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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Communicated by Robert J. Huebner, November 3, 1986

ABSTRACT In most human follicular lymphomas, the chromosome translocation (t14;18) occurs within two breakpoint clustering regions on chromosome 18, the major one at the 3' untranslocated 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' Cγ Sμμ JH 5':5' bcl-2 3' (where C = constant, S = switch, and JH = 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 Sau3AI and DNA fragments between 15 and 23 kb were collected. DNA inserts were ligated with λEMBL3A phage vector DNA cut with BamHI 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 JH (J segment of the heavy chain locus), Sμμ, and Cγ regions of the IgH locus, as determined based on the hybridization results (not shown) using the respective probes for these regions and the 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 Jγ4 segment of the IgH locus. There is also a stretch of ~170 nucleotides between the JH 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 Cγ Sμμ JH 5':5' bcl-2 3'. This is of considerable interest because this

Abbreviations: bcl-2, B-cell lymphoma/leukemia 2; JH, joining segment of the heavy chain locus.
The breakpoint hot spots, a and b. The filled arrow indicates the breakpoint in FL989 cells. The restriction enzyme cleavage sites are shown by β (BamHI) and γ (HindIII).

gene order cannot be explained by a simple chromosome translocation event, since the genes for IgH variable genes (VH) 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' Cγ2 Sγ2 JH 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 JH and the chromosome 18-derived sequences might normally be located 3' to the IgH constant region gene.

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 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 \( \triangleright \) (BamHI), \( \triangledown \) (StI I), and \( \triangledown \) (HindIII). The initiation (ATG) and termination (TGA) codons for bcl-2 protein are shown. C, constant; S, switch; J, joining.

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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<sub>H</sub> region (J<sub>H</sub>) 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<sub>H</sub> sequences, and normal chromosome 18 sequences, respectively. The antisense sequences of J<sub>H</sub> are shown.

We thank Ms. Deborah Jiametti and Mr. Andrew E. Ochroch for excellent technical assistance. Ms. Charlotte Long for preparation of the manuscript, and Ms. Marina Hoffman for editing. This study was supported by Grant CA 39860 from the National Cancer Institute.