Diversity of Immunological Phenotypes of Lymphoblastic Lymphoma

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ABSTRACT

Eleven cases of lymphoblastic malignancy, presenting as lymphoma, were investigated for immunological and differentiation markers prior to the onset of therapy. Biopsy specimens exhibited the typical morphological features of lymphoblastic lymphoma (convoluted T-cell lymphoma). Intranuclear terminal deoxynucleotidyl transferase was detected in the neoplastic cells from each case by indirect antibody staining of cytocentrifuge preparations. Eight cases were T-cell type as evidenced by unsensitized sheep erythrocyte rosette formation and staining with the monoclonal antibody OKT11. Three T-cell cases were OKT4 positive, two were OKT8 positive, and none were positive with both OKT4 and OKT8. Three cases failed to react with any monoclonal antibodies specific for T-cells and did not form unsensitized sheep erythrocyte rosettes or stain for surface immunoglobulin. However, these three cases were la positive and J5 (common acute lymphoblastic leukemia antigen) positive. Cells from two of these erythrocyte rosette-negative, la-positive, common acute lymphoblastic leukemia-positive cases contained intracytoplasmic heavy chains and were therefore of pre-B-cell phenotype. These cases were histologically indistinguishable from the T-cell cases. However, clinically, they were distinguished by the absence of mediastinal masses and by a clinical presentation as isolated lytic lesions of bone in two of the three. OKT9 and OKT10 stained neoplastic cells from T-cell, as well as pre-B-lymphoblastic, lymphoma. Although morphologically homogeneous, lymphoblastic lymphomas are comprised of an immunologically diverse group of neoplasms which include cells of "common" and "mature" thymocyte, non-T, non-B, and pre-B phenotypes and are closely related to the cells of acute lymphoblastic leukemia. In addition, intratumor heterogeneity was observed in most instances and may reflect growth or differentiation differences between subpopulations of individual neoplastic clones.

INTRODUCTION

LBL is a tumor of lymphoid precursors with a lymphomatous rather than a leukemic presentation (2, 20, 26, 32, 33, 39). It is not only histologically unique but is also distinguished from other non-Hodgkin's lymphomas by the consistent presence of TdT and stained with a modified Wright's stain.

MATERIALS AND METHODS

Patient Material. Eleven previously untreated patients, ranging in age from 4 to 56 years, were included. All presented with tumors, usually lymph node, disease, and none of these patients was leukemic at initial diagnosis (Table 1). Three patients had bone marrow involvement, but peripheral blood was uninvolved. Tissue from involved sites was received immediately upon surgical removal, a representative portion was processed for routine histological examination, and viable single-cell suspensions were prepared from the remainder. In most instances, cells were frozen in 10% dimethyl sulfoxide (Fisher Scientific Co., Fair Lawn, N.J.) and stored in a viable state in the vapor phase of liquid nitrogen until use. Upon thawing, viabilities were always greater than 60% and usually greater than 90%.

Immunological Markers. Rosette assays using E-preparations and EAC were performed as described previously (8). Rosette preparations were stained with 1% trypan blue, and the percentage of viable rosette-forming cells was determined. Separate aliquots were cytocentrifuged and stained with a modified Wright's stain.


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3 The abbreviations used are: LBL, lymphoblastic lymphoma; TdT, terminal deoxynucleotidyl transferase; ALL, acute lymphoblastic leukemia; E, unsensitized sheep erythrocyte; EAC, antibody complement-coated sheep erythrocytes; Slg, surface immunoglobulin; C1g, intracytoplasmic immunoglobulin; ASC complex, avidin-biotin-peroxidase complex; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorter; CALLA, common acute lymphoblastic leukemia antigen.

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Slg was examined by direct immunofluorescence using affinity-purified IgG fractions of chain-specific goat anti-human  \( \gamma, \alpha, \mu, \delta, \kappa, \) and \( \lambda \) immunoglobulins conjugated to fluorescein isothiocyanate (Tago, Inc., Burlingame, Calif.). Clg was detected by direct immunofluorescence of cyto- globulins conjugated to fluorescein isothiocyanate (Tago, Inc., Burlingame, Calif.), according to the method of Vogler et al. (36). In addition, antibodies, anti-7, anti-k, and anti-X (Becton-Dickinson Monoclonal Antibodies, Sunnyvale, Calif.), according to the method of Vogler et al. (36). In addition, J5 antibody was provided by Dr. Jerome Ritz, from Dr. Patrick Kung, Ortho Pharmaceutical Corp., Raritan, N.J.) (25, 26). Additionally, typical lymphoblasts were identified in one of the cases studied (27-29, 35). In addition, OKT10, considered to be a marker of most thymocytes (27, 29). In Case 4, the OKT4-positive cells were small in size and concentration, i.e., simultaneously OKT4 positive and CD10 negative. OKT3, OKT4, OKT6, OKT8, and OKT9 showed variable staining of T-cell cases. In some cases, small percentages of cells stained with OKT4 (inducer-helper phenotype) and OKT8 (suppressor phenotype). Since a normal thymic cell may possess both the inducer and suppressor phenotype prior to the differentiation of these cells into 2 distinct populations (27), the question was raised whether the blasts from some cases of LBL could reflect this stage of differentiation, i.e., simultaneously OKT4 positive and OKT8 positive. In Case 4, the OKT4-positive cells were small in diameter relative to the entire cell population. In order to determine whether the small percentage of OKT4- and OKT8-positive cells were blasts, each preparation was sorted through the FACS and subsequently stained for TdT. In Cases 4 and 5, TdT-positive cells were present in the OKT4-positive but not the OKT8-positive fraction. More than 80% of TdT-positive cells, i.e., blasts, were from the OKT4-negative and OKT8-negative fractions. Conversely, many OKT4-positive and all OKT8-positive cells were TdT negative, were somewhat smaller, and presumably were nonneoplastic T-cells in these partially involved lymph nodes.

### RESULTS

Biopsies of involved tissues from all patients exhibited the characteristic histological features required for a diagnosis of LBL (2, 26). Additionally, typical lymphoblasts were identified in cytocentrifuge preparations, and in all 11 cases tested these cells contained TdT (Table 2) localized to the nucleus in a reticulated pattern. All cases were negative for surface immunoglobulin staining. Wright's-stained cytocentrifuge preparations of rosetted cells (E or EAC) demonstrated lymphoblasts forming rosettes with E in cases 1 to 8 and with EAC in Cases 1, 4, and 8. TdT staining of the rosette cytocentrifuge preparations confirmed that rosetted cells were blasts in these cases. Cells from all 8 E-rosette-positive cases also reacted with the pan-T-cell antibody, OKT11 (Table 2). All T-cell cases tested reacted with OKT11, considered to be a marker of most thymocytes (27, 29).

#### Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Mediastinal mass</th>
<th>Site studied</th>
<th>Initial WBC</th>
<th>Bone marrow blasts (%)</th>
<th>Treatment</th>
<th>Response</th>
<th>Survival (mos.)</th>
</tr>
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<td>1</td>
<td>24</td>
<td>M</td>
<td>+</td>
<td>Lymph node</td>
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<td>CHOP-M*</td>
<td>CR</td>
<td>12+ alive</td>
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<tr>
<td>2</td>
<td>16</td>
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<td>+</td>
<td>Pleural fluid</td>
<td>7,700</td>
<td>0</td>
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<td>CR</td>
<td>62+ alive</td>
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<td>52</td>
<td>M</td>
<td>+</td>
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<td>My, COP, B</td>
<td>PR</td>
<td>8 died</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>M</td>
<td>+</td>
<td>Lymph node</td>
<td>9,100</td>
<td>0</td>
<td>PM</td>
<td>CR</td>
<td>14 died</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>M</td>
<td>-</td>
<td>Lymph node</td>
<td>5,700</td>
<td>50</td>
<td>PM</td>
<td>CR</td>
<td>28 died</td>
</tr>
<tr>
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<td>20</td>
<td>M</td>
<td>+</td>
<td>Lymph node</td>
<td>4,300</td>
<td>0</td>
<td>CHOP-M</td>
<td>CR</td>
<td>20 died</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>F</td>
<td>+</td>
<td>Lymph node</td>
<td>4,800</td>
<td>64</td>
<td>PM</td>
<td>CR</td>
<td>12 died</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>M</td>
<td>+</td>
<td>Mediastinal</td>
<td>7,800</td>
<td>0</td>
<td>CHOP-M</td>
<td>CR</td>
<td>44+ alive</td>
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<tr>
<td>9</td>
<td>8</td>
<td>F</td>
<td>+</td>
<td>Tibil mass</td>
<td>5,200</td>
<td>0</td>
<td>R, CHOP, B</td>
<td>CR</td>
<td>62+ alive</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>M</td>
<td>+</td>
<td>Lymph node</td>
<td>1,900</td>
<td>63</td>
<td>D, As, O, P</td>
<td>CR</td>
<td>28+ alive</td>
</tr>
<tr>
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<td>F</td>
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<td>7,200</td>
<td>0</td>
<td>R, CHOP-M</td>
<td>CR</td>
<td>12+ alive</td>
</tr>
</tbody>
</table>

* CHOP-M, Cytoxan, Adriamycin, vincristine, prednisone, and methotrexate; CR, complete response (no evidence of disease); My, Myleran; COP, Cytoxan, vincristine, prednisone; B, bleomycin; PR, partial response; PM, ProMACE/MOPP (12); R, local irradiation; CHOP, Cytoxan, Adriamycin, vincristine, prednisone; D, daunomycin; As, L-asparaginase; O, vincristine; P, prednisone.

Leukemic reaction, 95% granulocytes without blasts.

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**Lymphoblastic Lymphoma**

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A more extreme example of partial involvement of a lymph node by LBL was observed in Case 4, in which the lymphoma infiltrated the paracortical T-cell zones and left residual germinal centers (B-cell areas) intact. Cell suspension studies of this lymph node demonstrated 2 cell populations. One was a T-cell population which was OKT11 positive (55%), E-rosette positive (36%), and TdT positive; many cells were also OKT3-, OKT4-, OKT9-, and OKT10-positive. A separate population of B-cells (S1g positive) comprised 42% of this suspension and expressed polyclonal heavy- and light-chain types. Some EAC-positive cells were TdT positive. EAC-positive, TdT-negative cells were also present, and these presumably were nonneoplastic germinal center cells.

Three cases (Cases 9, 10, and 11) did not demonstrate E-rosette formation by neoplastic cells. In Case 9, only la, J5 (CALLA) and TdT were positive and this case, therefore, exhibited the immunological features of non-T, non-B or "common" ALL. Cases 10 and 11 showed a staining pattern similar to that in Case 9 but, in addition, the cells in Case 10 also showed binding of OKT9 and OKT10. Cells from both Case 10 and Case 11 were consistently found to contain intracytoplasmic \( \mu \) heavy chains with both heteroantisera and monoclonal antibodies and by both indirect immunofluorescence and ABC techniques. No other immunoglobulin chains were detected, and therefore Cases 10 and 11 were pre-B-cell lymphomas, with an admixture of normal T-cells. These 2 non-T cases were histologically indistinguishable from T-cell LBL cases; some blasts had convoluted nuclei, and this was particularly evident in Case 11 (Fig. 1).

**DISCUSSION**

All cases of LBL in this study contained intranuclear TdT. This biochemical marker is unique to lymphoblastic malignancies and has not been found in other lymphomas (6, 11, 22). Thus, the single marker that was consistently found in LBL (convoluted T-cell lymphoma) was the presence of intranuclear TdT. Although all cases had the morphological features of LBL (convoluted T-cell lymphoma) and contained TdT, they were, nevertheless, heterogeneous with regard to cell surface phenotype. Phenotypic heterogeneity within this class of lymphomas has been recognized previously in studies using conventional antisera and rosette receptor techniques (19, 23). Although most of the cases of LBL in this series were of T-cell type (8 of 11), it is clear that non-T LBL also occurs. The 3 non-T LBL cases expressed markers typical of common ALL (la positive, CALLA positive, TdT positive) (Case 9) and pre-B ALL (la positive, CALLA positive, TdT positive, clg positive) (Cases 10 and 11).

The phenotypes seen in these cases of non-T LBL correlated with the known phenotypes of non-T ALL. Studies based on an analysis of immunoglobulin gene rearrangement in common ALL have indicated that all such cases are committed to B-cell differentiation (21) and that such cells may retain the capacity to differentiate into early B-cells (10, 24). Thus, Cases 9 to 11 probably represent precursor B-cell lymphomas. Although the presence of nuclear convolutions suggests a T-cell phenotype (40), in the present series T, pre-B, and non-T, non-B cases were histologically indistinguishable. The occurrence of nuclear convolutions in the blasts of pre-B-cell and non-T, non-B LBL points out the nonspecificity of this morphological feature as indicative of T-cell phenotype.

Those patients with non-T LBL had several distinguishing clinical features. Although they presented within the age distribution commonly seen in T-LBL, none of them manifested a mediastinal mass. Two patients presented with lytic lesions of bone involving the tibia but did not have evidence of generalized bone marrow involvement. In one case, the cells had a pre-B phenotype; whereas in the second, the phenotype was non-T, non-B, characteristic of common ALL. Although Bernard et al. (5) have reported cutaneous involvement by non-T, non-B childhood non-Hodgkin's lymphoma, this was not observed in the present series.

All LBL of T-cell type demonstrated E-rosette formation by neoplastic cells, as well as staining by OKT11, an antibody that appears to detect the E-receptor itself (35). OKT11 antibody allowed for the detection of more positive cells than did E-rosette formation in several cases and thus may be more sensitive in identifying cells expressing the E-receptor. Although a spectrum of phenotypes of T-LBL was observed, all cases appeared to fall within the intrathymic stage of T-cell differentiation since all were TdT positive and all were OKT11 positive, E positive, and either OKT4 positive or OKT8 positive. The simultaneous occurrence of both the inducer (OKT4) and suppressor (OKT8) phenotypes on individual thymocytes and on T-lymphoblastic malignancies has been reported (14, 15, 25, 27, 28); however, this was not observed in any of the 8 T-LBL cases in our series. Double staining of sorted OKT4-positive and OKT8-positive cells with anti-TdT demonstrated that only a small fraction of the lympho-
blasts were positive for OKT4 and all were OKT8 negative; thus, inclusion of normal T-cells within the lymph node cell suspensions accounted for the presence of cells staining with OKT4 and OKT8 in Cases 4 and 5. It is evident, therefore, that lymph nodes involved by LBL may contain residual nonneoplastic lymphocytes, and these latter cells should be excluded from the analysis of the immunological phenotype of the lymphoblasts to avoid the false impression that lymphoblasts express additional markers such as OKT4 or OKT8. OKT3 was absent in 3 of 8 cases of T-cell LBL. This determinant is found on most peripheral blood T-cells, is found on only a minority (10%) of thymocytes, and is considered to appear somewhat later than OKT11, probably at the mature thymocyte stage (27). Thus, the OKT3-negative cases may be composed of cells that are somewhat less mature. Two of the OKT3-negative cases expressed an unusual antigen for T-lymphoblastic malignancies, either CALLA (J5) or Ia-like antigens. The significance of the occurrence of these determinants in T-LBL is not known but may correspond to an early intrathymic stage of T-cell differentiation. J5 expression is seen in some cases of T-ALL (14, 30). The fact that the cells of a pre-B-cell LBL stained for T9 and T10 is further evidence that these 2 reagents are not specific for T-cells (1). The surface membrane determinant identified by OKT3 has been shown to be the receptor for transferrin (13) and is essential for cell proliferation (3, 34).

By using the monoclonal antibody phenotyping data, cases of T-cell LBL in this study can be correlated to discrete stages of T-cell, particularly intrathymic, differentiation. LBL of T-cell type appears to correspond to a somewhat later stage of thymocyte differentiation than that reported for T-cell ALL since OKT4 and OKT8 did not occur simultaneously in these cases, in contrast to normal thymocytes, most cases of T-ALL, and some reported cases of T-LBL (14, 27, 30). In addition, many of the T-LBL cases were OKT6 negative. A diagrammatic representation of T-cell differentiation and the relative position of T-cell lymphoblastic malignancies is presented in Chart 1. Also shown in Chart 1 are so-called "peripheral" T-cell lymphomas, where both node-based and cutaneous types (9, 16) exhibit a more mature phenotype corresponding to T-cells in the peripheral circulation and peripheral lymphoid tissue.

The cell-by-cell analyses performed in this investigation have documented that within each individual case of LBL many determinants were expressed by some, but not all, of the lymphoblasts. For example, not all lymphoblasts in the cases of pre-LBL contained detectable Clg. The same phenomenon has been reported for pre-B ALL (37). Individual cases of pre-B-ALL may also include multiple immunoglobulin isotypes (37), an observation suggestive of fluidity of differentiation within a single neoplastic clone. In the present series, T-LBL also displayed phenotypic heterogeneity within individual neoplastic populations as evidenced by the variable fraction of cells expressing each determinant. The existence of subpopulations in a neoplastic clone may reflect a range of differentiation that includes multiple stages rather than all cells "frozen" at a single isolated differentiation stage. Alternatively, this heterogeneity of intratumor cell surface phenotypes may be due to cell cycle-associated determinants. This may be a general phenomenon of lymphoid cancers, since cases of peripheral T-lymphoma (9) and B-cell lymphoma (38) display phenotypic heterogeneity, and selection of subpopulations following conventional therapy of lymphoid cancer has been reported (7). Future therapeutic trials using monoclonal antibody for malignant lymphomas should include a consideration of these potential selective factors.

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REFERENCES


Fig. 1. Case 11. Pre-B cells with convoluted nuclei indistinguishable from those of typical T-cell LBL. H & E, × 950.
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