

PREFERENTIAL LINKAGE OF BCL-2 TO
IMMUNOGLOBULIN LIGHT CHAIN GENE IN CHRONIC
LYMPHOCYTIC LEUKEMIA

BY MASAOKI ADACHI,* AYALEW TEFFERI,† PHILIP R. GREIPP,‡
THOMAS J. KIPPS,§ AND YOSHIHIDE TSUJIMOTO*

From *The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104; the †Mayo Clinic, Division of Hematology, Department of Internal Medicine, Rochester, Minnesota 55905; and the ‡Research Institute of Scripps Clinic, La Jolla, California 92037

Nonrandom chromosome aberrations are associated with human malignancies and believed to play a key role in the pathogenesis of these diseases by disturbing cellular genes involved in the control of cell growth. The human *bcl-2* gene was the first to be identified by the study of chromosome abnormalities associated with human neoplasms (1-3). About 90% of follicular B cell lymphoma and ~20% of large diffuse B cell lymphoma carry the t(14;18)(q32;q21) translocation (4), which directly involves the IgH locus on chromosome 14 and the *bcl-2* gene on chromosome 18. The t(14;18) translocation occurs nearly exclusively at two loci, a major breakpoint clustering region within the 3' noncoding region and the minor breakpoint clustering region within the 3' flanking region of the *bcl-2* gene (1, 5).

It has recently been reported that the *bcl-2* gene product plays a dominant role in cell survival (6-8), which could be a key step for neoplastic transformation of B lymphocytes. This biological function of the *bcl-2* gene product led us to speculate that the spectrum of *bcl-2* gene involvement in B cell malignancies might be broad. In fact, we have recently reported a case of chronic lymphocytic leukemia (CLL) with the *bcl-2*/Igλ juxtaposition (9). In this article we have extended this study and shown that a significant fraction of CLL carries the *bcl-2* rearrangements and that in all cases studied, rearrangements of the *bcl-2* gene were the results of juxtaposition of the 5' region of *bcl-2* gene and one of the IgL genes.

Materials and Methods

CLL Samples. CLL2 (IgMλ) and CLL26 (IgMκ) co-expressed CD5, CD19, and CD20 as assessed by flow cytometry. The circulating lymphocytes in both cases appeared morphologically mature and phenotypically characteristic of B cell CLL. Our trial of karyotyping of CLL2 and CLL26 was unsuccessful.

DNA Probes. The pB16 probe contains a part of the first exon of the *bcl-2* gene (10). The

This work was supported in part by grant CD359 from the American Cancer Society (Y. Tsujimoto) and by grants RR-00833 and CA-49870 from the National Institutes of Health (T. J. Kipps). T. Tsujimoto is a Leukemia Society of America Scholar and T. J. Kipps is supported in part as a Scholar of the Leukemia Society with funds from the Scott Helping Hand Fund.

Address correspondence to Dr. Yoshihide Tsujimoto, The Wistar Institute of Anatomy and Biology, 36th and Spruce Street, Philadelphia, PA 19104.

pB3 probe spans the major breakpoint clustering region of t(14;18) translocation, which is localized within the second exon of the *bcl-2* gene (10). The pFL-2 probe lies more than 20 kb downstream from the second exon of the *bcl-2* gene and detects most t(14;18) translocations, the breakpoints of which do not fall in the major breakpoint clustering region (5). DNA probes were ^{32}P labeled by nick translation.

Southern Blot Analysis. High molecular weight DNA, isolated from peripheral leukemia cells and from healthy human placenta as a control, was digested with restriction endonucleases, run on 0.7% agarose, and blotted to a nitrocellulose filter as described (9).

Construction of the Genomic DNA Libraries. DNAs were partially digested with *Sau3AI* enzyme, and DNA fragments between 15 and 23 kb were ligated with λ EMBL3A phage vector and packaged in vitro as described (9).

DNA Sequencing. Nucleotide sequences were determined by the dideoxy chain termination method as described (11).

Results and Discussion

32 fresh specimens of CLL cases were collected from the National Institutes of Health (two cases) (9), Scripps Clinic (20 cases), and the Mayo Clinic (10 cases), and screened for *bcl-2* gene rearrangement using Southern blot analysis. The *bcl-2* gene probes used include pB16 (10) (a part of the *bcl-2* first exon), pB3 (10) (the major breakpoint clustering region of the t(14;18) translocation), and pFL-2 (5) (the minor breakpoint clustering region). Of the 32 CLL samples, three cases (CLL1446 [9], CLL2, and CLL26) showed the rearrangements of the *bcl-2* gene that were detected with the pB16 probe. No case showed any rearrangement with the pB3 and pFL-2 probe. Fig. 1 shows a Southern blot analysis of CLL2 (Fig. 1 A) and CLL26 (Fig.

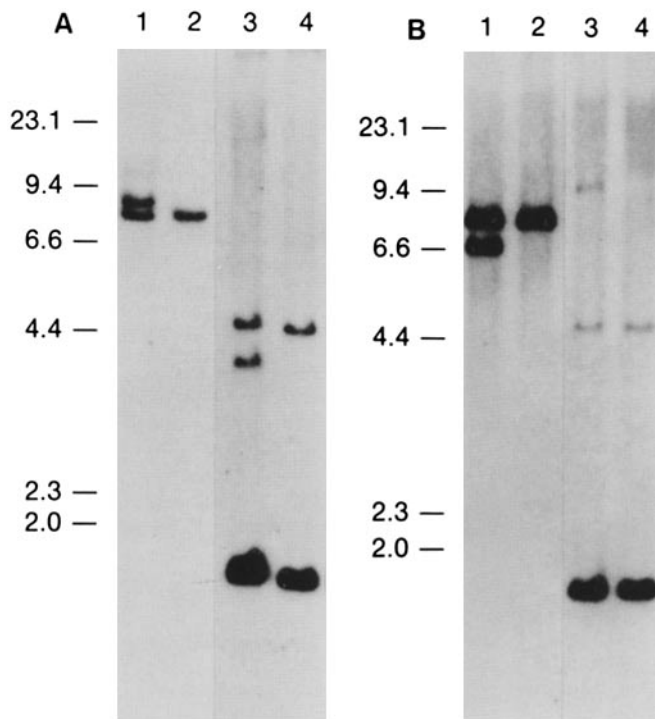


FIGURE 1. Rearrangement of the *bcl-2* gene in CLLs. The Southern blots of CLL2 (A, lanes 1 and 3), CLL26 (B, lanes 1 and 3), and human placenta DNA (A and B, lanes 2 and 4) digested with *Hind* III (A and B, lanes 1 and 2) and *Bam* HI (A and B, lanes 3 and 4) were hybridized with ^{32}P -labeled pB16 probe. CLL2 and CLL26 DNA digested with *Hind* III and *Bam* HI revealed additional hybridizing fragments that represent the rearranged *bcl-2* gene. The size is given in kilobases.

1 B) DNAs with the pB16 probe, in which both revealed distinct rearranged fragments with Bam HI and Hind III restriction enzymes. Thus, a significant fraction ($\sim 10\%$) of CLL involves the rearrangement of the *bcl-2* first exon region.

Genomic libraries were prepared from CLL2 and CLL26 DNAs using λ EMBL3A vector and screened with pB16 probe as described (9). The λ C2 and λ C26 were representative clones containing the rearranged *bcl-2* gene of CLL2 and CLL26, respectively (Fig. 2). A comparison of the restriction maps of these clones with maps of normal *bcl-2*, *Ig λ* , and *Ig κ* genes proved physical linkage between the *bcl-2* gene and *Ig λ* locus in λ C2 clone, and *Ig κ* locus in λ C26 clone, in a head-to-head configuration. We determined the nucleotide sequences in the vicinity of the breakpoints and the relevant portions of the *bcl-2* and *Ig*Ls loci (Fig. 3). The breakpoints on chromosome 18 were mapped within the 5' flanking region of the *bcl-2* gene ($\sim 1,100$ and 600 bp upstream from the 5' end of cDNA [10] in CLL2 and CLL26, respectively). In addition to these two CLL samples, a third (CLL1446) has been shown to carry the *bcl-2*/*Ig λ* rearrangement, the breakpoint of which was mapped ~ 50 bp upstream from the 5' end of cDNA (10), suggesting a hot spot for rearrangements in CLL at the 5' flanking region of *bcl-2* gene. The breakpoints on *Ig*L loci were mapped within the V λ (subclass III) of the V λ J λ joining product in CLL2 (Fig. 3 A) and within the 5' flanking region of J κ 4 in CLL26 (Fig. 3 B). Analysis of the reciprocal recombinant of the λ C26 clone has shown that a V κ gene rearranged to J κ 3 (Fig. 2). It is interesting that the breakpoints of the *bcl-2*/*Ig*L juxtapositions in CLL2 and CLL26 are located near (~ 20 and 150 bp, respectively) the VJ joining

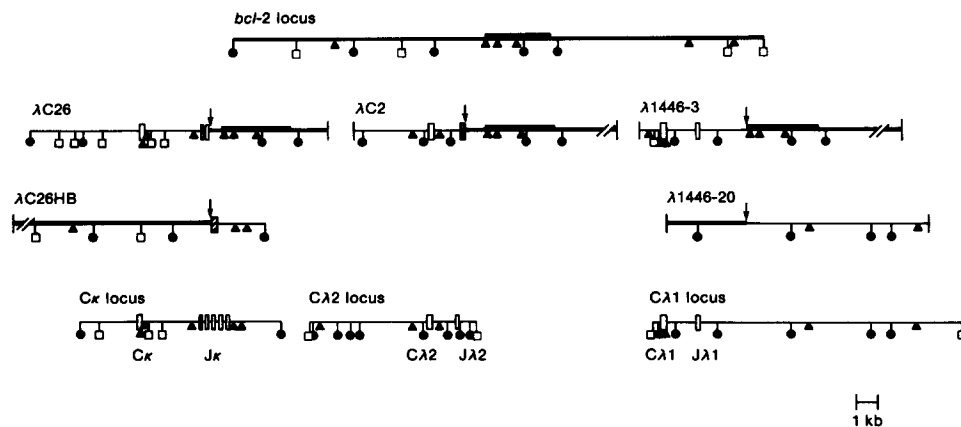


FIGURE 2. Juxtaposition of *bcl-2* and *Ig*L genes in CLLs. The top line represents the structure of the *bcl-2* gene. The first exon of the *bcl-2* gene is shown by the filled box. The transcription direction of the *bcl-2* gene is from left to right. The structures of *Ig*L loci, C κ (15), C λ 1 (16), and C λ 2 (16) in a germline are shown at the bottom. J segments and constant regions are indicated by open boxes. The *bcl-2* and *Ig*Ls loci are shown by thick and thin lines, respectively. Structures of the rearranged *bcl-2* gene in CLL, 26, and 1446 are shown in the middle. The λ C26 and λ C26HB (reciprocal recombinant) were derived from CLL26, and λ C2 was from CLL2. The λ 1446-3 (9) and λ 1446-20 (reciprocal recombinant) (11) were derived from CLL1446 and are shown for comparison. The stippled box in λ C2 and the cross-hatched box in λ C26HB indicate V λ J λ 2 and V κ J κ 3 joining product, respectively. The vertical arrows indicate the breakpoints. The restriction sites are shown by: Bam HI (\bullet), Eco RI (\square), and Sst I (\blacktriangle). All Sst I sites are not shown in C λ 1.

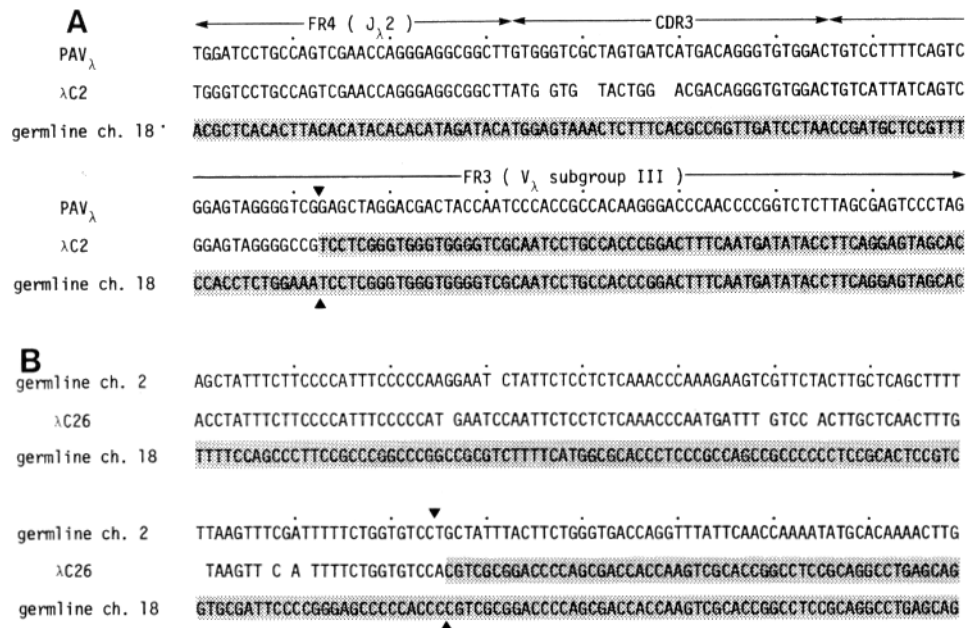


FIGURE 3. Nucleotide sequences of the regions involved in the *bcl-2*/IgL juxtaposition in CLL. The sequences derived from the germline chromosome 18 are shadowed. The triangles indicate the breakpoints. (A) In CLL2, the breakpoint of Ig λ locus is located within the FR3 of V λ subgroup III (17), which was aberrantly rearranged with J λ 2 (FR4), since the V λ J λ joining is out of the frame. The nucleotide sequences of the lower strand of the λ C2 clone are presented in 3' to 5' direction to show the reading frame of the V λ J λ joining product. (B) In CLL26, the breakpoint on Ig κ locus is located within the flanking region between J κ 3 (15) and J κ 4 (15).

sites. This might reflect that the VJ joining product could remain activated, and undergo further rearrangements with new V genes, thus producing additional variations in the VJ product.

As described above, no case of CLL showed any rearrangement with the *bcl-2* probes that detect the major and minor breakpoint clustering regions of the t(14;18) translocation. Conversely, in follicular lymphoma the *bcl-2* gene is commonly rearranged within these two breakpoint clustering regions (1, 12), except in one case (FL989) (13). In the case of FL989, the 5' flanking region of the *bcl-2* gene was rearranged but juxtaposed to IgH locus (13). These results suggest that rearrangement of *bcl-2* gene occurs preferentially at its 5' flanking region in CLL and results in the *bcl-2*/IgL juxtaposition. This situation seems to be characteristic of CLL because none of 22 cases of multiple myeloma samples tested thus far have shown the *bcl-2* gene rearrangement (Adachi, M., and Y. Tsujimoto, unpublished observation).

We are intrigued to find that two different B cell tumors, follicular lymphoma and CLL, are characterized by chromosome translocations involving the same *bcl-2* gene but different Ig loci. Several explanations can be advanced. The *bcl-2* gene might be activated in different ways by IgH and IgL genes at the transcriptional level or through mutations of the protein coding region. The second possibility is based on the timing of *bcl-2* gene activation. The *bcl-2* gene could be activated at

a pre-B stage in follicular lymphoma when IgH gene is rearranged (14) and at a more mature B cell stage in CLL when IgL gene is rearranged, which in turn produces the expanded pool of the prefollicular lymphoma or pre-CLL cells. The expansion of activated B cells by the bcl-2 gene could enhance the chance of acquiring additional genetic alterations of different genes that define the nature of B cell tumors.

Regardless of the mechanism of the bcl-2 gene activation, our finding demonstrates that the choice of breakpoint regions of an oncogene and of Ig genes as an activator can characterize different malignancies of the B cell lineage.

Summary

Most of human follicular lymphomas possess the t(14;18) chromosome translocation that juxtaposes the IgH gene to the 3' region of bcl-2 in a head-to-tail configuration. Here we show that the rearrangement of the bcl-2 gene occurs in a significant fraction (~10%) of B cell CLL. In all cases analyzed, breakpoints on chromosome 18 clustered at the 5' flanking region of the bcl-2 gene, and no rearrangements were found at the major or minor breakpoint clustering region (3' region of bcl-2 gene) typical of the t(14;18) chromosome translocation. All of the rearranged bcl-2 genes were juxtaposed with the Ig λ or κ genes in a head-to-head configuration. These results imply that the bcl-2 gene is preferentially linked to the IgL genes in CLL and could function in leukemogenesis.

We thank Dr. G. Rovera for critical reading of the manuscript, Ms. C. Borer and Mr. C. Ralphe for excellent technical assistance, Dr. L. Rassenti for preparing some of the CLL samples, and the Editorial Services Department of the Wistar Institute for preparing this manuscript.

Received for publication 28 August 1989 and in revised form 9 November 1989.

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