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Genetics

DNA rearrangements in human follicular lymphoma can involve the 5′ or the 3′ region of the *bcl-2* gene

(genetics of B-cell lymphomas/gene deregulation/immunoglobulin gene rearrangements)

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ABSTRACT **In most human follicular lymphomas, the chromosome translocation t(14;18) occurs within two breakpoint clustering regions on chromosome 18, the major one at the 3′ untranslated region of the *bcl-2* gene and the minor one at 3′ of the gene. Analysis of a panel of follicular lymphoma DNAs using probes for the first exon of the *bcl-2* gene indicates that DNA rearrangements may also occur 5′ to the involved *bcl-2* gene. In this case the IgH locus and the *bcl-2* gene are found in the order 3′ C_γ $S_{\gamma/\mu}$ J_H 5′::5′ bcl-2 3′ (where C = constant, S = switch, and J_H = joining segment of the heavy chain locus), suggesting that an inversion also occurred during the translocation process. The coding regions of the *bcl-2* gene, however, are left intact in all cases of follicular lymphoma studied to date.**

More than 80% of human follicular lymphomas carry a t(14;18)(q32;q21) chromosome translocation (1, 2). This translocation directly involves the immunoglobulin heavy chain (IgH) locus and the *bcl-2* (B-cell lymphoma/leukemia 2) gene (3, 4). By comparing the structures of *bcl-2* cDNA clones and genomic DNA clones, we have shown that the human *bcl-2* gene consists of at least two exons separated by an intron of >50 kilobases (kb) of DNA (5). The first exon is transcribed into a 3.5-kb mRNA (5) (also see Fig. 1). This exon contains a splicing donor signal so that *bcl-2* mRNA is spliced to the second exon to produce a 5.5-kb and an 8.5-kb mRNA (5) (also see Fig. 1). The 5.5-kb and 3.5-kb mRNA code for the *bcl-2* α and β protein, respectively, which are identical except for the carboxyl-terminal portion (5). We have shown that ≈60% of the breakpoints of the t(14;18) translocation on chromosome 18 are tightly clustered in the 3′ noncoding region of the *bcl-2* gene (3–5) and ≈10% are clustered at a region 3′ to the *bcl-2* gene (3, 4) (also see Fig. 1). We have also shown that the association of the *bcl-2* gene with the heavy chain locus results in high levels of *bcl-2* expression (3). In all cases we have analyzed previously, the immunoglobulin heavy chain (IgH) locus, including the IgH enhancer, is 3′ to the involved *bcl-2* open reading frames (3–5), suggesting that the IgH locus is responsible for the *bcl-2* activation in the follicular lymphomas.

MATERIALS AND METHODS

Gel Electrophoresis and Southern Transfer. High molecular weight DNA was digested with restriction endonucleases and 5-μg samples were fractionated on 0.7% agarose gels and transferred to nitrocellulose filters essentially as described by Southern (6).

Construction of Genome 2 DNA Library. DNA extracted from the follicular lymphoma FL989 was partially digested with restriction enzyme *Sau*3AI and DNA fragments between 15 and 23 kb were collected. DNA inserts were ligated with λEMBL3A phage vector DNA cut with *Bam*HI and packaged *in vitro* (7, 8). Two recombinant phage clones, containing a fragment corresponding in size to the rearranged *bcl-2* first exon sequences, were obtained by screening the library with the *bcl-2* first exon probe pB16 (5).

DNA Sequencing. Nucleotide sequences were determined by the method of Sanger *et al.* (9). Both strands of DNA were sequenced.

RESULTS

To identify additional breakpoint cluster regions involving the *bcl-2* locus, we used different DNA probes surrounding the first exon to screen a panel of 17 follicular lymphoma DNAs for rearrangement (3). We have shown previously that 9 of the 17 randomly collected follicular lymphomas have the breakpoints within the cluster region a and one has the breakpoint within the cluster region b (Fig. 1) (3). The cell line 380, from which we initially cloned the t(14;18) chromosome breakpoint, and a follicular lymphoma sample, LN128, which have been shown to carry the t(14;18) translocation, have breakpoints within cluster region b (3, 7). Interestingly, one follicular lymphoma, FL989, showed rearrangement of the *bcl-2* locus, as detected using a first exon probe (Fig. 2). Good metaphases were not obtained from this lymphoma for analysis of the chromosomal abnormality. We prepared a genomic DNA library from the FL989 DNA in the λ phage vector EMBL3A as described in *Materials and Methods* and screened the recombinant clones with a probe for the first exon of the *bcl-2* gene. Fig. 3 shows the structure of the germ-line and rearranged *bcl-2* first exon and also presents the order of the J_H (J segment of the heavy chain locus), $S_{\gamma/\mu}$, and C_γ regions of the IgH locus, as determined based on the hybridization results (not shown) using the respective probes for these regions and the phage clones containing the rearranged *bcl-2* gene. The nucleotide sequence encompassing the breakpoint is shown in Fig. 4. The nucleotide sequence of the rearranged *bcl-2* first exon shows homology to normal chromosome 18 DNA and to the J_H segment of the IgH locus. There is also a stretch of ≈170 nucleotides between the J_H DNA sequences and the chromosome 18 DNA sequences that does not seem to be derived from either the heavy chain locus or chromosome 18. We have demonstrated the presence of extranucleotides, N regions (11), at joining sites between chromosomes 14 and 18 in several follicular lymphomas (4). However, the stretch of extranucleotides in FL989 is too long to be simply an N region. As shown in Fig. 3, the IgH locus is joined to the *bcl-2* gene in the order C_γ $S_{\gamma/\mu}$ J_H 5′::5′ bcl-2 3′. This is of considerable interest because this

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Abbreviations: *bcl-2*, B-cell lymphoma/leukemia 2; J_H, joining segment of the heavy chain locus.

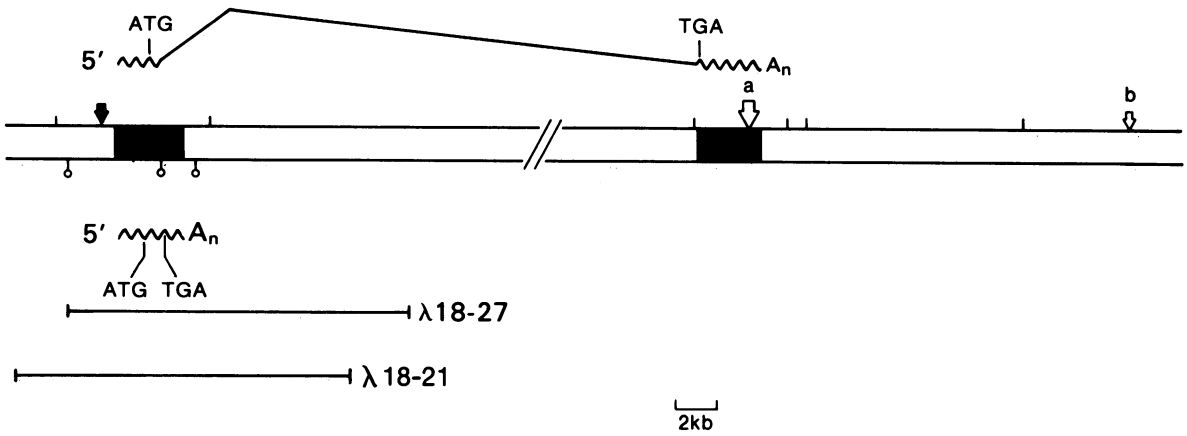

FIG. 1. Genomic structure of human *bcl-2* gene. Two exons are shown by filled boxes. The distance between these exons on the genomic DNA is not yet known nor is the position of the *bcl-2* gene 5' end. The upper and lower wavy lines indicate the 5.5- and 3.5-kb mRNA species, respectively. The 3.5-kb-long mRNA is the unspliced form of mRNA, and the 5.5-kb mRNA is the spliced form consisting of two exons. The 8.5-kb mRNA (not shown) seems to use a different poly(A) signal than that used by the 5.5-kb mRNA. The initiation and termination codons for *bcl-2* α (on 5.5-kb mRNA) and β (on 3.5-kb mRNA) proteins are also indicated on the mRNAs. Genomic clones λ18-21 and λ18-27, isolated by screening the 380-cell genomic library with the pB16 (5) first exon probe, are shown below the *bcl-2* gene. Empty arrows indicate two breakpoint hot spots, a and b. The filled arrow indicates the breakpoint in FL989 cells. The restriction enzyme cleavage sites are shown by ◊ (*Bam*HI) and I (*Hin*dIII).

gene order cannot be explained by a simple chromosome translocation event, since the genes for IgH variable genes (V_H) are distal to the constant region genes (12) and the 5' end of the *bcl-2* gene is distal to its 3' end (5). A simple chromosome translocation should give rise to the order 3' C_γ $S_{\gamma/\mu}$ J_H 5'::3' bcl-2 5' that is commonly observed in follicular lymphomas with the t(14;18) chromosome translocation (4, 5). Thus, the gene order on the translocation chromosome of FL989 cells suggests that the translocation involved the inversion of either the heavy chain locus or, less likely, of the *bcl-2* locus. It has been shown that the loci for the κ light chain of the immunoglobulin and β chain of the T-cell receptor may undergo inversion during the process of gene rearrangement (13, 14). Thus, the DNA stretch of unknown origin between J_H and the chromosome 18-derived sequences might normally be located 3' to the IgH constant region gene.

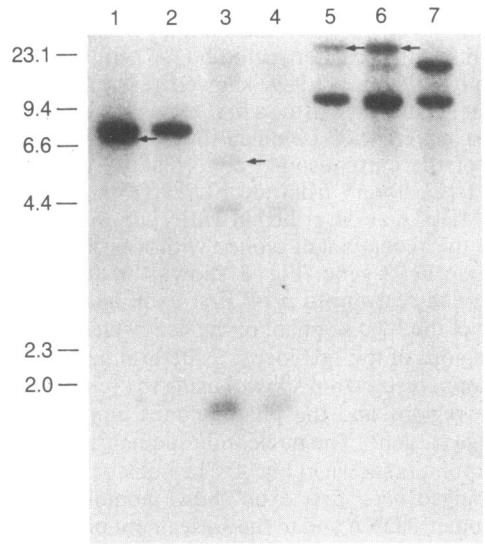

FIG. 2. Rearrangement of the *bcl-2* first exon sequences in FL989. DNAs from FL989 and from GM607 human lymphoblastoid cells were digested with several restriction enzymes, separated on a 0.7% agarose gel, and analyzed by Southern blot hybridization with a *bcl-2* cDNA clone, pB16 (5), which corresponds to about the 1.6-kb 3' part of the first exon. Arrows indicate the rearranged restriction fragments. The *Eco*RI fragment shows restriction enzyme length polymorphism. Since the pB16 probe has a *Bam*HI site (see Fig. 1), *Bam*HI digestion shows two bands for the germ-line configuration. Two *Eco*RI digests of FL989 (lanes 5 and 6) were derived from two sequential biopsies of the same patient. Size is given in kb. Lane 1, FL989/*Hin*dIII digest; lane 2, GM607/*Hin*dIII; lane 3, FL989/*Bam*HI; lane 4, GM607/*Bam*HI; lanes 5 and 6, FL989/*Eco*RI; lane 7, GM607/*Eco*RI.

DISCUSSION

We have shown here one case of follicular lymphoma in which the 5' region of the *bcl-2* gene is rearranged with IgH locus. In most cases of follicular lymphoma the 3' region of the *bcl-2* gene is involved in the t(14;18) chromosome translocation. Since, in the case of FL989, karyotype analysis did not succeed, the juxtaposition of the *bcl-2* gene to IgH locus can be explained by either chromosome translocation t(14;18) or transposition of IgH sequences or *bcl-2* sequence.

The finding of a *bcl-2* rearrangement 5' to the *bcl-2* open reading frames parallels the findings in Burkitt lymphomas where the rearrangements involving the *myc* locus may occur 5' to 3' to the involved *myc* locus (15). In Burkitt lymphomas with the t(8;14) chromosome translocation, the rearrangements occur 5' to the *myc* open reading frame, whereas in Burkitt lymphomas with the t(8;22) or the t(2;8) chromosome translocations, the rearrangements occur 3' to the *myc* locus. Similarly, the chromosomal breakpoints in follicular lymphoma define the chromosomal position of the open reading frames of the gene involved in the neoplastic process.

Since we have never observed a case of follicular lymphoma with the t(14;18) chromosome where the entire DNA region comprising the enhancer and constant region genes is not associated with the *bcl-2* locus, it seems likely that both of these DNA segments can be either upstream or downstream of the *bcl-2* gene in order to result in *bcl-2* deregulation. In addition, molecular analysis of different cases of follicular lymphoma indicates that the rearrangements most commonly involve either the 3' exon or are downstream of it. Thus, since the distance between the 5' exon and the 3' exon is at least 50 kb and since the exons are ≈8.5 kb in length, the distance between the *bcl-2* promoters and the heavy chain element must be >60 kb. Thus, the in cis influence of the heavy chain locus on the expression of the gene involved in the malignant process must occur over considerable chromosomal distances.

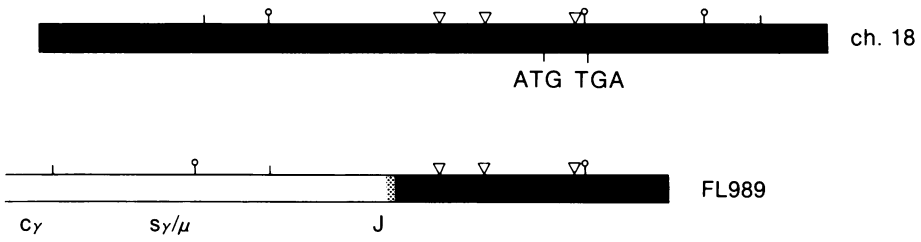

FIG. 3. Rearranged fragment of the *bcl-2* first exon in the follicular lymphoma. The upper and lower lines represent the germ-line and rearranged configuration, respectively, of *bcl-2* first exon sequences in FL989. Filled and open bars indicate chromosomes 18 and 14, respectively. Stippled areas indicate the DNA sequences of unknown origin (see text). The restriction enzyme cleavage sites are shown by ♀ (*Bam*HI), ▽ (*Sst* I), and | (*Hin*dIII). The initiation (ATG) and termination (TGA) codons for *bcl-2* protein are shown. C, constant; S, switch; J, joining.

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I  TGG CCAGTCCGCCCAGGTCCC GTCGGAACAAGGCCCGAGG ACCACCCTTCAA GGCTCAGGAAACCCCACAGGCAGTAGCAGAAAACAAGGGCCCTA
II TGGACCAGTCCG CCAGGTCCCCCATGGA CATGCCCCGAGGCACCAACCG CAATGGCGCAGGAAACCCCACAGGCAGTAGCAGAAAACAAAGGCCCTA

I  GAGTGGCCATTTTTACTTAAGAATGCCACGACCCCACAGGCAACAACTGGCGCCCCCCAACGTCCCTGGGACCCTCTCAGACTGAGCCCGGGGAGGGCCC
II GAGTGGCCATTCTTACCTGAGGAGACGGTGACCGTGGTCCCTTGCCCCC
                   ◄── J6

I  GGGGGTTATTGGGCATTGGACCCCAGAGGCCTAGGGTGGCCCTGGCAAGAGAGACCCATCGTAATTTGGACTCAGGAGGAAGAATTGTCATCCAGGCGAC
III                GCGGCCGAGCGCCGGTTAACACAACGTGTGTGTGTGTAGCGCGTGTACACACTCTCATACACGGCTAGAAAGGGTCCAGGCGAC

I  ACACACATTGCCACAAAGACGCC AGAAAAGAACCAGTCGACACACACACACACACACACATAGATTTTT CACACACACTTATACGACCA AAAGGGT
III ACACACACTCCCACATACACGGCCAGAAAAGGTCCAGGCGTCACACACACACACACACACACACACACACTCCACACACACTCACACGGCCAGAAAGGGT

I  CG          CGGCGCTTCTCCAGACCC TCCGCCCGGCCCGGCCGCGTCTTTTCGTGGCGCTCCCTCCCGCCAGCCGCCCCCCTCCGCACTCCGTCG
III CCAGGCGGTTGGCGGCGCTTTTCCAG CCCTTCCGCCCGGCCCGGC GCGTCTTTTCATGGCGCACCCTCCCGCCAGCCGCCCCCCTCCGCACTCCGTCG

I  TGCGATTTTTCGGGAGCCCCCACCCCGTCGCGGACCCCAGCGACCACGAAGTCGCACCGGCCTCCGCAGGCCTGAGCAGAAGGCCCCGCGCACACCCCCG
III TGCGATTCCCCGGGAGCCCCCACCCCGTCGCGGACCCCAGCGACCACCAAGTCGCACCGGCCTCCGCAGGCCTGAGCAGAAGGCCCCGCGCACACCCACG
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FIG. 4. Nucleotide sequences encompassing the breakpoint of the chromosome translocation in FL989. The nucleotide sequences of normal and rearranged *bcl-2* first exon and a part of the J_H region (J_6) of the IgH locus (10) are shown. Vertical lines indicate the sequences identical in the two parts. Lines 1, 2, and 3 represent breakpoint sequences, J_H sequences, and normal chromosome 18 sequences, respectively. The antisense sequences of J_H are shown.

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