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Genetics

Variant translocation of the *bcl*-2 gene to immunoglobulin λ light chain gene in chronic lymphocytic leukemia

(chromosome translocation/immunoglobulin λ light chain gene/oncogene)

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ABSTRACT **The *bcl*-2 gene has been identified as a gene directly involved in the consistent chromosome translocation t(14;18), which is found in ≈90% of human follicular lymphoma cases, and is a prime candidate for the oncogene playing a crucial role in follicular lymphomagenesis. In this paper, we describe a case of chronic lymphocytic leukemia showing the juxtaposition of the *bcl*-2 gene on chromosome 18 to immunoglobulin λ light chain (Igλ) gene on chromosome 22 in a head-to-head configuration. Sequencing analysis of the joining site of the *bcl*-2 gene and Igλ gene has shown that the breakpoint is within the 5′ flanking region of the *bcl*-2 gene and about 2.2 kilobases 5′ to the joining segment of Igλ locus in a germ-line configuration. The extranucleotide, commonly appearing at the joining site of the t(14;18) translocation involving the IgH locus, is absent from the joining site of *bcl*-2 and Igλ. The lack of extranucleotide suggests that the juxtaposition of the *bcl*-2 and Igλ genes occurred during physiological rearrangement of the Igλ gene since it has been shown that the rearrangement of the Igλ locus is not accompanied by extranucleotides.**

Chromosomal abnormalities, predominantly translocations, deletions, and inversions, are present in most hematopoietic malignancies (1). Involvement of the immunoglobulin genes in chromosomal translocations is observed in various types of B-cell malignancies (2–4). Burkitt lymphoma carries one of three different chromosome translocations—t(8;14) (nearly 80%), t(8;22) (15%), and t(2;8) (5%)—in all of which the c-*myc* gene is juxtaposed to one of the three immunoglobulin loci, heavy chain (IgH), light chain λ (Igλ), and κ chain (Igκ), respectively (3). Although the breakpoints vary among cases in the c-*myc* gene locus as well as immunoglobulin gene loci, a consequence of these translocations is the deregulation of c-*myc* gene expression. This deregulation is due to the cis effect of elements of the immunoglobulin loci (3).

Approximately 90% of the cases of follicular lymphoma, one of the most common hematopoietic malignancies in the United States and Europe, carry the t(14;18) (q32;q21) chromosome translocation that juxtaposes the *bcl*-2 gene on chromosome 18 to the 5′ region of the joining segment of IgH locus (4–7). The *bcl*-2 gene is a prime candidate for the oncogene involved in this translocation because all t(14;18) translocations studied thus far occur within or in close proximity to the *bcl*-2 gene (4–8), and the steady-state level of the *bcl*-2 mRNA is elevated in cells carrying the t(14;18) translocation (8).

Although the c-*myc* gene has been shown to be involved in various types of human tumors, including both hematopoietic malignancies and solid tumors, the *bcl*-2 gene has been implicated specifically in follicular lymphoma and diffuse large cell lymphoma. We began to elucidate the possible involvement of the *bcl*-2 gene in other types of hematopoietic neoplasmas, including chronic lymphocytic leukemia (CLL) and multiple myeloma. In this paper, we describe a case of a patient with CLL cells carrying a *bcl*-2-Igλ rearrangement, representing a variant translocation involving the *bcl*-2 gene.‖

MATERIALS AND METHODS

Construction of the Genomic DNA Libraries. DNA extracted from leukemia cells of a CLL patient was partially digested with the restriction enzyme *Sau*3AI, and DNA fragments between 15 and 23 kilobases (kb) were collected. DNA inserts were ligated with the phage vector λEMBL3A DNA digested with *Bam*HI, and the library was screened with the radiolabeled *bcl*-2 probe by nick-translation using α-^{32}P-labeled dNTPs.

DNA Sequencing. Nucleotide sequences were determined by using the Sanger dideoxy nucleotide protocol for M13 single-stranded DNA or double-stranded DNA (9). Sequences were analyzed by using the University of Wisconsin Genetics Computer Group software (10).

Southern Blot Analysis. High molecular weight DNAs were cleaved with appropriate restriction enzymes, subjected to electrophoresis on 0.8% agarose gels, and transferred to nitrocellulose sheets as described (11).

DNAs. The pB16 probe (12) is a *bcl*-2 cDNA clone that corresponds to the 1.6-kb 3′ part of the first exon. The Huλ-5 clone (13), which contains the normal constant region and joining segment of the Igλ light chain locus (Cλ1 and Jλ1), was kindly supplied by Philip Leder (Harvard Medical School, Boston). The λ18-21 (14) is a genomic clone containing the normal first exon of the *bcl*-2 gene.

Diagnosis of a Patient. Leukemia cells 1446 were obtained by leukopheresis from a patient who was 59 years old and diagnosed as having CLL (1984) with ≈40,000 peripheral leukocytes per mm^3. The leukemia cells had the classic morphology of CLL and produced IgM, IgD, and Igκ as immunologically characterized by flow cytometry (15). The leukemia cells were also CD5 positive, a typical marker of CLL.

RESULTS

Rearrangement of the *bcl*-2 Gene. We and others have previously shown that breakpoints of the t(14;18) transloca-

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Abbreviations: CLL, chronic lymphocytic leukemia; Igλ, immunoglobulin λ light chain; IgL, immunoglobulin light chain; Jλ and Cλ, joining segment and constant region of the Igλ light chain locus, respectively.

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‖The sequences reported in this paper are being deposited in the EMBL/GenBank data base (accession nos. J04529 for the breakpoint and J04530 for chromosome 22).

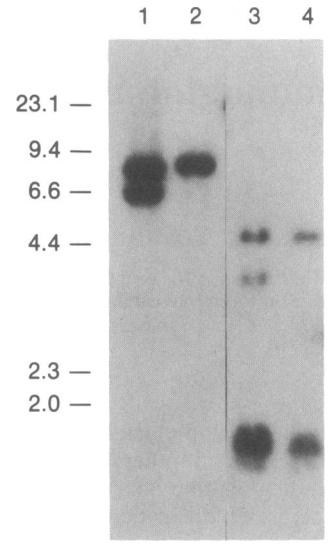

FIG. 1. Southern blot analysis of CLL 1446 DNA. DNA from CLL 1446 cells (lanes 1 and 3) and from a human B-lymphoblastoid cell line (GM607) (lanes 2 and 4) was digested with *Hin*dIII (lanes 1 and 2) or *Bam*HI (lanes 3 and 4). The Southern blot was hybridized with the pB16 probe containing the 3′ part of *bcl*-2 first exon. Size markers are in kb.

tion in follicular lymphoma cluster in several regions, the major one at the 3′ untranslated region of the *bcl*-2 gene and the minor ones 3′ to the gene (4, 8, 16). In addition, we have also described one case of follicular lymphoma that showed the rearrangement at the 5′ end of the *bcl*-2 first exon juxtaposed with the IgH locus (14). During a survey of a panel of malignant B-cell DNAs, including CLL and multiple myeloma, using different regions of *bcl*-2 as DNA probes, one case of CLL (CLL 1446) was found that showed the rearrangement of the first exon of the *bcl*-2 gene. As shown in Fig. 1, a B-lymphoblastoid cell line (GM607) was used as a control that showed fragment(s) in a germ-line configuration in both *Bam*HI and *Hin*dIII digestions, whereas CLL 1446 DNA revealed the distinct rearranged fragments in both enzymes.

Molecular Cloning of the Rearranged *bcl*-2 Allele. A genomic DNA library was prepared from the CLL 1446 DNA in the λEMBL3A vector and screened with a probe, pB16, that corresponds to the first exon of the *bcl*-2 gene (12). The clone λ1446-3 containing the rearranged *bcl*-2 fragment was

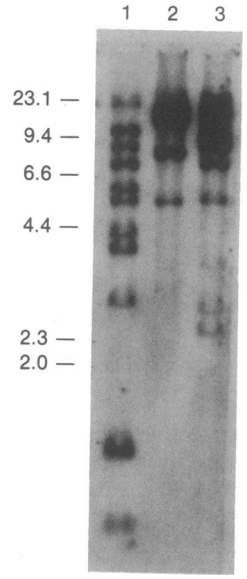

FIG. 3. Hybridization analysis of human placental DNA with the pCλ from the λ1446-3 clone. The human placental DNA was digested with *Bam*HI (lane 1), *Eco*RI (lane 2), and *Hin*dIII (lane 3). The probe recognizes restriction fragments corresponding to the Cλ family (13). The lowest signal in lane 1 indicates the 0.7-kb *Bam*HI fragment, which is identical to pCλ. Size markers are in kb.

selected and subjected to further analysis. The structures of the germ-line clone λ18-21 (14) and clone λ1446-3 are illustrated in Fig. 2. By comparison of λ1446-3 DNA with λ18-21 DNA, the breakpoint on chromosome 18 was located close to the 5′ end of the *bcl*-2 gene as shown in Fig. 2.

To elucidate the origin of the DNA sequences rearranged to the 5′ end of the *bcl*-2 gene, we subcloned the repeat-sequence free 0.7-kb *Bam*HI fragment of the clone λ1446-3 into pUC19 (pCλ) (Fig. 2). A Southern blot analysis probed with pCλ demonstrated that pCλ recognizes multiple fragments in human placental DNA (Fig. 3), which are similar to the Igλ constant region genes (13). In fact, the restriction map of the region beyond the breakpoint in λ1446-3 perfectly fits to the 7.6-kb *Eco*RI–*Sac* I fragment derived from a clone, Huλ5, containing the normal Cλ locus (Fig. 2). The nucleotide sequences were also determined from the 3′ *Bam*HI site of pCλ and showed >98% homology with the sequences of Cλ previously published (13) (data not shown). Thus, rear-

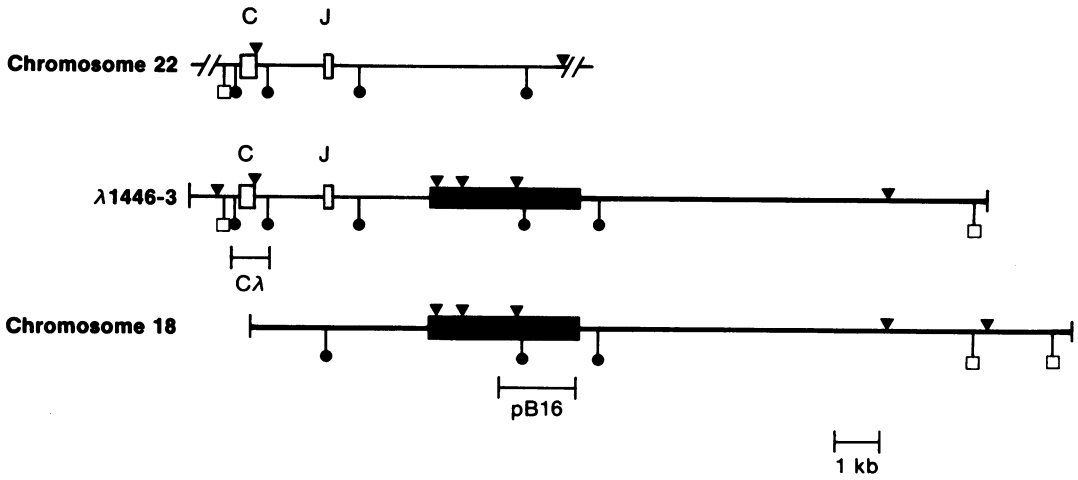

FIG. 2. Restriction map of the rearranged *bcl*-2 gene in CLL 1446. The Huλ5 clone contains the genomic Cλ1 and Jλ1 locus and the λ18-21 clone contains the genomic first exon of the *bcl*-2 gene. The λ1446-3 clone carries the joining region of chromosomes 18 and 22. Chromosome 18- and chromosome 22-derived regions are designated by thick and thin lines, respectively. The closed boxes represent the first exon of the *bcl*-2 gene and the open boxes represent the Jλ1 and Cλ1 exons. Restriction sites are shown by ● (*Bam*HI), □ (*Eco*RI), and ▼ (*Sst* I).

FIG. 4. Nucleotide sequences encompassing the joining region of chromosomes 18 and 22. Arrowheads indicate the sites of breakage. Sequence identity is shown by vertical lines.

rangement of the *bcl*-2 allele was caused by the juxtaposition of the *bcl*-2 gene on chromosome 18 to the Igλ gene on chromosome 22, representing a variant translocation.

Sequence Analysis of the Breakpoint. The nucleotide sequences around the joining region of the *bcl*-2 and Igλ locus were determined and are shown in Fig. 4. The breakpoint on chromosome 18 is located in the 5′ flanking region of the *bcl*-2 gene and is close to the cap site (12, 17). The nucleotide sequences beyond the breakpoint are identical to those of normal Igλ locus. The breakpoint on chromosome 22 is at about 2.2 kb 5′ to the Jλ1 sequence. Comparison of the detailed restriction maps indicates that the Igλ locus is in a germ-line configuration. Note also that no extranucleotide is found at the joining site, the *bcl*-2 and Igλ locus; this is in contrast to the t(14;18) translocation, in which the presence of extranucleotides is more common (5–7).

DISCUSSION

It has been shown that the c-*myc* gene can rearrange at the 5′ and 3′ regions of the gene with IgH and immunoglobulin light chain (IgL) genes, respectively. In this paper, we have described a case of CLL in which the 5′ end of the *bcl*-2 gene rearranges with the Igλ locus. Thus, the *bcl*-2 gene can also rearrange at the 5′ and 3′ regions with the IgL and IgH genes, respectively. The interesting feature of these translocated *bcl*-2 and c-*myc* genes is that these genes are always located 5′ to immunoglobulin constant loci. This configuration might be crucial for activation of these genes (3, 14). We have previously shown a case of follicular lymphoma (no. 989) in which the 5′ region of the *bcl*-2 gene rearranged with the IgH locus (14). The orientation of the IgH locus, however, is not what is expected from the orientation of the *bcl*-2 and IgH genes with respect to the centromere but is opposite, and the *bcl*-2 gene is situated in front of the IgH locus. This observation strongly suggests the requirement of localization of the genes to be activated 5′ to constant loci of immunoglobulin genes. This type of activation is not compatible with characteristics of IgH enhancer, which is orientation independent, and thus suggests the existence of a cis element different from the conventional IgH enhancer in immunoglobulin constant loci with orientation dependency for gene activation.

Sequencing analysis of the joining point of the *bcl*-2 and Igλ genes has shown that these two sequences were joined without any extranucleotides. This is in contrast to the situation of the rearrangement of the *bcl*-2 gene with the IgH locus, in which the addition of extranucleotides is more common. This might reflect the physiological situation whereby rearrangement of the IgH gene (VDJ joining, where V = variable, D = diversity, and J = joining segments) is often associated with the presence of extranucleotides (N region) (18), whereas rearrangement of IgLs (VJ joining) is not (19). This might suggest that the rearrangement of *bcl*-2 and Igλ genes in CLL 1446 occurred during the physiological rearrangement of IgL gene. In this case, however, DNA sequences similar to the 7-mer–9-mer sequences recognized by V(D)J recombinase are not found around the joining site, making it unlikely that V(D)J recombinase is directly involved in the rearrangement of the *bcl*-2 to Igλ locus in CLL 1446.

It has been shown that Burkitt lymphomas carrying the variant translocation t(2;8) and t(8;22) always produce Igκ chain and λ chain, respectively (20). In one exceptional case of Burkitt-like lymphoma (PA 682), derived from a homosexual patient with acquired immunodeficiency syndrome, the t(8;22) translocation was present but produced Igκ chain (21). The CLL 1446 represents another example of this nonconcordance with respect to IgL expression. These exceptions might suggest that rearrangement of the Igλ gene could occur even after productive rearrangement of the Igκ chain gene or that rearrangement of the Igλ gene could precede rearrangement of the Igκ chain gene as a rare event. Alternatively, rearrangement of the *bcl*-2 gene/Igλ seen in CLL 1446 might not be totally dependent on physiological rearrangement of IgL.

The *bcl*-2/Igλ rearrangement described in this paper is a very rare event in CLL, but it might represent a subclass of CLL. Although the *bcl*-2/Cλ rearrangement has not been described in follicular lymphoma, the cases described to show rearrangement of the *bcl*-2 first exon (16) might carry this type of rearrangement. The *bcl*-2 gene is involved in low-grade (not acute) B-cell malignancies, such as follicular lymphoma and CLL, suggesting that additional oncogenetic events are crucial in higher-grade malignancies such as acute lymphoblastic leukemia. Coactivation of c-*myc* and *bcl*-2 genes, for example, has been described in B-cell acute leukemias (22, 23). These data imply that the *bcl*-2 gene might have a lower tumorigenic potential than the c-*myc* gene. This is consistent with our recent observation that the overproduction of the *bcl*-2 gene product enhances growth of an Epstein–Barr virus-transformed B-cell line but does not confer tumorigenicity (24).

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