Genomic Organization of IgH Gene Compared With the Expression of Bcl-2 Gene in t(14;18)-Positive Lymphoma


In three lymphoma cell lines carrying t(14;18), named FL-18, FL-218, and FL-318, the genomic organization of IgH gene was compared with the expression of bcl-2 gene; the t(14;18) of the FL-18 cells occurred downstream from the major breakpoint cluster region (mbr) of a bcl-2 gene, and that of the FL-218 and FL-318 cells within the mbr. The FL-318 expressed the normal-sized bcl-2 transcript of 8.5-kb mRNA having the noncoding region 3’ to the mbr, which was found in the FL-18, and the FL-218 lacking the intact bcl-2 gene did not. This finding suggests that in t(14;18)-positive lymphoma having the breakpoint within the mbr, transcription of the nontranslocated bcl-2 allele is not necessarily silent. In addition, the FL-218 and FL-318 expressed aberrant bcl-2 transcripts and heterogenous IgH transcripts lacking the VH region, and the bcl-2 transcripts each comigrated with parts of the sterile IgH mRNAs. The FL-318, which did not exhibit switch recombination on either IgH allele, contained abundant amounts of lµ mRNAs, a prerequisite for the recombination into the Cy locus. One of the lµRNA species comigrated with the aberrant bcl-2 transcript. The FL-18 and FL-218 lacking the lµ mRNAs had completed switch recombination of both Igh alleles. This result raises a possibility that deregulated bcl-2 transcription caused by t(14;18) is capable of playing a role in class switch recombination of IgH gene.

MATERIALS AND METHODS

Cells. The three follicular lymphoma cell lines used in this study, FL-18, FL-218, and FL-318, carry a specific t(14;18) (q32.3; q21.3) translocation (Fig 1A). The breakpoint of a bcl-2 gene involved in the translocation of the FL-18 cells was previously localized downstream from the major breakpoint cluster region (mbr), but within the 7.8-kb Sst I fragment containing the mbr, and that of the FL-218 and FL-318 cells within the mbr. The FL-18 cells had surface IgG and κ-light chain, the FL-218 cells had surface IgG and λ-light chain, and the FL-318 cells expressed IgM and κ-light chain on their surface. These FL cells were negative for Epstein-Barr virus (EBV) nuclear antigens. As controls, we used nine other cell lines that do not have the t(14;18): one EBV-immortalized clonal lymphoblastoid cell line, named LCL-Amakawa (AM), having a normal male karyotype, and eight B-lymphoma cell lines showing abnormal karyotypes.

Southern and Northern blot analysis. High molecular weight genomic DNA extracted from cells was digested with appropriate restriction endonucleases, including EcoRI, BamHI, HindIII, and Sst I, separated on a 0.7% agarose gel, denatured with alkali, and transferred to a nylon membrane filter. DNA and RNA filters were hybridized with a nick-translated probe in a mixture containing 50% formamide, 5 × Denhardt’s solution, 0.1% sodium dodecyl sulfate (SDS), 50 mmol/L phosphate buffer (pH 6.5), and heat-denatured salmon sperm DNA (250 μg/mL) at 42°C for 24 hours. The filter washing was done with 2 × SSC-0.1% SDS at room temperature and then with 0.1 × SSC-0.1% SDS at 50°C for 30 minutes.

DNA probes. The probe used for chromosome 18-specific DNA (bcl-2 gene) is a 2.8-kb Sst I-HindIII fragment, named “probe b1,” that spans the mbr of bcl-2 gene (Fig 1B). A 3’ bcl-2 probe that is a 1.0-kb HindIII fragment was also used for detection of the noncoding region sequences just 3’ to the mbr. The following DNA fragments were used as probes for the IgH loci.

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0004-4971/91/7709-0124$3.00/0

IGH AND BCL-2 GENES IN t(14;18)

Fig 1. (A) G-banded karyotypes of chromosomes 14 and 18 in the FL-18, FL-218, and FL-318 cell lines carrying a t(14;18)(q32.3;q21.3). The normal chromosome in each pair is on the left. These FL cells have a normal chromosome 14 and a 14q+ (arrowhead) derived from the t(14;18). The FL-18 and FL-318 cells retain a normal chromosome 18 and the FL-218 cells lack the normal chromosome. The FL-18 cells have an 18q- (*), the FL-218 cells have an extra 18q-, and the FL-318 cells have an extra 18q- and an 18q- isochromosome. (B) Physical map of bcl-2 gene on chromosome 18, showing location of "probe b" for the mbr and sites of breakpoints of the FL-18, FL-218, and FL-318 cell lines. (.) The open reading frame and (01 the 3' noncoding region of the third exon. A 3' bcl-2 probe can detect the noncoding region just downstream of the mbr, where polyadenylation consensus sequences are conserved. Restriction sites of BamHI, SstI, and HindIII are shown by *, V, and I, respectively. The first and the second exons are not shown on this map.

probe for joining (J) region of IgH gene is the 3.4-kb EcoRI-HindIII fragment containing JH-3, 4, 5, and 6. The Cμ probe for constant (C) region is the 1.2-kb EcoRI-EcoRI fragment containing the CH1, CH2, CH3, and a part of the CH3 exons of the Cμ gene. The Cy1 probe is the 0.6-kb Pst I-Sma I fragment containing the CH1, CH2, and part of the CH3 exons of the Cy1 gene. The VH probe for variable (V) region is a mixture of VH-1, VH-II, VH-III, VH-IV, VH-V, and VH-VI probes. The Iγ1 probe is the 1.4-kb HindIII-BamHI fragment containing the I, II, and III exons of the Iγ1 regions. The region designated Iγ3 consists of segments that are localized close to the 5' end of the Cy1 and Cy3 switch regions (Fig 2B).

RESULTS

Genomic organization of IgH gene. Genomic bcl-2 organization of three follicular lymphoma cell lines carrying t(14;18), named FL-18, FL-218, and FL-318, has already been reported elsewhere.16,17 and the profile is briefly described here. When the DNAs from the FL cells were digested with EcoRI, BamHI, HindIII, and Sst I, Southern blot analysis using probe b showed that the breakage of a bcl-2 gene involved in the translocation of the FL-18 cells occurred at the 3' region, slightly distant from the entire 4.3-kb HindIII fragment containing the mbr. The FL-218 and FL-318 cells had the breakpoint within the mbr. An intact bcl-2 gene was lost in the FL-218 cells missing the normal chromosome 18 (Fig 1). In the hybridization study using the JH probe, two rearranged JH fragments were observed in all the digest of the FL-18 and FL-218 cells. The FL-318 cells appeared to have one rearranged JH-containing BamHI fragment. However, after digestion with other enzymes, two rearranged JH fragments were observed. These FL cells did not contain the germline JH fragment, and one of the two rearranged JH fragments comigrated with the rearranged bcl-2 fragment detected by probe b. This result indicates that the t(14;18) resulted in the juxtaposition of the truncated part of a bcl-2 gene on chromosome 18 with the IgH gene locus on chromosome 14.

Their filters were hybridized with DNA probes to examine the rearrangement profile of the IgH gene locus. Figure 3 shows representative results of the Southern blot analysis in the BamHI digest (Fig 3A through C); the restriction enzyme is useful in the study of class switch recombination because each Cγ, Cα, and Ce gene locus, including the switch region, is present on a single BamHI fragment in the genome. In the FL-18 and FL-218 cells, the Cμ probe did not detect any fragments, indicating that the Cμ loci had been deleted from both IgH alleles. In these cell lines, the
Cy1 probe that cross-hybridizes with all the Cy subclass genes showed deletion and rearrangement of certain Cy subclass genes, and the rearranged Cy1 fragment comigrated with the rearranged JH fragment that did not hybridize with probe b. This finding suggests that, corresponding to the expression of IgG on the surface of the FL-18 and FL-218 cells, the JH and Cy segments were physically linked in the genome on a normal chromosome 14. In the FL-218 cells, a BamHI fragment detected by the Cy1 probe located at the germline position also hybridized with probe b and JH probe, suggesting the linkage of bcl-2, JH, and Cy genes on the translocated IgH allele. Thus, the linkage of bcl-2, JH, and Cy genes occurs on the translocated allele, and the physical linkage of JH and Cy genes on the productive allele. The BamHI fragment for certain Cy subclass genes may be polymorphic (asterisk in Fig 3C); however, the HindIII sites flanking the Cy genes are conserved (data not shown).

The Ca2 probe that cross-hybridizes with Ca1 gene detected no rearranged fragments in the BamHI digests of the FL-218 and FL-318 cells, while the probe detected one rearranged BamHI fragment that hybridized with probe b and JH probe in the FL-18 cells, suggesting that the linkage of bcl-2, JH, and Cy genes occurred on the translocated IgH allele of the FL-18 cells. Schemata deduced from these findings are given in Fig 4, showing that both IgH alleles of the FL-18 and FL-218 cells underwent class switch recombination events, while those of the FL-318 cells did not.

Expression of bcl-2 and IgH genes. When poly(A)' RNAs obtained from the FL-18, FL-218, and FL-318 cells were fractionated in agarose gel, Northern blot analysis using probe b disclosed active transcription of a bcl-2 gene (Fig 5). Eight t(14;18)-negative B-lymphoma cell lines expressed little or no detectable bcl-2 mRNA. The FL-18 cells expressed an abundant amount (8.5 kb) of the bcl-2 transcript, the same size as the major species of normal bcl-2 transcripts in the EBV-immortalized LCL-AM cells used as control. The 8.5-kb mRNA was also abundantly expressed in the FL-318 cells. The normal-sized transcript in the FL-18 and FL-318 cells, as well as in the LCL-AM cells, hybridized with a 3' bcl-2 probe for the 3' noncoding region just downstream of the mbr. The FL-218 cells lacking an intact bcl-2 gene did not express the 8.5-kb bcl-2 mRNA. The FL-218 and FL-318 cells contained abnormal-sized bcl-2 transcripts that were not labeled with the 3' bcl-2 probe.

Transcripts of the IgH gene in the FL cells were compared with the isotypes expressed on the cell surface and the genomic organization (Fig 6). The FL-18 and FL-218 cells expressing the µ-chain contained two species of Cy-chain mRNA of 3.5 and 1.8 kb. The FL-318 cells expressing the µ-chain had two species of µ-chain mRNA of 2.4 and 2.1 kb on the short time exposure autoradiogram. These two species of Cy- or µ-containing mRNAs hybridized to the VH probe (data not shown), indicating that they are the membranous-bound and the secretory forms of mature IgH mRNAs, respectively. Surprisingly, the FL-318 cells that did not exhibit switch recombination of both IgH genes also continuously expressed abundant amounts of Cy-containing mRNAs, which consisted of four explicit species of 3.5, 3.0, 1.8, and 1.5 kb. These mRNAs were not hybridized with a mixture of probes for six VH gene families. This finding suggests that they could be "sterile" transcripts lacking the VH region. In addition, the 3.5- and 1.8-kb mRNAs were labeled with the Iy1 probe that spans the Iy region located 5' to the switch region of Cy1 locus (Fig 2), confirming that they are germline Cy transcripts. Such a constitutional expression of the Iy1 mRNAs was not observed in eight t(14;18)-negative B-lymphoma cell lines (data not shown), including four cell lines that had µ-chain on the surface. The FL-18 and FL-218 cells that had
completed switch recombination of both IgH alleles did not express any germline Cγ transcripts. The long exposure of the autoradiograms disclosed that the FL-218 and FL-318 cells expressed other species of IgH genes, some of which comigrated each other; they were JH-, Cκ-, Cγ-, and/or Cα-containing mRNAs in the FL-218 cells, and JH-, Cκ-, Cγ-, and/or Cα-containing mRNAs in the FL-318 cells. These transcripts were abnormally large and heterogeneous, and lacked the VH region. Two species of the less amount of sterile Cγ mRNAs found in the FL-318 cells were also labeled with the Jγ1 probe (Fig 2). The abnormal-sized bcl-2 transcripts expressed in the FL-218 and FL-318 cells each comigrated with parts of the sterile JH and/or CH transcripts. One of the two minor Jγ mRNA species was present in a comigration line of the sterile IgH transcripts with the aberrant bcl-2 transcript (Figs 2A and 6). In contrast, none of these sterile transcripts in the FL-318 cells comigrated with the normal-sized bcl-2 transcript of 8.5-kb mRNA.

**DISCUSSION**

The FL-18 cells having t(14;18) that occurred downstream from the mbr of a bcl-2 gene expressed the normal-sized bcl-2 transcript of 8.5-kb mRNA. The bcl-2 transcript hybridized with a 3' bcl-2 probe for the noncoding region just 3' to the mbr, where the polyadenylation consensus sequences are conserved. Thus, the transcription of a bcl-2 gene involved in the t(14;18) probably cannot proceed across the breakpoint junction. The FL-218 and FL-318 cells having t(14;18) that occurred within the mbr expressed abnormal-sized bcl-2 transcripts that did not hybridize with
Corresponding to the isotype expressed on the cell surface and the genomic organization of the nontranslocated IgH allele, the FL-18, FL-218, and FL-318 cells had the membrane-bound and the secretory forms of the IgH mRNAs. In addition, the FL-218 and FL-318 cells contained heterogenous IgH transcripts that did not hybridize with the VH mixture probe. Therefore, these are "sterile" transcripts that cannot be translated to produce Ig heavy chain. These sterile transcripts are of two types: comigrated and noncomigrated with the abnormal-sized bel-2 transcript. The noncomigrated JH- and/or CH-containing mRNAs could be the differentially spliced products of the long comigrated IgH mRNAs. In support of this interpretation are the findings that neither the FL-18 cells nor the EBV-immortalized LCL-AM cells expressed any sterile IgH transcripts similar to those in the FL-218 and FL-318 cells.

Among the sterile transcripts of the IgH locus, multiple species of Cy containing mRNAs found in the FL-318 cells, which did not undergo class switch recombination on either IgH allele, are especially noteworthy. Hybridization with the Iyl probe gave strong evidence that the four Cy transcripts, including two minor species, are of germline origin, because the segments designated Iy are localized upstream of the switch region of each Cy subclass gene in the human genome. A similar pseudo exon upstream of the Sy2b region is present in Abelson Murine virus-transformed pre-B cells. In the murine system, class switching on both the productive and the nonproductive alleles has been reported to occur after induction of germline γ2b transcripts by treatment with lipopolysaccharide, leading to the hypothesis that the transcription of a certain unrearranged CH region, named I region, would open the chromatin structure surrounding the adjacent switch region and modulate the accessibility of the corresponding switch region to a putative common switch recombinase. According to the accessibility model, these germline Cy mRNAs found in the FL-318 cells reflect the status preceding a class switch.
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Fig 6. Northern blot analysis of three FL cell lines. Three micrograms of poly(A)+ RNA were loaded into each lane. The filter each for the FL-18, FL-218, and FL-318 cells was serially hybridized with probe b, JH, Cµ1 probe, and that of FL-318 cells was also hybridized with Cµ2 probe. The FL-18 and FL-218 cells produced membranous-bound (3.5 kb) and secretory (1.8 kb) forms of Cµ transcripts. The FL-318 cells produced those forms of Cµ transcripts (2.4 kb and 2.1 kb) that were estimated on the short time exposure autoradiogram. The FL-318 cells expressed abundant Cγ-containing mRNA species of 3.5, 3.0, 1.8, and 1.5 kb. In addition, the FL-218 and FL-318 cells expressed heterogenous species of IγH mRNAs, some of which comigrated each other. The FL-218 and FL-318 cell lines contained aberrant bcl-2 transcripts, most of which were comigrated with parts of the IγH mRNAs. Horizontal lines between gel lanes denote representative comigrated mRNAs in FL-218 and FL-318 cells. The dotted lines in the FL-318 cells show comigration of JH-, Cµ-, and Cγ-mRNAs with the bcl-2 transcript.

As shown in the FL-18 and FL-218 cells, a group of t(14;18)-positive lymphoma often exhibit class switch recombination of the translocated IγH allele5,24,25 whereas the switch recombination occurs with extreme rarity in B-cell lymphoma having a 14q+ translocation each with other chromosomes.26,27 Transcription of a bcl-2 gene involved in t(14;18) proceeds in a direction to the breakpoint junction and the transcriptional orientation is the same as that of the IγH gene involved in the translocation. Therefore, the bcl-2 transcription can proceed across the breakage junction into the IγH gene locus.28 In contrast, the transcription of three genes involved in other 14q+ translocations, named interleukin-3 (IL-3),29 c-myc,29,30 and bcl-3,31 proceeds in a direction away from the breakpoint, and the translocated IγH allele does not undergo a class switch event. The FL-318 cells that did not exhibit switch recombination of both IγH alleles continuously expressed abundant amounts of the Iγ mRNAs. One of the minor species was observed in a comigration line of sterile JH, Cµ, and Cγ transcripts with the abnormal-sized bcl-2 transcript. Such a constitutional expression of the germline transcripts was not detected in all t(14;18)-negative B-lymphoma cell lines examined, including four cell lines having μ-chain on the surface. Taken together, the bcl-2 transcription across the breakpoint junction into the IγH gene locus may be capable of yielding the processed germline form of sterile IγH transcripts, a prerequisite for class switch recombination of both IγH alleles. The finding that the FL-18 cells underwent switch recombination on both the translocated and the nontranslocated IγH alleles leads us to an intriguing hypothesis that deregulated expression of an intact bcl-2 gene also plays a role in the switch recombination of IγH gene.

ACKNOWLEDGMENT

We are indebted to Drs Tsujimoto and Croce (Wister Institute) and Drs Cleary and Sklar (Stanford University), who kindly provided “probe b” and “3’ probe,” respectively.

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