Expression of bcl-3 in Chronic Lymphocytic Leukemia Correlates With Trisomy 12 and Abnormalities of Chromosome 19

Ellen Schlette, MD, George Z. Rassidakis, MD, PhD, Ozlem Canoz, MD,* and L. Jeffrey Medeiros, MD

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A b s t r a c t

The bcl-3 gene at chromosome 19q13 encodes a member of the IκB family involved in regulating the nuclear factor κB pathway. Originally identified by its involvement in the t(14:19)(q32;q13), bcl-3 expression recently has been reported in 12% of non-Hodgkin lymphomas and 41% of Hodgkin lymphomas. Because the t(14:19) is detected most commonly in chronic lymphocytic leukemia (CLL), we assessed for bcl-3 expression using immunohistochemical analysis in 72 CLL cases with immunophenotypic and cytogenetic data. Of 72 CLL cases, 12 (17%) were bcl-3+. Expression of bcl-3 correlated with an atypical immunophenotype, defined using the World Health Organization scoring system. Expression also correlated with trisomy 12 and chromosome 19 abnormalities but was not limited to cases with the t(14:19)(q32;q13). Although the mechanism of bcl-3 expression is unclear, these results raise the possibility that bcl-3 may be involved in the pathogenesis of this subset of tumors and could be a potential target for investigational therapies.

The bcl-3 gene, located at chromosome 19q13, originally was identified by its involvement in the t(14;19)(q32;q13). In this translocation, bcl-3 is juxtaposed with the immunoglobulin heavy chain gene locus at 14q32, and bcl-3 expression is up-regulated. The t(14;19) originally was described in 1983,1 and the bcl-3 gene first was cloned by McKeithan and colleagues2 in 1987.

The bcl-3 gene encodes a member of the IκB protein family, and the members of this family are known to regulate the transcription factor nuclear factor (NF) κB.3 The mammalian NF-κB family consists of 5 known members: p50/p105, p52/p100, RelA (p65), c-Rel, and RelB.3 These proteins normally are intracytoplasmic and are complexed with inhibitory IκB molecules, but when IκB proteins are degraded, free NF-κB family proteins can translocate to the nucleus and activate expression of a variety of genes. The IκB family is composed of 5 members: bcl-3, IkBα, IkBβ, IkBε, and IkBγ. In this family, bcl-3 is unique because it is located predominantly in the nucleus, it interacts with p50 and p52 homodimers of NF-κB, and it binds to other nuclear proteins. The cellular functions of bcl-3 remain obscure. It has been suggested that bcl-3 is an adapter protein between members of the NF-κB family, particularly p50 and p52, and other transcriptional regulators (eg, JAB1, Pirin). bcl-3 can form stable complexes with p50 or p52 on NF-κB DNA binding sites to activate transcription or disassociate these molecules from DNA. In addition, the phosphorylation status or concentration of bcl-3 also can affect its interaction with p50 and p52.4

The t(14;19) or bcl-3 gene rearrangement has been reported rarely in hematologic neoplasms, most often in cases of chronic lymphocytic leukemia/small lymphocytic...
lymphoma (CLL/SLL), but in fewer than 5% of cases based on conventional cytogenetics and molecular genetic analysis.5-9 The t(14;19) or bcl-3 gene rearrangement is even more rare in other types of non-Hodgkin lymphoma and acute leukemias.5,9-14 Although the t(14;19) is rare, bcl-3 expression is more common and has been shown in 12% of non-Hodgkin lymphomas, 6% of B-cell and 23% of T-cell lineage, and 41% of Hodgkin lymphomas.15

In previous studies of CLL/SLL, the t(14;19) has been associated with atypical morphologic features and trisomy 12. For the present study, we hypothesized that bcl-3 expression also might be associated with atypical morphologic features and trisomy 12. Thus, we studied bcl-3 expression in 72 cases of CLL for which immunophenotypic and cytogenetic data were available.

Materials and Methods

Study Group

Bone marrow aspirate smears and clot or biopsy specimens from 72 patients with the diagnosis of CLL and for which the results of flow cytometry immunophenotypic and conventional cytogenetic analysis were available were collected from the files of the Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston, from September 1999 through December 2001. The diagnosis of CLL was based on morphologic and immunophenotypic criteria outlined in the World Health Organization (WHO) classification.16

There were 47 men and 25 women; the median age was 57 years (range, 24-79 years). Fifteen patients had been treated with chemotherapy before the bone marrow sample included in this study was obtained. The clinical and laboratory data for these patients are summarized in Table 1.

Morphologic Examination

The morphologic features of all cases were examined using Wright-Giemsa–stained bone marrow aspirate smears and were classified as atypical or typical using adapted criteria for atypical morphology on peripheral blood smears in CLL, as described by Matutes et al.17 Specifically, cases with more than 15% lymphoplasmacytoid cells or clefted nuclear contours were considered atypical. Cases with more than 10% prolymphocytes were classified as CLL/prolymphocytic leukemia, not atypical CLL in agreement with the WHO classification.16

In each case, the percentage of each lymphocyte type was determined by a manual 100-cell differential count.

Immunohistochemical Methods

Tumors were analyzed using full tissue sections (n = 30) or 2 tissue microarrays (n = 42). The tissue microarrays included duplicate cores from each tumor and were constructed using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD) as described previously.15 All cases were fixed in 10% buffered formalin, processed routinely, and embedded in paraffin.

Tissue or microarray sections (3 or 4 µm thick) were deparaffinized in xylene and rehydrated in a graded series of ethanols. Heat-induced epitope retrieval was performed by placing sections in plastic Coplin jars containing preheated target retrieval solution (DAKO, Carpinteria, CA) heated in a household vegetable steamer (model Sunbeam 4713/5710, 900 W; Sunbeam-Oster, Boca Raton, FL) for 35 minutes. Sections then were allowed to cool at room temperature for at least 15 minutes. We used a monoclonal antibody specific for bcl-3 (clone 1E8, Novocastra, Newcastle upon Tyne, England) at a dilution of 1:50. Subsequent steps of the immunostaining procedure were performed in a humidity chamber as previously described.15

Table 1

Summary of Clinical and Laboratory Information*

<table>
<thead>
<tr>
<th></th>
<th>CLL With Trisomy 12 (n = 22)</th>
<th>CLL Without Trisomy 12 (n = 50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis (y)</td>
<td>55 (range, 27-73)</td>
<td>56 (range, 24-79)</td>
<td>.87†</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/9</td>
<td>33/17</td>
<td>.6‡</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>19/22 (86)</td>
<td>36/47 (77)</td>
<td>.52‡</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>6/22 (27)</td>
<td>19/45 (42)</td>
<td>.29‡</td>
</tr>
<tr>
<td>Median WBC count, /µL (× 10³)</td>
<td>68,300 (68.3); range, 4,000-226,000</td>
<td>34,600 (34.6); range, 3,800-299,500</td>
<td>.075†</td>
</tr>
<tr>
<td>Median absolute lymphocyte count, /µL (× 10³)</td>
<td>65,400 (65.4) range, 2,400-225,500</td>
<td>29,500 (29.5) range, 1,300-287,500</td>
<td>.12†</td>
</tr>
<tr>
<td>β₂-Microglobulin, µg/mL (nmol/L)</td>
<td>2.6 (221); range, 1.2-5.8 (102-493)</td>
<td>3.6 (306); range, 1.6-10.7 (136-910)</td>
<td>.072†</td>
</tr>
<tr>
<td>Median time from diagnosis to last follow-up, mo</td>
<td>47.5; range, 28-149</td>
<td>58; range, 15-156</td>
<td>—</td>
</tr>
<tr>
<td>Follow-up</td>
<td>Died, 3; alive, 15</td>
<td>Died, 12; alive, 24</td>
<td>—</td>
</tr>
</tbody>
</table>

* Data are given as number/total (percentage) unless otherwise indicated.
† Mann-Whitney test.
‡ Fisher exact test.
Scoring of Positive Cells

Only nuclear staining was considered positive for bcl-3, irrespective of intensity, because the protein has been shown to be located predominantly within the nucleus. Positive was arbitrarily defined as 20% or more positive cells. However, almost all negative tumors were completely negative or tumors had fewer than 5% positive cells and positive tumors showed a range of positive cells, always more than 20% and, in several cases, more than 50%.

Flow Cytometry Immunophenotypic Analysis

The samples were assessed using 3- or 4-color flow cytometric analysis and a FACScan (or FACScalibur) instrument (Becton Dickinson, San Jose, CA). Lymphocytes were gated for analysis using CD45 expression and right-angle light scatter. Fluorescein isothiocyanate and phycoerythrin-conjugated IgG1 and IgG2 antibodies were used for negative control experiments, and cursors were set to include more than 95% of events as negative. The panel of antibodies, conjugated to fluorescein isothiocyanate, phycoerythrin, or allophycocyanin, included reagents specific for CD3, CD5, CD11c, CD19, CD20, CD22, CD23, CD38, CD79b, FMC-7, and immunoglobulin κ and λ light chains (Becton Dickinson). The intensity of expression of CD20 and immunoglobulin light chains was also assessed.

As described in the WHO classification, the typical CLL immunophenotype is defined as a monotypic B-cell neoplasm positive for CD5 and CD23 with low intensity (“dim”) positivity for CD20 and surface immunoglobulin and negative for CD22, CD79b, and FMC-7.

Conventional Cytogenetics

Conventional G-band karyotype analysis was performed on bone marrow aspirate specimens from all patients. Cells were placed in 10 mL of Ham F10 medium with 20% fetal calf serum at a concentration of 2 to 4 × 10^6 nucleated cells per milliliter. The culture was incubated overnight at 37°C (approximately 24 hours). Standard harvesting procedures were used. Colcemid (0.1 mL/10 mL) was added to the culture for 30 minutes. For the hypotonic treatment, a 0.075-mol/L concentration of potassium chloride was used for 30 minutes at room temperature. The fixation procedure consisted of 3 changes of methanol:glacial acetic acid (3:1) with a 10-minute waiting period between each change. The Thermaton drying chamber (Thermaton Industries, Holland, MI) was used for slide preparation. Slides were placed in a 60°C oven overnight in preparation for Giemsa trypsin G-banding. A maximum of 30 metaphases was analyzed. The karyotypes were written using the International System for Human Cytogenetic Nomenclature.

Results

Expression of bcl-3 in CLL

Of 72 cases of CLL, 12 (17%) were bcl-3+. The majority of positive cases had 50% or more neoplastic cells that were positive, and staining intensity was moderate to strong. No difference in pattern or expression was noted between cases assessed by tissue microarrays and those assessed by full tissue sections. No significant association was
found between bcl-3 expression and age, sex, laboratory findings, or history of chemotherapy.

**Correlation of bcl-3 Expression With Morphology and Immunophenotype**

In bone marrow aspirate smears, atypical morphologic features were detected in 10 cases (14%). One of 10 atypical CLL cases compared with 9 of 62 typical CLL cases were bcl-3+ (P = 1; Fisher exact test).

All cases tested were positive for monotypic immunoglobulin light chain, CD5, CD19, and CD20 and were negative for CD3. Of 70 CLL cases, 66 (94%) were positive for CD23 and 11 (16%) were positive for FMC-7. Most cases assessed expressed CD11c (54/61 [89%]). CD38 was positive in 25 (38%) of 66 cases assessed.

For 58 patients, sufficient immunophenotypic data were available to generate a score using the antigens and system described in the WHO classification. Of 11 bcl-3+ cases, 6 (55%) had a score of 3 or less (ie, atypical for CLL) compared with 8 (17%) of 47 bcl-3– cases (P = .017; Fisher exact test). Expression of bcl-3 also correlated with CD79b positivity (P = .008) and the combination of CD11c and CD22 (P = .026; Fisher exact test).

**Correlation Between bcl-3 Expression and Cytogenetic Findings**

Based on the karyotype, the 72 CLL cases in this study can be divided into 3 groups: diploid cases (n = 24), cases with trisomy 12 and/or chromosome 19, including 1 with the t(14;19) and trisomy 12, while 13 (28%) of 47 bcl-3– CLL cases had trisomy 12. In addition, 3 of 5 CLL cases with abnormalities of chromosome 19, including 1 with the t(14;19) and trisomy 12, were positive for bcl-3, also showing a positive correlation (P = .03; Fisher exact test). One tumor with a diploid karyotype and 1 with cytogenic abnormalities other than trisomy 12 or chromosome 19 were positive for bcl-3 (Tables 2 and 3).

**Table 2**

Expression of bcl-3 Correlated With Immunophenotypic and Cytogenetic Findings

<table>
<thead>
<tr>
<th>Immunophenotypic Score</th>
<th>bcl-3+ Cases (n = 12)</th>
<th>bcl-3– Cases (n = 60)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD79b</td>
<td></td>
<td></td>
<td>.008</td>
</tr>
<tr>
<td>Positive</td>
<td>7/10 (70)</td>
<td>11/47 (23)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3/10 (30)</td>
<td>36/47 (77)</td>
<td></td>
</tr>
<tr>
<td>CD11c/CD22</td>
<td></td>
<td></td>
<td>.026</td>
</tr>
<tr>
<td>Positive</td>
<td>6/11 (55)</td>
<td>10/51 (20)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>5/11 (45)</td>
<td>41/51 (80)</td>
<td></td>
</tr>
<tr>
<td>Immunophenotypic Score</td>
<td></td>
<td></td>
<td>.017</td>
</tr>
<tr>
<td>≤3</td>
<td>6/11 (55)</td>
<td>8/47 (17)</td>
<td></td>
</tr>
<tr>
<td>4 or 5</td>
<td>5/11 (45)</td>
<td>39/47 (83)</td>
<td></td>
</tr>
<tr>
<td>Trisomy 12</td>
<td></td>
<td></td>
<td>.003</td>
</tr>
<tr>
<td>Present</td>
<td>9/12 (75)</td>
<td>13/60 (22)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>3/12 (25)</td>
<td>47/60 (78)</td>
<td></td>
</tr>
<tr>
<td>Chromosome 19 abnormalities</td>
<td></td>
<td></td>
<td>.03</td>
</tr>
<tr>
<td>Present</td>
<td>3/12 (25)†</td>
<td>2/60 (3)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>9/12 (75)</td>
<td>58/60 (97)</td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as number/total (percentage).
† Fisher exact test.
‡ Includes 1 case with t(14;19) that was bcl-3+.

**Table 3**

Karyotypes of bcl-3+ Chronic Lymphocytic Leukemia Cases

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47,XX,+12,t(14;19)(q32;q13)[7]</td>
</tr>
<tr>
<td></td>
<td>idem,add(13)(q34)[6]</td>
</tr>
<tr>
<td></td>
<td>idem,–5,–18[cp5]</td>
</tr>
<tr>
<td>2</td>
<td>48,XY,+12,+19[19]</td>
</tr>
<tr>
<td>3</td>
<td>47,XX,+12,t(13;19)(q21;q13),+19[1]</td>
</tr>
<tr>
<td>4</td>
<td>45,XY,del(1)(p34),add(6)(p25),del(10)(p12),add(13)(p12)[1]</td>
</tr>
<tr>
<td>5</td>
<td>47,XX,+12[2]</td>
</tr>
<tr>
<td>6</td>
<td>idem,del(1)(p34),add(6)(p25),del(10)(p12),add(13)(p12)[1]</td>
</tr>
<tr>
<td>7</td>
<td>47,XX,del(1)(p34),add(6)(p25),del(10)(p12),add(13)(p12)[1]</td>
</tr>
<tr>
<td>8</td>
<td>47,XX,+12[3]</td>
</tr>
<tr>
<td>9</td>
<td>47,XX,+12[9]</td>
</tr>
<tr>
<td>10</td>
<td>43,47,XX,add(13)(q29),del(3)(p24),del(6)(q21),del(11)(q14q23),–17,+18,add(19)[p13.3],+mar[cp17]</td>
</tr>
<tr>
<td>11</td>
<td>46,47,XY,add(14)(q32)[cp24],[5],[6]</td>
</tr>
<tr>
<td>12</td>
<td>46,XY[20]</td>
</tr>
</tbody>
</table>
closely in cell proliferation and survival. In 1 study, transgenic mice expressing bcl-3 were found to have an expansion of B cells in vivo, further suggesting a role for bcl-3 in cell proliferation or survival. In addition, bcl-3 can bind to the promoter of cyclin D1, enhancing progression of the cell cycle from G1 to S phase. Furthermore, although bcl-3–deficient mice appear developmentally normal, they show defects in B- and T-cell responses to antigens, suggesting a role of bcl-3 in maintaining B cells in wild-type mice. Thus, bcl-3 expression might confer a survival advantage for CLL cells.

Previous studies have reported that the t(14;19) in CLL is associated with atypical morphologic features and immunophenotype and trisomy 12. In a review of 23 cases of lymphoproliferative disorders with the t(14;19) by Michaux and colleagues, 17 B-cell CLL cases were described. Young patient age and progressive disease were common, and atypical lymphocyte morphologic features were reported in 41% of cases. McKeithan and colleagues also reported an association between young age or progressive disease and the t(14;19) or bcl-3 gene rearrangements. On the basis of these data, we hypothesized that bcl-3 expression also might correlate with trisomy 12 and atypical morphologic and immunophenotypic features, and this seems to be true, in part.

Atypical morphologic features in CLL have been associated with various factors, including atypical immunophenotype, abnormal cytogenetic findings, and, in particular, trisomy 12, and have been reported by others as an indicator of poor prognosis. In the present study, we did not find an association between atypical morphology and bcl-3 expression, as only 1 (8%) of the 12 bcl-3+ CLL cases had atypical morphology.

In the present study, 12 (17%) of 72 CLL cases expressed bcl-3, and expression correlated with trisomy 12, present in 9 (75%) of 12 bcl-3+ cases. Trisomy 12 is a common cytogenetic abnormality in CLL, reported in 15% to 30% of cases in various studies, and has previously been associated with atypical morphology, atypical immunophenotype, other karyotypic abnormalities, and poorer prognosis.

Expression of bcl-3 also correlated with atypical immunophenotype (score ≤3) using the immunophenotypic scoring system for CLL described in the WHO classification and based on earlier studies by Matutes et al and Moreau and colleagues. Fifty-eight cases had the prerequisite antigens needed to generate this score, which is based on expression of CD5 (1 point), CD23 (1 point), low (dim) levels of surface immunoglobulin (1 point), absent or weak expression of CD79b/CD22 (1 point), and FMC-7 (1 point). Cases with scores of 4 or 5 are considered typical for CLL, whereas scores of 3 or less are atypical and more often occur in other types of B-cell leukemia. In the present study, 6 (55%) of 11 bcl-3+ CLL cases had a score of 3 or less compared with 8 (17%) of 47 bcl-3– CLL cases (P = .017, not significant).

Specific antigens associated with bcl-3 expression were CD79b and the combination of CD11c and CD22. CD79b expression previously has been associated with trisomy 12 in CLL. When CD11c and CD22 expression were analyzed separately, no significant correlation with bcl-3 expression was identified for either antigen. Expression of bcl-3 in CD22+ CLL might have clinical importance because anti-CD22 monoclonal antibody therapy may be considered as a treatment for patients with CD22+ CLL. However, no clinical data using this antibody are available in CLL.

Although only a small number of cases had karyotypic abnormalities involving chromosome 19 and these abnormalities were associated with trisomy 12, chromosome 19 abnormalities correlated with bcl-3 expression. A single CLL/SLL case with t(14;19) was included in the study. This case showed strong expression of bcl-3. In CLL/SLL, the prevalence of t(14;19) is low, less than 1% by conventional cytogenetic analysis and up to 2% by molecular genetic analysis. Thus, the frequency of the t(14;19) in the present study is consistent with earlier studies.

Because bcl-3 expression is not limited to cases with the t(14;19), other mechanisms might up-regulate bcl-3 in positive cases or the translocation may be present in a subset of the neoplastic cells that was not detected by conventional cytogenetic analysis. In a study by Lishner and colleagues using fluorescence in situ hybridization (FISH), 4 (20%) of 20 consecutive CLL patient samples had evidence of bcl-3/IgH fusion sequences in 6% to 93% (median, 16%) of nuclei counted. This study suggests that the t(14;19) is identified more commonly by FISH than by conventional cytogenetics and can be present in only a subset of cells. Because the present study used cytogenetic data obtained retrospectively, FISH for t(14;19) was not attempted.

In summary, we performed an immunohistochemical study for bcl-3 in 72 cases of CLL. Our results indicated that a subset of CLL cases, most of which have trisomy 12 or chromosome 19 abnormalities, express bcl-3. The relatively high frequency of bcl-3 expression in these cases raises the possibility that bcl-3 has an oncogenic role in development of these neoplasms and might be a potential target for investigational therapies. While the t(14;19) has been reported to be associated with atypical morphologic features and progressive disease requiring early treatment, in this study bcl-3 expression did not correlate with atypical morphologic features or overall survival. Expression of bcl-3 did, however, correlate with atypical immunophenotypic findings, as has been suggested by others.

From the Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston.
References


