>5</td>
<td>ALL</td>
<td>9/32</td>
</tr>
</tbody>
</table>
contribution to serum LD may come from nonleukemic sources.

Our results suggest that intracellular isoenzyme analysis may be a useful adjunct in defining the biochemical profile of normal and malignant lymphoid cells.

ACKNOWLEDGMENT

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REFERENCES

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