Intermediate Lymphocytic Lymphoma: Immunophenotypic and Cytogenetic Findings

By Dennis D. Weisenburger, Warren G. Sanger, James O. Armitage, and David T. Purtill

A detailed immunohistologic and cytogenetic analysis of 12 cases of intermediate lymphocytic lymphoma was performed. The characteristic immunophenotype of intermediate lymphocytic lymphoma was: surface IgM and IgD+, BA1+, BA2-, B2-, B4-, Leu 14+, Leu 1+, HLA-DR+, and common acute lymphocytic leukemia-associated (CALLA) antigen negative. Clonal chromosome abnormalities were identified in ten cases, with structural or numerical abnormalities of chromosomes 11 or 12 in nine cases. Five cases had structural abnormalities involving the long arm of chromosome 11; three of these had translocations with chromosome 14 at band q32. Three cases had trisomy 12, and one case had a translocation involving the long arm of chromosome 12. The tenth case had a translocation involving the long arms of chromosomes 7 and 9. These characteristic immunophenotypic and cytogenetic findings suggest a close linkage relationship between intermediate lymphocytic lymphoma and small lymphocytic (well differentiated) lymphoma/chronic lymphocytic leukemia. Their differing clinical, cytologic, and architectural features suggest, however, that intermediate lymphocytic lymphoma should be considered a separate category of lymphocytic lymphoma in the International Working Formulation.

MATERIALS AND METHODS

Lymph node biopsy specimens were obtained from 12 patients being studied and treated by the Lymphoma Study Group at the University of Nebraska Medical Center. All lymph nodes were processed for histologic, immunologic, and cytogenetic studies according to a standard protocol. Portions of each lymph node specimen were fixed in B5 and formalin for routine histologic processing, and hematoxylin and eosin-stained sections were prepared. A diagnosis of intermediate lymphocytic lymphoma was made on the basis of previously established histologic criteria.1,2 The clinical records were reviewed, and the patients were staged according to the Ann Arbor classification.3,4 The type of therapy, response to therapy, status at termination of the study, survival in months, and cause of death were recorded. The response to therapy was classified as a complete remission, a partial remission, or no response. A diagnosis of intermediate lymphocytic lymphoma was formed. The characteristic immunophenotype of intermediate lymphoma is usually diffuse, although small cleaved cell lymphoma, with a median survival of only clinical presentation and course similar to that of diffuse appears to be a relatively indolent form of intermediate symptoms as a complete remission, a partial remission, or no response. A cause of intermediate lymphocytic lymphoma have been reported, however. This study reports the immunophenotypic and cytogenetic findings in 12 cases of intermediate lymphocytic lymphoma.

Intermediate lymphocytic lymphoma is a histologically distinctive subtype of non-Hodgkin's lymphoma with cytologic features between those of small lymphocytic (well differentiated) lymphoma and small cleaved cell (poorly differentiated) lymphoma.1,2 The architectural pattern of intermediate lymphoma is usually diffuse, although some cases have a follicular mantle-zone pattern (mantle-zone lymphoma).3,4 Diffuse intermediate lymphoma has a clinical presentation and course similar to that of diffuse small cleaved cell lymphoma, with a median survival of only 30 to 31 months.5,6 In contrast, mantle-zone lymphoma appears to be a relatively indolent form of intermediate lymphocytic lymphoma.3,4 Immunologic studies of a small number of cases of intermediate lymphoma have demonstrated a B cell origin.1,3,5,9 No cytogenetic studies of intermediate lymphoma have been reported, however. This study reports the immunophenotypic and cytogenetic findings in 12 cases of intermediate lymphocytic lymphoma.

Immunohistochecmical stains were performed with a three-stage immunoperoxidase technique6 using unconjugated mouse antibody in the first stage, followed by biotin-conjugated goat anti-mouse F(ab')2 antibody, and then by avidin-conjugated horseradish peroxidase (Vector Laboratories, Burlingame, CA). Primary mouse antibodies to human immunoglobulins (Igs) G, A, M, D, x and (Dako, Santa Barbara, CA), B cell antigens B1, B2, B4 (Coulter Immunology, Hialeah, FL), BA1, BA2 (Hybritech, San Diego), and Leu 14 (Becton Dickinson, Mountain View, CA), and T cell antigens OKT 4, 8, 11 (Coulter), and Leu 7 (Becton Dickinson) were used. Primary antibodies to common acute lymphocytic leukemia associated (CALLA) antigen (Coulter) and HLA-DR (Becton Dickinson) were also used. A case was considered positive for a specific cell surface antigen if most neoplastic cells stained clearly positive (weak or strong) for the antigen.

Flow cytometric analysis of peripheral blood mononuclear cells was also performed in four cases. The mononuclear cells were separated from heparinized peripheral blood by density centrifugation on Ficoll-Paque. The mononuclear cells were stained with unconjugated primary mouse antibodies to human x and light chains, B1, BA2, Leu 1, HLA-DR and CALLA antigens. The cells were then washed with cold phosphate-buffered saline (PBS), incubated with FITC-conjugated goat anti-mouse F(ab')2 antibody, washed, and quantitated with an Ortho 50 H cytofluorograph equipped with a Data General 2150 computer for data analysis.

Lymph node biopsy specimens involved by lymphoma were also received by the cytogenetics laboratory and processed within 1 hour after the biopsy procedure, or the specimens were similarly received, cultured, and processed through the fixation stage by a nearby

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RESULTS

Clinical features. The clinical features of the 12 patients are given in Table 1. The median age of the patients at the onset of disease was 60 years (range 42 to 80 years). The male to female ratio was 1:1. All patients had advanced disease. Splenomegaly and bone marrow involvement by lymphoma were commonly present (75% and 73%, respectively). Five patients (cases 2, 3, 5, 6, and 12) were considered leukemic at the time of initial diagnosis. The lymphoma cells in the peripheral blood and bone marrow were atypical prolymphocytes, as previously described.11 The patients were treated with various chemotherapies. Four patients achieved a complete remission; two of these (cases 7 and 11) are presently alive and free of disease; the other two patients (cases 1 and 8) had prolonged disease-free survivals before the lymphoma recurred. Eight other patients are alive with or have died of lymphoma.

Histologic features. In 11 patients, the initial lymph node biopsy specimen showed effacement of the normal nodal architecture by a diffuse lymphoid infiltrate (diffuse intermediate lymphoma).2 In one patient (case 5), the lymphoid cells infiltrated as wide mantles around benign-appearing germinal centers (mantle-zone lymphoma).3 The infiltrating cells were atypical small lymphocytes with slightly irregular and indented nuclear contours (Fig 1A). There were too few cells with the uniformly round nuclei typical of small lymphocytic (well differentiated) lymphoma or the markedly angulated and cleaved nuclei of small cleaved cell (poorly differentiated) lymphoma to justify these diagnoses.

Immunologic features. Immunohistochemical stains revealed that all 12 cases of intermediate lymphocytic lymphoma were of B cell type (Table 2). Typically, intermediate lymphoma was positive for surface IgM and HLA-DR, and the antigens BA1, B4, Leu 14, and Leu 1 (Fig 1B). Seven cases expressed monoclonal k light chains and five cases expressed monoclonal l light chains. Variable positive staining was present for surface IgD (8 cases), IgG (2 cases), B1 (9 cases), and B2 (2 cases). The neoplastic cells did not stain for surface IgA, B2 or CALLA antigens, or the T cell antigens OKT 4, 8, and 11. Moderate numbers of benign T cells were admixed, however, with a helper:suppressor cell ratio of 2 to 3:1.

Flow cytometric analysis of peripheral blood mononuclear cells was also performed in four cases (cases 2, 3, 5, and 10). A monoclonal B cell population was identified in three cases (cases 2, 3, and 5; Table 3); the other case (case 10) was normal. In the three monoclonal cases, the immunophenotypes of the circulating lymphoma cells were identical to those of the corresponding lymph node cells.

Cytogenetic features. Cytogenetic studies revealed a clonal chromosome abnormality in 10 of the 12 cases (Table 4). Nine cases had structural or numerical abnormalities of chromosomes 11 or 12. Five cases had structural abnormalities involving the long arm of chromosome 11; three of these had translocations involving bands q11-13 and chromosome 14 at band q32. Three cases had trisomy 12, and one case had

| Table 1. Clinical Features of Intermediate Lymphocytic Lymphoma |

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)/Race/Sex</th>
<th>Splenomegaly</th>
<th>Absolute Lymphs/μL</th>
<th>Bone Marrow</th>
<th>Initial Therapy</th>
<th>Response</th>
<th>Survival (mo)</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54/W/M +</td>
<td>+</td>
<td>2.035</td>
<td>IV B</td>
<td>COP</td>
<td>CR</td>
<td>D + 113</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>2</td>
<td>56/W/M +</td>
<td>+</td>
<td>32.951</td>
<td>+</td>
<td>IV A</td>
<td>CL, P</td>
<td>NR D + 21</td>
<td>Lymphoma, bacterial pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>75/W/M +</td>
<td>+</td>
<td>1.925</td>
<td>+</td>
<td>IV B</td>
<td>CAP-BOP</td>
<td>ED D + 2</td>
<td>Lymphoma, herpes simplex pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>63/W/F +</td>
<td>+</td>
<td>1.122</td>
<td>III A</td>
<td>COPP</td>
<td>PR</td>
<td>A + 87</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>63/W/M +</td>
<td>+</td>
<td>5.490</td>
<td>+</td>
<td>IV A</td>
<td>CLOP</td>
<td>NR D + 9</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>6</td>
<td>80/W/F –</td>
<td>–</td>
<td>10.586</td>
<td>+</td>
<td>IV A</td>
<td>CAP-BOP</td>
<td>PR A + 18</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>53/W/F +</td>
<td>+</td>
<td>930</td>
<td>+</td>
<td>IV A</td>
<td>COP, M</td>
<td>CR A – 60</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>59/W/F +</td>
<td>+</td>
<td>5.850</td>
<td>+</td>
<td>IV A</td>
<td>CL</td>
<td>CR A + 204</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>67/W/F +</td>
<td>+</td>
<td>5.214</td>
<td>+</td>
<td>IV B</td>
<td>COP, BCNU</td>
<td>PR A + 32</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>51/W/M –</td>
<td>–</td>
<td>2.376</td>
<td>+</td>
<td>IV A</td>
<td>CLOP</td>
<td>PR A + 40</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>60/W/F –</td>
<td>–</td>
<td>1.032</td>
<td>ND</td>
<td>IV B</td>
<td>CHOP-B</td>
<td>CR A – 27</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>42/W/M +</td>
<td>+</td>
<td>25.230</td>
<td>+</td>
<td>IV A</td>
<td>CL</td>
<td>PR A + 85</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: Lymphs, lymphocytes; W, white; +, present/positive; –, absent/negative; ND, not done; NA, not applicable; COP, cytoxan, vincristine, prednisone; CL, chlorambucil; P, prednisone; CAP-BOP, cytoxan, doxorubicin, procarbazine, bleomycin, vincristine, prednisone; COPP, cytoxan, vincristine, prednisone, procarbazine; CLOP, chlorambucil, vincristine, prednisone; M, methotrexate; BCNU, bischloroethyl nitrosourea; CHOP-B, cytoxan, doxorubicin, vincristine, prednisone, bleomycin; CR, complete remission; PR, partial remission; NR, no response; ED, early death; D +, dead with disease; A +, alive with disease; A –, alive without disease.
INTERMEDIATE LYMPHOCYTIC LYMPHOMA

Table 2. Immunophenotypic Features of Intermediate Lymphocytic Lymphoma

<table>
<thead>
<tr>
<th>Surface Antigen-Positive Cases (n)</th>
<th>IgM</th>
<th>IgD</th>
<th>IgG</th>
<th>IgA</th>
<th>k</th>
<th>λ</th>
<th>BA1</th>
<th>B1</th>
<th>BA2</th>
<th>B2</th>
<th>B4</th>
<th>Leu 14</th>
<th>Leu 1</th>
<th>HLA-DR</th>
<th>CALLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

Abbreviation: CALLA, common acute lymphocytic leukemia associated.

Discussion

Intermediate lymphocytic lymphoma is a common form of lymphocytic lymphoma, comprising 8.4% of all non-Hodgkin’s lymphomas in the Nebraska Lymphoma Registry.3 The clinical and pathologic features of the patients in this study are similar to those reported previously.2,5 Patients with intermediate lymphoma are usually elderly and have advanced disease. Splenomegaly and bone marrow involvement by lymphoma are common. The clinical features of intermediate lymphoma are different from those of small lymphocytic (well differentiated) lymphoma, in which leukemia, usually with a high lymphocyte count, monoclonal gammapathy, autoimmune hemolytic anemia, and an indolent clinical course are common.2,4,6,16 Although five (42%) of the patients in this study were leukemic at the time of initial diagnosis, the highest lymphocyte count was only 32,951/μL, and the cells were clearly atypical prolymphocytes rather than small lymphocytes. We believe, however, that an accurate diagnosis of intermediate lymphocytic lymphoma can only be made from well-fixed lymph node biopsy specimens using established histologic criteria.2,3

In this study, we determined the characteristic immunophenotypic and cytogenetic features of intermediate lymphocytic lymphoma. Using frozen section immunohistochemical stains, the cells of intermediate lymphoma typically bear surface IgM, usually with IgD, and the pan-B cell antigens B1, BA1, B4, and Leu 14. The cells also bear the T cell antigen Leu 1 and lack CALLA antigen. Our findings differ from those of Cossman and colleagues7 who, using lymph node suspensions analyzed by flow cytometry, reported the expression of CALLA and BA2 antigen on most cases of intermediate lymphoma. However, Swerdlow and co-workers,17 using a frozen section immunohistochemical technique similar to ours, reported that only 1 of 6 cases was clearly positive for BA2 antigen, and none were positive for CALLA antigen. However, Swerdlow and co-workers18 did detect the weak expression of CALLA antigen on 1 of 4 cases studied by flow cytometry. These differing findings suggest that frozen section immunohistochemistry may fail to detect weakly expressed surface antigens.

Our findings indicate that the immunophenotype of intermediate lymphoma is identical to that of small lymphocytic (well differentiated) lymphoma/chronic lymphocytic leukemia.1,8,16 The immunophenotype of intermediate lymphoma contrasts with that of the follicular center cell lymphomas, which usually bear surface IgG or IgA and CALLA antigen and lack IgD and Leu 1 antigen.20,21 These findings suggest a closer lineage relationship of intermediate lymphoma with small lymphocytic lymphoma/chronic lymphocytic leukemia than with the follicular center cell lymphomas. Small lymphocytic lymphoma/chronic lymphocytic leukemia appears
Table 4. Cytogenetic Abnormalities in Intermediate Lymphocytic Lymphoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Normal Cells (n)</th>
<th>Abnormal Cells (n)</th>
<th>Abnormal Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54/M</td>
<td>1</td>
<td>34</td>
<td>46,XY,del(11q14)</td>
</tr>
<tr>
<td>2</td>
<td>56/M</td>
<td>9</td>
<td>9</td>
<td>46,XY,t(11;14)(q13;q32), del(13)(q22)</td>
</tr>
<tr>
<td>3</td>
<td>75/M</td>
<td>0</td>
<td>4</td>
<td>46,XY,t(11;14)(q12;q32), del(13)(q22)</td>
</tr>
<tr>
<td>4</td>
<td>63/F</td>
<td>0</td>
<td>21</td>
<td>46,XX,t(1;7)(q32;?), t(6:13)(q33;q31), -11, +der(11),t(11;7)(q22;?), t(11;14)(q11;q32)</td>
</tr>
<tr>
<td>5</td>
<td>63/M</td>
<td>10</td>
<td>15</td>
<td>45,XY,t(2;9)(p14;p13), -4, +t(4;5)(q34;q21), t(8;11)(p12;q26), -9, del(13)(q13),t(13;7)(q34;?)</td>
</tr>
<tr>
<td>6</td>
<td>80/F</td>
<td>0</td>
<td>4</td>
<td>47,XX + 12</td>
</tr>
<tr>
<td>7</td>
<td>53/F</td>
<td>5</td>
<td>10</td>
<td>49,XX + 12, +19, +21</td>
</tr>
<tr>
<td>8</td>
<td>59/F</td>
<td>0</td>
<td>2</td>
<td>48,XY + 12, +mar</td>
</tr>
<tr>
<td>9</td>
<td>67/F</td>
<td>0</td>
<td>4</td>
<td>46,XX, +r(4)(16q31), t(4;6)(q31;q26), t(10;12)(q24;q22),t(12;?), (13;7),del(14)(q21)</td>
</tr>
<tr>
<td>10</td>
<td>51/M</td>
<td>0</td>
<td>22</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>60/F</td>
<td>20</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>42/M</td>
<td>6</td>
<td>0</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Same clone found in the bone marrow.

to arise from an immature, bone marrow derived, virgin B cell.22 The cell of intermediate lymphoma appears to correspond to a slightly more mature B cell that homes to and resides in primary lymphoid follicles and the mantle zones of secondary follicles.3,4,21 In normal human ontogeny, by the seventeenth week of gestation, Leu 1-, IgM-, and IgD-positive B cells form the primary follicles of lymph nodes in close association with follicular dendritic cells.23 Therefore, the intermediate lymphocyte appears to be the precursor cell of the normal germinal center.23,24

The cytogenetic abnormalities found in intermediate lymphoma are also similar to those reported for small lymphocytic (well differentiated) lymphoma/chronic lymphocytic leukemia.24-32 Our studies revealed a clonal chromosome abnormality in 10 of the 12 cases. In the two cases without clonal abnormalities, the normal karyotypes were probably those of proliferating normal lymphoid cells within the tumor. Abnormalities of chromosome 11 are common in small lymphocytic lymphoma/chronic lymphocytic leukemia, the most common of these being a t(11;14) or deletion of 11q.24-32 In small lymphocytic lymphoma/chronic lymphocytic leukemia, the break in chromosome 11 usually occurs in the region of band 11q13.26,28,31,32; this was also true in four of our five cases of intermediate lymphoma. Band 11q13 appears to be a fragile site prone to frequent breaks in the normal lymphocytes of patients with small lymphocytic lymphoma.25 The t(11;14) in small lymphocytic lymphoma/chronic lymphocytic leukemia and intermediate lymphoma causes juxtaposition of the cellular oncogene bcl-1 (11q13) with the immunoglobulin heavy chain gene (14q32).33 Translocation- or deletion-mediated deregulation or activation of the bcl-1 oncogene may play an important role in neoplastic transformation and/or excessive cellular proliferation in the lymphocytic malignancies.33 Trisomy 12 is also a common abnormality in small lymphocytic lymphoma/chronic lymphocytic leukemia.24,32 Three of our cases of intermediate lymphoma also had trisomy 12, and one case had a translocation involving chromosome 12 at band q22. The cellular ki-ras-2 oncogene has been localized to chromosome 12 at bands p12 and q24.34 Increased gene dosage or activation of the ki-ras-2 oncogene as a result of these abnormalities may result in an elevation of oncogene product, leading to neoplastic transformation and/or cellular proliferation.34 None of our cases had the t(14;18) commonly seen in the follicular center cell lymphomas.24,33,34 Thus, characteristic abnormalities involving chromosomes 11 and 12 have been demonstrated in intermediate lymphocytic lymphoma. These findings also suggest a close lineage relationship between intermediate lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukemia.

In conclusion, the diffuse and mantle-zone variants of intermediate lymphocytic lymphoma are distinctive forms of non-Hodgkin's lymphoma with characteristic clinical, cytologic, architectural,1,5 immunologic,1,6,9,17,20 and cytogenetic features. These findings suggest that intermediate lymphoma should be considered a separate category of lymphocytic lymphoma in the International Working Formulation.36,37

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