Rearrangement of the Chromosome 11 bcl-1 Locus in Centrocytic Lymphoma: Analysis With Multiple Breakpoint Probes

By Michael E. Williams, Timothy C. Meeker, and Steven H. Swerdlow

Centrocytic lymphoma is a B-cell non-Hodgkin’s lymphoma (NHL) composed of lymphocytes resembling cleaved follicular center cells (centrocytes). Previous studies have suggested an association between t(11;14) chromosomal translocations and bcl-1 rearrangement in centrocytic and related intermediate lymphocytic lymphomas. To further characterize the association between bcl-1 and centrocytic lymphoma, Southern blot analysis was performed on samples from 23 patients using four separate bcl-1 breakpoint probes spanning 63 kb of the chromosome 11 bcl-1 locus. Rearrangements were identified in six patients with the major translocation cluster (MTC) probe and in another six with probe p94PS, located about 24 kb 5′ of MTC. Eleven of these 12 cases showed comigration of rearranged bcl-1 and Ig heavy chain-joining genes, consistent with the t(11;14) chromosomal translocation. No rearrangements were observed with the bcl-1 locus probes p210 or p11E9 located 5′ of p94PS, nor with bcl-2 or c-myc oncogene probes. No bcl-1 rearrangements were identified in B-cell follicular NHL (15), small noncleaved cell (Burkitt’s and non-Burkitt’s) NHL (8), T-cell NHL (4), multiple myeloma (14), and pre-B-cell acute lymphoblastic leukemia (9). One of 23 B-cell NHL of large cell type and one of 19 chronic lymphocytic leukemias or small lymphocytic NHL had MTC rearrangement. Thus, bcl-1 rearrangement occurred at MTC or p94PS in 12 of 23 centrocytic lymphomas (52%), confirming a nonrandom association and suggesting a pathogenetic role for the bcl-1 locus in this immunohistologic subtype of NHL.

The bcl-1 LOCUS was originally identified as a breakpoint site on chromosome 11 from cell lines of a B-cell non-Hodgkin’s lymphoma (NHL) and a B-cell lymphocytic leukemia containing t(11;14) chromosomal translocations. The site was designated bcl-1, an acronym for “B cell lymphoma/leukemia-1,” because it was felt that a putative oncogene existed at this breakpoint site analogous to those observed in other hematopoietic neoplasms. Furthermore, work in several laboratories, while identifying additional breakpoints, has failed to identify a messenger RNA (mRNA) gene product from this site. Because of the lack of an identified gene product, and because the t(11;14) translocation and rearrangement of the bcl-1 locus seemed relatively uncommon in lymphoid malignancies, work on this potentially important site lagged behind that of other more prevalently altered oncogenes. However, recent studies have identified histologic subtypes of NHL with a high frequency of bcl-1 rearrangement using the major translocation breakpoint probe, raising the possibility that a pathogenetically important gene exists at this locus.

To more completely characterize bcl-1 locus rearrangements in centrocytic lymphoma, we now report an extension of our original report using additional cases of centrocytic lymphoma and five separate bcl-1 locus probes. Twelve of 23 centrocytic lymphomas were found to have bcl-1 rearrangements involving the major translocation cluster (MTC) or a second breakpoint site approximately 24 kb 5′ of the MTC designated p94PS. These results confirm a strong association between centrocytic histology and bcl-1 rearrangement, suggesting a role for this locus in the pathogenesis of these tumors.

MATERIALS AND METHODS

Specimen selection and case description. Twenty-seven specimens from 23 patients were selected on the basis of a histologic diagnosis of centrocytic lymphoma and the availability of snap-frozen tissue for molecular analysis. Cytogenetic data was not available for any of these centrocytic cases. Only one eligible case was excluded due to failure to obtain adequate DNA (case 89-89). Eighteen of these samples from 14 patients were included in a previous report. The histologic diagnosis was made using the criteria of Tolksdorf et al with B5-fixed sections stained with hematoxylin and eosin, periodic acid-Schiff hematoxylin, and methyl green pyronin. The growth pattern was diffuse in most cases, or in some cases showed a “mantle zone” pattern due to growth around reactive follicular centers. Cytologic criteria included the presence of lymphocytes resembling centrocytes (cleaved cells) without centroblasts (transformed or noncleaved follicular center cells). Cell suspension immunophenotypic studies were performed as previously reported. All cases were monoclonal B cell (κ 15, λ 12). Twenty-five of the 27 samples were tested for the expression of CD5 (Leu 1); 23 of the 25 showed CD5 expression, with strong expression on greater than 80% of apparent B cells in 15 (Table 1). CD10 (common ALL antigen) was negative in each of the 24 cases tested.

A variety of other hematopoietic malignancies were also analyzed for bcl-1 locus rearrangements, selected on the basis of a confirmed morphologic and phenotypic diagnosis and adequate DNA for Southern blot analysis. Cases included B- and T-cell NHL and acute and chronic lymphoid leukemias (ALL and CLL), as well as multiple myeloma and acute myelogenous leukemia (AML).

DNA studies. Southern blot analysis was performed as previously reported. Blots were serially hybridized with 32P-labeled probes from the MTC designated p94PS, located about 24 kb 5′ of MTC. These results confirm a strong association between centrocytic histology and bcl-1 rearrangement, suggesting a role for this locus in the pathogenesis of these tumors.


© 1991 by the American Society of Hematology.

From the Departments of Internal Medicine and Pathology, and the Diagnostic Molecular Genetics Laboratory, University of Virginia School of Medicine, Charlottesville, VA; Department of Medicine, University of California, San Francisco, CA; and the Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, OH.


Supported in part by National Cancer Institute Grant CA46723 (to M.E.W.), and National Institutes of Health Grant CA01102 and an American Cancer Society Research Grant (to T.C.M.). M.E.W. is the recipient of an American Cancer Society Clinical Oncology Career Development Award.

Address reprint requests to Michael E. Williams, MD, Hematology/Oncology Division, Box 502, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.
probes for Ig heavy chain-joining (JH) and μ switch genes, κ light chain-joining (Jκ), and λ light chain constant (Cλ) genes (kindly provided by Dr P. Leder, Harvard Medical School, Boston, MA), major (pFL-1) and minor (pFL-2) chromosome 18 breakpoint probes from the bcl-2 locus of the t(14;18) translocation\(^2\) (kindly provided by Drs Y. Tsujimoto, Wistar Institute, Philadelphia, PA, and C. Croce, Fels Institute, Temple University, Philadelphia, PA). Three additional probes from cell lines containing the t(11;14) breakpoint were isolated by SstI-digested DNA of centrocytic lymphoma. Five cases of these samples from 14 patients was previously reported.\(^5\) All cases of centrocytic lymphoma had clonally rearranged Ig genes. No TCRβ chain, γ chain, bcl-2, or c-myc rearrangements were identified (Table 1). In patients for whom two or more samples were available, the same pattern of Ig gene rearrangement was identified in each. Overall, 12 of the 23 centrocytic lymphoma patients (52%) showed bcl-1 locus rearrangements (Table 1, Figs 1 through 3). In each case, rearrangements were identified on three or more of the five restriction digests, except case 90-121, which was rearranged only on EcoRI and SstI digests. Six rearrangements involved the MTC, while another six were identified with the p94 probe. Restriction map localization of these cases showed the MTC rearrangements to be clustered near the MTC site, whereas those associated with p94 were more scattered (Fig 4). Rehybridization of SstI-digested DNA with the pRc probe localized breakpoints within an approximately 2.5-kb SstI fragment containing pRc in four cases (Figs 3 and 4). The breakpoint in case 89-85 showed rearrangements larger than germline on SstI digest with both the MTC and pRc probes, suggesting that the breakpoint eliminated the SstI site between these probes (Fig 4).

### RESULTS

A total of 27 tissue samples from 23 patients were studied. The results of MTC rearrangement analysis for 18 of these samples from 14 patients was previously reported.\(^5\) All cases of centrocytic lymphoma had clonally rearranged Ig genes. No TCRβ chain, γ chain, bcl-2, or c-myc rearrangements were identified (Table 1). In patients for whom two or more samples were available, the same pattern of Ig gene rearrangement was identified in each. Overall, 12 of the 23 centrocytic lymphoma patients (52%) showed bcl-1 locus rearrangements (Table 1, Figs 1 through 3). In each case, rearrangements were identified on three or more of the five restriction digests, except case 90-121, which was rearranged only on EcoRI and SstI digests. Six rearrangements involved the MTC, while another six were identified with the p94 probe. Restriction map localization of these cases showed the MTC rearrangements to be clustered near the MTC site, whereas those associated with p94 were more scattered (Fig 4). Rehybridization of SstI-digested DNA with the pRc probe localized breakpoints within an approximately 2.5-kb SstI fragment containing pRc in four cases (Figs 3 and 4). The breakpoint in case 89-85 showed rearrangements larger than germline on SstI digest with both the MTC and pRc probes, suggesting that the breakpoint eliminated the SstI site between these probes (Fig 4).
Fig 1. Southern blot autoradiograms for DNA from placental control (C) and centrocytic lymphoma cases 89-79 to 89-93 (see Table 1). Jₜ rearrangements are present in all cases. Comigrating Jₜ/MTC rearrangements are present in case 89-86 (arrows) but not for MTC rearrangements in cases 89-85, 87, or 92 (dash marks). Comigrating Jₜ/p94 rearrangements are present in cases 89-83 and 89-91A, B, and C (arrowheads); comigration was not present for the p94 rearrangement in case 89-94 (dash mark).

Fig 2. Southern blot autoradiograms for DNA from placental control (C) and centrocytic lymphoma cases 90-120 through 90-128 (see Table 1). Jₜ rearrangements are present in all cases. No Jₜ/MTC rearrangement comigration was present (dash marks), whereas all three cases with p94 rearrangement (cases 90-121, 122, and 127) showed Jₜ comigration (arrowheads). BamH1 restriction digest (left panel) and EcoR1 digest (right panel) were used. See the legend for Fig 1 for BamH1 germline band sizes for Jₜ, MTC, p94, and p11; p210 germline band, approximately 4.4 kb. Approximate EcoR1 germline band sizes are: Jₜ, 17 kb; MTC, 12.5 kb; p94, 18.5 kb; p11, 2.2 kb; and p210, 6.5 kb.

Eighteen of the 23 (78%) centrocytic lymphoma cases showed rearrangement with the MTC probe. Histologic review of this sample confirmed a diffuse sclerosing large cell lymphoma, CD5-negative, k-positive, with no cleaved cell component to suggest follicular center cell origin. Of 19 CLL cases or small lymphocytic lymphomas, one (5%) showed MTC rearrangement; review of this case showed diffuse marrow replacement by tumor cells. Many of the peripheral blood and marrow cells had clefted nuclei and nucleoli suggestive morphologically of prolymphocytic leukemia, with a CD5 and surface IgM A-positive phenotype. No bcl-1 rearrangements were detected in follicular NHL, multiple myeloma, T-cell NHL, ALL, CML lymphoblast crisis, or AML. There was no evidence of polymorphism at any restriction sites studied with these bcl-1 probes. Twelve of 15 follicular NHL (80%) had rearrangement of the bcl-2 locus, and five of eight small noncleaved cell NHL (Burkitt’s and non-Burkitt’s) had c-myc rearrangement.
DISCUSSION

Specific chromosomal translocations and oncogene rearrangements are strongly associated with morphologic subtypes of NHL. Small non-cleaved cell (Burkitt’s and non-Burkitt’s) NHL shows rearrangement of the chromosome 8q24 oncogene c-myc in most cases, while greater than 80% of follicular lymphomas and up to 30% of diffuse large cell NHL show rearrangement of the chromosome 18q21 bcl-2 oncogene. Both of these oncogenes are reciprocal partners in translocations with Ig gene loci, especially the chromosome 14q32 heavy chain-joining or switch genes, which appear to be mediated by recombinase enzyme activity. Another translocation breakpoint designated bcl-1 was cloned in 1984 from t(11;14) (q13;q32)-containing B-cell tumors. Centrocytic lymphoma is defined in the Kiel classification as an NHL composed of lymphocytes resembling small cleaved cells (centrocytes) without the presence of transformed lymphocytes. “Centrocytic lymphoma of intermediate differentiation” as defined in recent reports is closely related. The growth pattern of centrocytic lymphoma is diffuse or vaguely nodular due to “mantle zone” growth around reactive follicular centers. The neoplastic cells are of B-cell origin and are usually CD5-positive and CD10-negative.

The results of the present study confirm a strong association between centrocytic lymphoma and rearrangement of the bcl-1 locus. Using probes isolated from t(11;14) (q13;q32)-containing B-cell tumors, which span 63 kb of the 11q13 bcl-1 locus, DNA from 12 of 23 centrocytic lymphomas (52%) demonstrated rearrangements. Rearrangements were detected with the MTC and pRc probes and the p94 probe, but not with the p11 and p210 probes. Using a panel of five separate restriction enzyme digestions, it was possible to perform limited restriction mapping of these rearrangements; those at MTC and pRc appeared to be tightly clustered while p94 rearrangements were more widely scattered. Eleven of the 12 bcl-1 rearrangements showed comigration with Jh consistent with a t(11;14) translocation (Figs 1 through 3). Demonstration of comigration required the pRc probe in five of the 12 cases (Fig 3). The tight clustering of translocations at this site should permit relatively straightforward identification and characterization by polymerase chain reaction (PCR) methodology. Furthermore, because the bcl-1 locus is as yet incompletely mapped, and because karyotype data were not available for these centrocytic cases, it is possible that additional cases contain t(11;14) breakpoints that are undetected by presently available probes.

Previous studies of bcl-1 in human lymphoid neoplasms have shown a low frequency of rearrangement with probes from the MTC region, usually less than 5% to 10% of cases. Our findings in non-centrocytic lymphomas and other hematopoietic malignancies is consistent with this, as only two of 118 tumors showed rearrangement (Table 2). One tumor was a diffuse large cell lymphoma of B-cell

![Fig 3. Southern blot autoradiograms for placental control (C) and centrocytic lymphoma DNA hybridized with the Jh and pRc probes. Comigrating rearranged bands are noted at approximately 7.5 kb (arrowheads). The similar sizes of the rearranged pRc and comigrating Jh bands indicate nearly identical breakpoint sites. Sst I restriction digest was used. Germline Jh band, approximately 11 kb; germline pRc band, 2.5 kb.](image-url)

![Fig 4. Partial restriction map of the chromosome 11 bcl-1 locus illustrating probe locations. Not all BalmHI, Bcl I, or Sst I sites are shown. Localization of centrocytic lymphoma breakpoint sites is identified for each case with rearrangement (see Table 1).](image-url)
BCL-1 IN CENTROCYTIC LYMPHOMA

non-cleaved cell (Burkitt’s, non-Burkitt’s).

phases of centrocytic/intermediate lymphocytic lymphoma.2

unusual; some of these cases may represent leukemic origin that lacked centrocytic features, and the other a CLL probe, Medeiros et al found bcl-1 rearrangement in 10 of 19

and bcl-1 rearrangement in classical CLL appear to be


translocation. Science 224:1403, 1984

of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation. Blood 76:2086, 1990


of bcl-1 rearrangements with lymphocytic lymphoma of inter-

unlike the low frequency of bcl-1 rearrangement in these tumors, centrocytic lymphoma and lymphocytic lymphoma of intermediate differentiation show rearrangement in about half of the cases54 (present study). Using the MTC probe, Medeiros et al found bcl-1 rearrangement in 10 of 19

lymphocytic lymphomas of intermediate differentiation,6 a higher frequency than observed with the same probe in our 23 centrocytic cases. Vandenberghe et al recently reported 18 lymphoid tumors with t(11;14) or bcl-1 rearrangement, including 14 diffuse small cleaved cell lymphomas.33

To date, the nature of the putative oncogene bcl-1 remains undefined.14 Genes encoding CD5, CD20, SEA oncogene, and protein phosphatase 1-α localize to chromosome 11q12-13. However, mapping these genes by radiation hybrids demonstrates that bcl-1 is distinct from these entities.34 The genes for hst and int-2, members of the fibroblast growth factor family, also localize to 11q12-13, but are likewise distinct from bcl-1.34,35 Interestingly, bcl-1 is amplified without rearrangement in about a third of head and neck squamous cell carcinomas and a small number of squamous cell lung carcinomas, and is coamplified with hst and int-2 in a subset of human breast carcinomas.36,39

Thus, the nature of the proposed bcl-1 gene and the potential regulatory or structural disruption introduced by rearrangement and translocation in lymphoid tumors, as well as its possible interactions with the linked genes noted above, remain intriguing areas for investigation. Centrocytic lymphoma provides an important model for characterizing the bcl-1 locus and, potentially, for identifying the putative bcl-1 gene itself. Furthermore, the present study verifies the non-random association of bcl-1 rearrangement and centrocytic lymphoma that, like c-myc in Burkitt’s lymphoma and bcl-2 in follicular NHL, likely plays an important pathogenetic role.

ACKNOWLEDGMENT

The authors thank Drs Carlo Croce, Philip Leder, Takis Papas, Jeffrey Sklar, and Yoshhide Tsujimoto for providing probes used in this study. We also acknowledge the expert technical assistance of Patricia Ennis and Holly Dressman, and thank Lisa Morris and Christine Peterson for assistance in preparation of the manuscript.

REFERENCES


Table 2. bcl-1 Rearrangements in Hematopoietic Malignancies

<table>
<thead>
<tr>
<th>Tumor</th>
<th>n</th>
<th>Ig</th>
<th>TCR</th>
<th>bcl-1 Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML, follicular*</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>ML, diffuse, large cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cell</td>
<td>23</td>
<td>23</td>
<td>21</td>
<td>3/17 1 0 0 0</td>
</tr>
<tr>
<td>T cell</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>ML, SNCC†</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>CLL/ML, lymphocytic</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>1/15 1 0 0 0</td>
</tr>
<tr>
<td>Myeloma</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>ND 0 0 0/13 ND</td>
</tr>
<tr>
<td>ALL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-B</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>1 0 0 0 0 0</td>
</tr>
<tr>
<td>T cell‡</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>7 0 0 0 0 0</td>
</tr>
<tr>
<td>CML</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoblast crisis</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>AML</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not done; ML, malignant lymphoma; SNCC, small non-cleaved cell (Burkitt’s, non-Burkitt’s).

*Twelve cases (80%) showed bcl-2 rearrangement (9 pFL-1, 3 pFL-2).

†Five cases (63%) showed c-myc rearrangement.

‡All 9 T-cell ALL cases had clonal TCR γ gene rearrangement.

§HindIII digest only.
13. Meeker TC, Sellers W, Harvey R, Withers D, Carey K, Xiao H, Block A, Dadey B, Han T: Cloning of the t(11;14) (q13;q32) translocation breakpoints from two human leukemia cell lines. (submitted)


19. Tsujimoto Y, Louie E, Bashir MM, Croce CM: The reciprocal partners of both the t(14;18) and the t(11;14) translocations involved in B-cell neoplasms are rearranged by the same mechanism. Oncogene 2:347, 1988


35. Richard C, Withers D, Meeker TC, Maure S, Evans G, Myers RM, Cox DR: A radiation hybrid map of the proximal long arm of human chromosome 11 containing the MEN 1 and bcl-1 disease loci. (submitted)


Rearrangement of the chromosome 11 bcl-1 locus in centrocytic lymphoma: analysis with multiple breakpoint probes

ME Williams, TC Meeker and SH Swerdlow