Genotypic Characterization of Centrocytic Lymphoma: Frequent Rearrangement of the Chromosome 11 bcl-1 Locus

By Michael E. Williams, Cindy D. Westermann, and Steven H. Swerdlow

Centrocytic lymphomas are defined in the Kiel classification as B-cell lymphomas composed exclusively of cells resembling cleaved follicular center cells (FCC). These lymphomas have been shown to be histologically, immunophenotypically, and clinically distinct from other cleaved FCC lymphomas. DNA from 18 centrocytic lymphomas (14 patients) was analyzed using Southern blotting and probes for immunoglobulin heavy (JH) and kappa light chain (Jk) joining gene, T-cell receptor beta chain constant gene (Cβ), bcl-1, bcl-2, and c-myc gene rearrangements. All of the lymphomas had JH and Jk rearrangements, confirming their B-cell origin. None of the specimens had detectable Cμ.

CENTROCYTIC LYMPHOMA is defined by Tolksdorf et al.1 as a malignant lymphoma composed exclusively of cells resembling the centrocytes (cleaved cells) of reactive germinal centers. Although composed of cells resembling cleaved cells, these lymphomas are distinguished from other cleaved follicular center cell (FCC) lymphomas on the basis of their lack of transformed cells, their usual diffuse or vaguely nodular growth pattern, frequent immunophenotypic differences (CD5+, CD10−), and their general lack of transformation to noncleaved or centroblastic types of FCC lymphomas.1-4 Centrocytic lymphomas are recognized only as a distinct entity in the Kiel classification.5 Nevertheless, these lymphomas are included in the cleaved FCC categories in the Lukes/Collins classification, and the diffuse small cleaved cell (or less commonly diffuse large cell) category in the Working Formulation.6,7 Using a modified Rappaport classification, a moderate number of centrocytic lymphomas would be included in the category of intermediate lymphocytic lymphoma, although others fulfill the criteria for the poorly differentiated lymphocytic category.7 Both of these categories also include lymphomas that would not be considered centrocytic. Thus, the precise relationship of centrocytic lymphomas to other cleaved FCC lymphomas remains uncertain.

To genotypically characterize centrocytic lymphomas, 18 samples from 14 patients were studied by Southern blot analysis. Analyses were performed with immunoglobulin (Ig), bcl-2, and c-myc gene probes, and with a chromosome 11 bcl-1 breakpoint probe. While no sample had demonstrable bcl-2 or c-myc gene rearrangement, tumor DNA from 4 of the 14 patients showed rearrangement of the bcl-1 locus. The lack of bcl-2 rearrangement genotypically distinguishes centrocytic lymphomas from other cleaved FCC lymphomas; furthermore, a subset of these lymphomas contains detectable bcl-1 rearrangement, suggesting a possible role in the pathogenesis of some of these tumors.

MATERIALS AND METHODS

Specimen selection and case description. Eighteen specimens were selected on the basis of a histologic diagnosis of centrocytic lymphoma and the availability of a snap-frozen tissue sample for molecular biologic studies. An additional case (no. 89-89) was initially selected, but was excluded due to the failure to obtain adequate DNA from the very small amount of frozen tissue. The tissue sources included lymph nodes (12 specimens), spleen (2 specimens), lid/orbit (3 specimens), and rectal (1 specimen). Multiple specimens were studied in three patients; in one patient a cervical lymph node was studied, followed by a right orbital mass excised 10 months later (cases 89-81A and B; Table 1). The latter specimen showed evidence of histologic transformation. Another patient had a cervical node excision followed by a left upper eyelid biopsy 26 months later (cases 89-80A and B; Table 1). The third patient had an axillary node biopsy followed by spleen and a splenic hilar node excision 10 months later (cases 89-91A, B, and C; Table 1).

The histologic diagnosis was made using the criteria of Tolksdorf et al.1 with B5-fixed sections stained with hematoxylin and eosin, periodic acid-Schiff hematoxylin, and methyl green pyronin. Morphologic criteria included the presence of centrocytes (cleaved cells), without centroblasts, with a diffuse growth pattern. At times a vaguely nodular pattern was present due to growth around reactive follicular centers ("mantle zone" growth pattern). Cell suspension immunophenotypic studies were performed as previously reported4; these showed monoclonal B cells in all cases (kappa 12, lambda 6). CD5 (Leu 1) was on greater than 80% of apparent B cells in eight cases, on a smaller proportion of the apparent B cells in seven cases, apparently only on T cells in one case, and not tested in two cases (Table 1). CD10 (common ALL antigen) was negative (less than 5%) in the 15 cases in which it was tested.

DNA studies. Southern blot analysis was performed as previously reported8,9. Blots were serially hybridized with 32P-labeled probes from the Ig heavy chain joining gene (JH), kappa light chain joining gene (Jk), and T-cell receptor beta chain constant gene (Cβ).

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Table 1. Immunophenotype and Gene Rearrangement Analysis in Centrocytic Lymphomas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tissue</th>
<th>% CD5 on B Cells</th>
<th>Ig</th>
<th>bcl-2</th>
<th>c-myc</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>JH</td>
<td>Jk</td>
<td>pFL-1</td>
</tr>
<tr>
<td>89-79</td>
<td>LN</td>
<td>NT</td>
<td>mclA</td>
<td>2R</td>
<td>2R</td>
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<tr>
<td>89-80A</td>
<td>LN</td>
<td>+</td>
<td>mclA</td>
<td>1R</td>
<td>1R</td>
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<tr>
<td>-80B</td>
<td>Eyelid</td>
<td>+</td>
<td>mclA</td>
<td>1R</td>
<td>1R</td>
</tr>
<tr>
<td>89-81A</td>
<td>Orbit</td>
<td>+</td>
<td>mclA</td>
<td>2R</td>
<td>3R</td>
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<tr>
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<td>LN</td>
<td>+</td>
<td>mclA</td>
<td>2R</td>
<td>3R</td>
</tr>
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<td>LN</td>
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<td>89-83</td>
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<td>+</td>
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<td>2R</td>
</tr>
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<td>+</td>
<td>mclA</td>
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<td>1R</td>
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<td>89-86</td>
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<td>+</td>
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<td>89-87</td>
<td>Spleen</td>
<td>+</td>
<td>mclA</td>
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<td>LN</td>
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<td>mclA</td>
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<td>1R</td>
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<td>LN</td>
<td>NT</td>
<td>mclA</td>
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<td>2R</td>
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<tr>
<td>-91B</td>
<td>LN</td>
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<td>mclA</td>
<td>2R</td>
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<tr>
<td>89-92</td>
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<td>mclA</td>
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</tbody>
</table>

Abbreviations: LN, lymph node; Ig, immunoglobulin; JH, heavy chain joining gene; Jk, kappa light chain joining gene; pFL-1, bcl-2 major breakpoint; pFL-2, bcl-2 minor breakpoint; C, T-cell receptor beta chain constant gene; GL, germline; R, rearrangement; NT, not tested; +, >80% apparent B cells positive; +, <80% apparent B cells positive; -, not on apparent B cells; mcl, monoclonal.

(OnCor, Gaithersburg, MD). Other probes included the major (pFL-1) and minor (pFL-2) chromosome 18 breakpoint probes from the bcl-2 locus of the t(14;18) translocation, a 2.1-kilobase (kb) Sst I fragment from the chromosome 11 breakpoint of the bcl-1 locus of the t(11;14) translocation, and c-myc exon I and III probes previously described.23 BamHI, EcoR1, Bcl-1 and HindIII DNA restriction digests were used for the JH probe; BamHI and EcoR1 for Jk; BamHI and HindIII for c-myc exon I, pFL-1, and pFL-2; EcoR1 and HindIII for c-myc exon III; and BamHI for C, bcl-1 rearrangement was assessed on BamHI, EcoR1, and Bcl-1 digests.

RESULTS

A total of 18 tissue samples from 14 patients were studied. Clonal rearrangements of the Ig JH and Jk genes were identified in each sample (Table 1, Fig 1). Specimens from each patient with multiple biopsies showed the same Ig gene rearrangement pattern among samples from that individual (Fig 1). Two patients (cases 89-81A and B, and Case 89-92; Table 1) showed three rearranged Jk bands (Fig 1). The C, probe showed a germline unarranged configuration in each case.

No rearrangement of the chromosome 18 bcl-2 locus was detected using the pFL-1 and pFL-2 probes (Fig 2). The c-myc exon I and III probes also showed no rearrangement (Fig 2). Four cases showed rearrangement of the chromosome 11 bcl-1 locus on BamHI, EcoR1, and Bcl-1 digests (Fig 3). Comigration of rearranged JH and bcl-1 fragments suggestive of a t(11;14) chromosomal translocation could not be documented on the multiple enzyme digests used, being
observed only on BamHI digest in case 89-86. Similarly, no J_{5}/bcl-1 comigration suggestive of a t(2;11) was identified. Each bcl-1 positive patient demonstrated CD5 expression on tumor cells (Table I).

**DISCUSSION**

Genotypic characterization of the non-Hodgkin's lymphomas is being increasingly used to better classify these neoplasms and improve understanding of their pathogenesis. The present study failed to find a single centrocytic lymphoma with bcl-2 gene rearrangement, and supports the separation of these lymphomas from other cleaved FCC lymphomas (Table I, Fig 2). Furthermore, it suggests that the centrocytic lymphomas develop via different oncogenic mechanisms than the other FCC lymphomas. It is of interest that the centrocytic lymphomas are also distinguishable from most other cleaved FCC lymphomas by their general lack of CD10 expression, and the frequent presence of CD5.24

There was no evidence of c-myc oncogene rearrangement in any case using exon I and III probes (Table I, Fig 2). c-myc is frequently rearranged in high-grade lymphoma, and rearrangement has been observed in non-Hodgkin's lymphoma progressing from low- to high-grade histology.10,13

Rearrangement of the bcl-1 gene was present in 4 of 14 patients (28.6%) in this study. This rearrangement has been correlated with the t(11;14) (q13;q32) chromosomal translocation, and in most cases juxtaposes the bcl-1 locus from 11q13 with the Ig heavy chain joining locus at 14q32.12,14 Rearrangement of bcl-1 has been identified in cell lines derived from human chronic lymphocytic leukemia,13 diffuse large cell lymphoma,13 centroblastic/centrocytic diffuse lymphoma,13 and B-cell prolymphocytic leukemia and multiple myeloma.16,17 Analysis of 50 uncultured human B-cell lymphomas showed rearrangement in three cases using a bcl-1 probe identical to that utilized in the present study; two were diffuse small lymphocytic and one a diffuse large cell lymphoma that appeared to have evolved from a small lymphocytic lymphoma.18 A recent report of 29 B-cell lymphomas with gastrointestinal tract involvement (including six with low-grade small cell histology) found no case with bcl-1 rearrangement.19 Another report found bcl-1 rearrangements in 2 of 42 chronic lymphocytic leukemias...
and 4 of 114 B-cell non-Hodgkin’s lymphomas, including two small lymphocytic and two diffuse small cleaved cell type.20 Prior studies in this laboratory have not identified bcl-1 rearrangements or polymorphisms in 11 patients with chronic lymphocytic leukemia or 14 patients with multiple myeloma. Finally, Medeiros et al21 found that 7 of 22 (32%) lymphocytic lymphomas of intermediate differentiation had bcl-1 but not bcl-2 rearrangement. These findings are consistent with the results of the present study, because many intermediate lymphocytic lymphomas would be classified as centrocytic lymphomas in the Kiel classification.7 While a potentially new oncogene has been proposed for the 11q13 breakpoint locus, B-cell leukemia/lymphoma-1 (bcl-1), no gene has as yet been identified.21,22 Although most chromosome 11 breakpoints identified to date have occurred in the so-called major translocation cluster (MTC), additional breakpoints distant from the MTC have been identified.14,15 Most translocations also appear to involve the Ig heavy chain joining gene locus on chromosome 14, and it has been suggested that the translocation occurs during diversity-joining (D-J) recombination catalyzed by the V-D-J recombinase and using recombination-like signal sequences in the so-called major translocation cluster (MTC), additional breakpoints distant from the MTC have been identified.14,15 Most translocations also appear to involve the Ig heavy chain joining gene locus on chromosome 14, and it has been suggested that the translocation occurs during diversity-joining (D-J) recombination catalyzed by the V-D-J recombinase and using recombination-like signal sequences on chromosome 11.22 In this study no case showed clear evidence of a t(11;14) by virtue of comigrating rearranged bcl-1 and Jg bands on two or more restriction enzyme digests; case 89-86 showed comigrating rearranged Jg/bcl-1 bands on BamHI digest only. Because karyotypic data were not available for the cases in this study, identification of a translocation involving chromosome 11p sequences adjacent to the rearranged bcl-1 fragment is unconfirmed. Therefore, it is uncertain whether the observed bcl-1 rearrangements resulted from bcl-1 locus deletions, insertions, non-Jg or -Jk translocations, or Ig gene translocations distant from these joining gene loci.

Thus, centrocytic lymphomas appear to be distinct from other cleaved FCC lymphomas by immunophenotype and by the absence of bcl-2 rearrangement. Furthermore, the chromosome 11 bcl-1 locus is rearranged in 28% of cases as compared with less than 5% in other B-cell lymphomas, suggesting a potential role in pathogenesis. Additional studies of centrocytic and centrocytic-like lymphomas using multiple breakpoint probes and analysis of breakpoints over a larger chromosome 11 span, eg, by pulse-field gel technology, will be of interest. Centrocytic lymphoma may also prove to be a useful tumor model for the identification and characterization of the putative bcl-1 oncogene product.

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