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bcl-2 Gene Rearrangement Analysis in Japanese B Cell Lymphoma; Novel *bcl*-2 Recombination with Immunoglobulin κ Chain Gene

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The rearrangement of *bcl*-2 gene was studied in 56 Japanese B cell lymphoma cases to investigate the contribution of *bcl*-2 gene to lymphomagenesis in Japan. Ten out of 56 cases showed *bcl*-2 gene rearrangement; it was detected in only 5 out of 16 follicular lymphoma cases (31%) and in 5 out of 40 diffuse B cell lymphoma cases (13%). The incidence of *bcl*-2 gene involvement in Japanese follicular lymphomas was lower than those reported in the United States. This might contribute to the lower incidence of follicular lymphoma cases in Japan. Novel recombination between *bcl*-2 and Ig κ genes at the 5′ region of *bcl*-2 and $J_κ4$ segment was observed in one follicular lymphoma case, suggesting that *bcl*-2 gene is transcriptionally activated by Ig κ enhancer. It was also suggested that this case had originated from a more differentiated B cell than most follicular lymphomas with *bcl*-2-Ig H recombination.

Key words: *bcl*-2 — Immunoglobulin κ — B cell lymphoma

Since chromosomal abnormalities have been associated with neoplastic cells in certain types of malignancy, attempts have been made to employ such information for understanding oncogenesis and also for diagnosis. Recent developments in molecular biology have revealed the association of translocation sites with oncogenes or putative oncogenes. The t(14;18)(q32;q21) translocation has been associated particularly with follicular lymphoma in the United States.[1] Molecular studies demonstrated that this translocation involves immunoglobulin heavy chain (Ig H)[5] gene and *bcl*-2 gene.[2–4] *bcl*-2 rearrangements were detected in most follicular lymphoma cases in the United States.[2,3,5,6] Bakhshi *et al.*[7] demonstrated that the translocation event occurred during D-J joining, the first step of Ig rearrangement.

In Japan, follicular lymphoma accounts for about 10% of malignant lymphoma, in contrast to 30% in the United States,[8] and t(14;18) is rather rare.[9] In this study, we analyzed *bcl*-2 rearrangement among B cell lymphomas to try to understand the lower incidence of follicular lymphoma and t(14;18) translocation in Japan. We found *bcl*-2 rearrangement in only 31% of follicular lymphoma cases. One case was found to possess a novel recombination of *bcl*-2 gene with Ig κ chain (Ig κ) gene.

Lymph node samples from Japanese patients with non-Hodgkin's lymphomas, 16 cases of follicular lymphoma and 40 cases of diffuse B cell lymphoma, were selected. Histological classification was carried out based on the National Cancer Institute study on non-Hodgkin's lymphoma.[10] High-molecular-weight DNA was extracted and digested with restriction endonuclease *Bam*HI, *Hin*dIII, or *Pst*I. Conditions for Southern blot study were as described previously.[11] The *bcl*-2 probes used were 1) *bcl*-2 cDNA probe #58,[12] which contains part of exons II and III, 2) genomic 5′ *bcl*-2 probe,[12] which contains part of exons I and II, 3) mbr (major breakpoint region[7]) (provided by Dr. S. J. Korsmeyer), and 4) mcr (minor breakpoint cluster region) probe pFL-2[5] (provided by Dr. J. Sklar)(Fig. 1). The other probes used were human Ig H joining region (J) J_H probe (provided by Dr. J. V. Ravetch), human Ig κ J probe $J_κ$[13] (Fig. 1)(provided by Dr. P. Leder, and Dr. T. Honjo), and human Ig λ constant region $C_λ$ probe (provided by Dr. S. J. Korsmeyer).

Fifty-six Japanese cases with B cell lymphoma were studied by Southern blot analysis using *bcl*-2 probes (Table I). These probes have been shown to detect *bcl*-2 rearrangement in 89% of follicular lymphoma cases in the United States.[5] In 16 follicular lymphoma cases, five (31%) showed *bcl*-2 rearrangement. The breakpoints of *bcl*-2 in these five cases are shown in Table I. In particular, one case (Case HN) had a breakpoint at the 5′ region, detected with 5′ *bcl*-2 probe (Fig. 2).

In diffuse B cell lymphoma, *bcl*-2 rearrangements were detected in only two cases out of 27 diffuse large cell

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[5] Abbreviations used in this paper; Ig, immunoglobulin; Ig H, Ig heavy chain; Ig κ, Ig κ chain; J, joining region; mbr, major breakpoint region; mcr, minor breakpoint cluster region; RSS, recombination signal sequence.

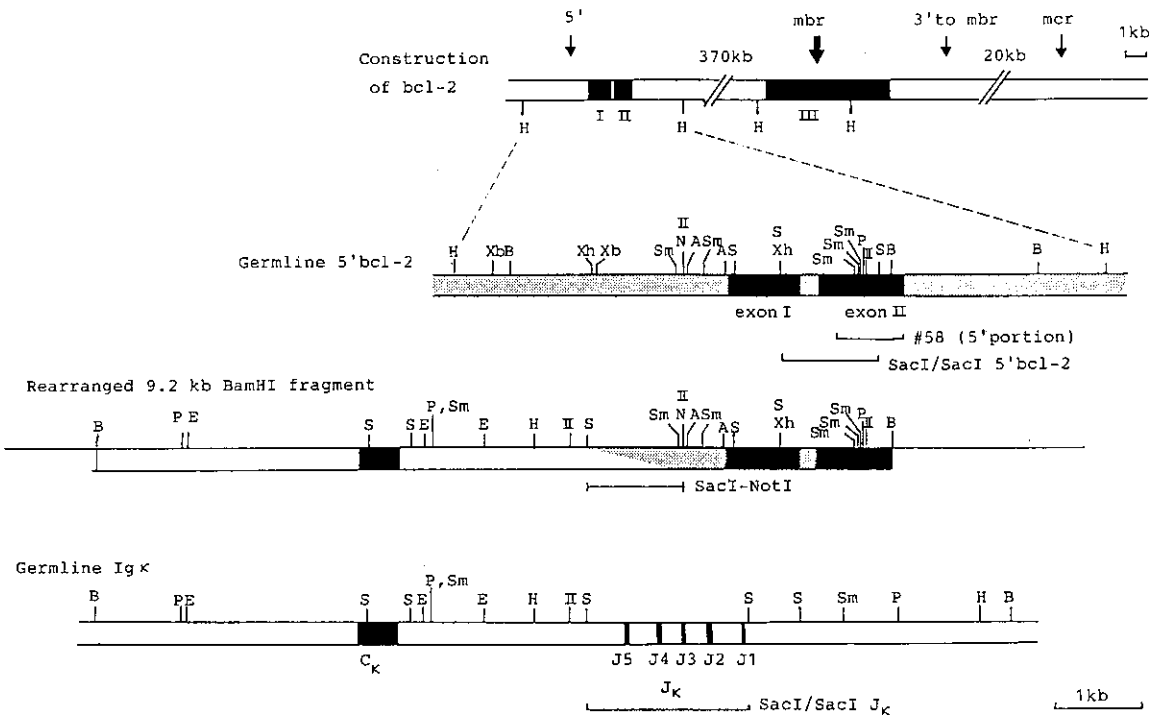

Fig. 1. Restriction enzyme map of the rearranged 9.2 kb *Bam*HI fragment cloned from Case HN. The rearranged fragment is shown along with the 5′ region of germline *bcl*-2[12] and Ig κ gene.[13] The construction of *bcl*-2 gene and each breakpoint are also shown at the top. The shaded and open areas of the 9.2 kb fragment are homologous to *bcl*-2 5′ region and Ig κ gene, respectively. Solid boxes represent exons of *bcl*-2 and Ig κ genes. *bcl*-2 gene is recombined with Ig κ gene in head-to-head orientation within the *Sac*I-*Not*I region (underlined), which was subcloned for further analysis. The regions corresponding to the probes used are also indicated. A, *Apa*I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Not*I; P, *Pst*I; S, *Sac*I; Sm, *Sma*I; Xb, *Xba*I; Xh, *Xho*I; II, *Sac*II.

Table I. Incidence of *bcl*-2 Gene Rearrangement in Follicular and Diffuse B Cell Lymphomas

Histological diagnosis	No. of case	*bcl*-2 gene rearrangement				
			Breakpoint region[a]			
		Total	5′	mbr	3′mbr	mcr
Follicular lymphoma	16	5 (31%)	1	2	1	1
Mixed	13	3	1[b]	1		1
Small cleaved	1	1		1		
Large	2	1			1	
Diffuse B cell lymphoma	40	5 (13%)		5		
Small cleaved	13	3 (23%)		3[c]		
Large	27	2 (7%)		2		
Total	56	10 (17%)	1	7	1	1

a) See Fig. 1.
b) This is Case HN, the breakpoint of which was studied (see Figs. 1–3).
c) One (Case JA) of these cases did not show comigration between *bcl*-2 and J_H rearranged fragments.

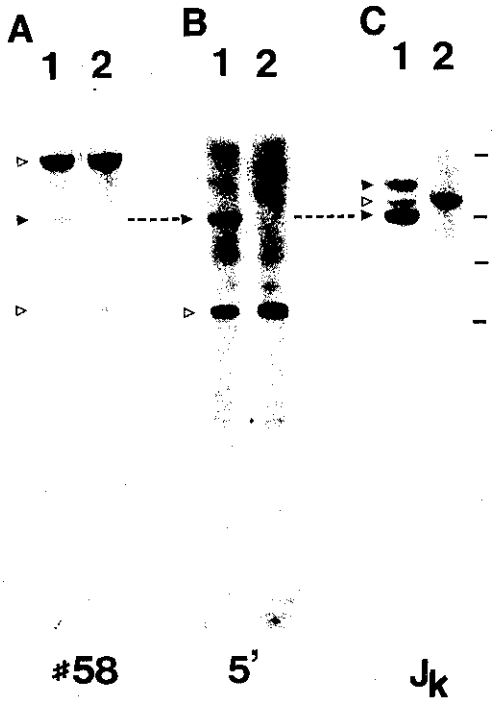

Fig. 2. Southern blot analysis of Case HN. DNA from Case HN (lane 1) and normal liver (lane 2) was digested with *Bam*HI and subsequently hybridized with #58 cDNA probe (A), 5′ probe (B), and J_κ probes (C). Both #58 cDNA probe and 5′ probe detect a 9.2 kb rearrangement band, which co-migrates with one of two rearrangement bands of Ig κ gene (indicated by broken lines). The open and solid arrowheads indicate germline and rearrangement bands, respectively. Dashes on the right side indicate the size markers (23.1, 9.4, 6.6, and 4.4 kb).

lymphoma cases (7%). In diffuse small cleaved cell lymphoma, three of 13 cases (23%) displayed the rearrangement. All diffuse lymphoma cases with *bcl*-2 rearrangements had the breakpoints within the mbr.

All cases were studied also with J_H probe to analyze translocation events. Rearrangements of J_H were observed in all cases, confirming B cell lineage of these tumors. Eight of ten cases with *bcl*-2 rearrangement showed comigration of *bcl*-2 and J_H rearranged fragments, indicating that the rearrangement of *bcl*-2 to Ig H gene had taken place in these lymphomas. Two cases, Case HN (follicular lymphoma) and Case JA (diffuse small cleaved cell lymphoma), did not show comigration of *bcl*-2 and J_H fragments. By analogy to c-*myc*-Ig light chain translocation in Burkitt lymphoma, *bcl*-2 gene may also be recombined with light chain genes. Therefore, these two cases were further examined with J_κ and C_λ probes. The *bcl*-2 rearranged fragment of Case HN was demonstrated to comigrate with one of the J_κ rearranged fragments in each endonuclease digestion (the result with *Bam*HI digestion is shown in Fig. 2). The results suggested that *bcl*-2 gene is translocated to the J_κ region. In Case JA, *bcl*-2 rearranged fragment did not show comigration with either J_κ or C_λ fragment (data not shown).

To investigate the mechanism of this *bcl*-2 translocation to Ig κ gene in Case HN, we have constructed a genomic library of *Bam*HI fragments with EMBL3 vector, screened it with 5′ *bcl*-2 and J_κ probes, and obtained a recombinant clone, which had the rearranged 9.2 kb *Bam*HI fragment of *bcl*-2 gene. A restriction enzyme map of this fragment was made and compared with those of the 5′ region of germline *bcl*-2[12] and Ig κ gene.[13] As shown in Fig. 1, one side of this fragment had homology with the 5′ region of germline *bcl*-2 up to an *Sma*I site 5′ to the *Not*I site (shaded area in Fig. 1), and the other portion (open area) is homologous with Ig κ gene when Ig κ gene is reversely aligned, suggesting that *bcl*-2 and Ig κ genes are joined in head-to-head orientation. The breakpoint region (*Sac*I-*Not*I fragment) was subcloned and further examined. A finer restriction map indicated that the breakpoint is in the vicinity of $J_\kappa 4$ (data not shown). Thus, this breakpoint region was sequenced by the dideoxy method (Sequenase, USB, Cleveland, OH).

As shown in Fig. 3, the complementary sequence of $J_\kappa 4$ was interrupted at the heptamer-nonamer recombination signal sequence (RSS), implying cleavage by Ig recombinase at the J_κ region. Five nucleotides (AGGGA) of unknown origin similar to N segment were present at the recombination site, followed by identical sequences to the 5′ *bcl*-2 region. Three sets of RSS heptamer-like sequence CACACTC were observed near the breakpoint in germline *bcl*-2 sequence, although the RSS nonamer motif did not exist. Downstream of the breakpoint, a stretch of alternating cytosine and adenine residues forming a poly (CA) family may form Z-DNA.[14]

The present study showed a relatively low incidence (31%) of *bcl*-2 gene involvement in follicular lymphoma in Japan. Amakawa *et al.* recently reported a similar result.[15] Therefore, it is possible that the low incidence of follicular lymphoma in Japan might be attributed partly to the low incidence of *bcl*-2 involvement.

In diffuse large cell lymphoma, the *bcl*-2 rearrangement was significantly lower (7%) than in follicular lymphoma (31%) in this study, and also lower than that in diffuse large cell lymphoma (28%) in the United States. The ratio between these two types of lymphomas in Japan (7% versus 31%) is comparable with that in the United States (28% versus 89%[5]).

Maseki *et al.* reported the frequency of t(14;18) in follicular lymphoma (22%) and diffuse large cell

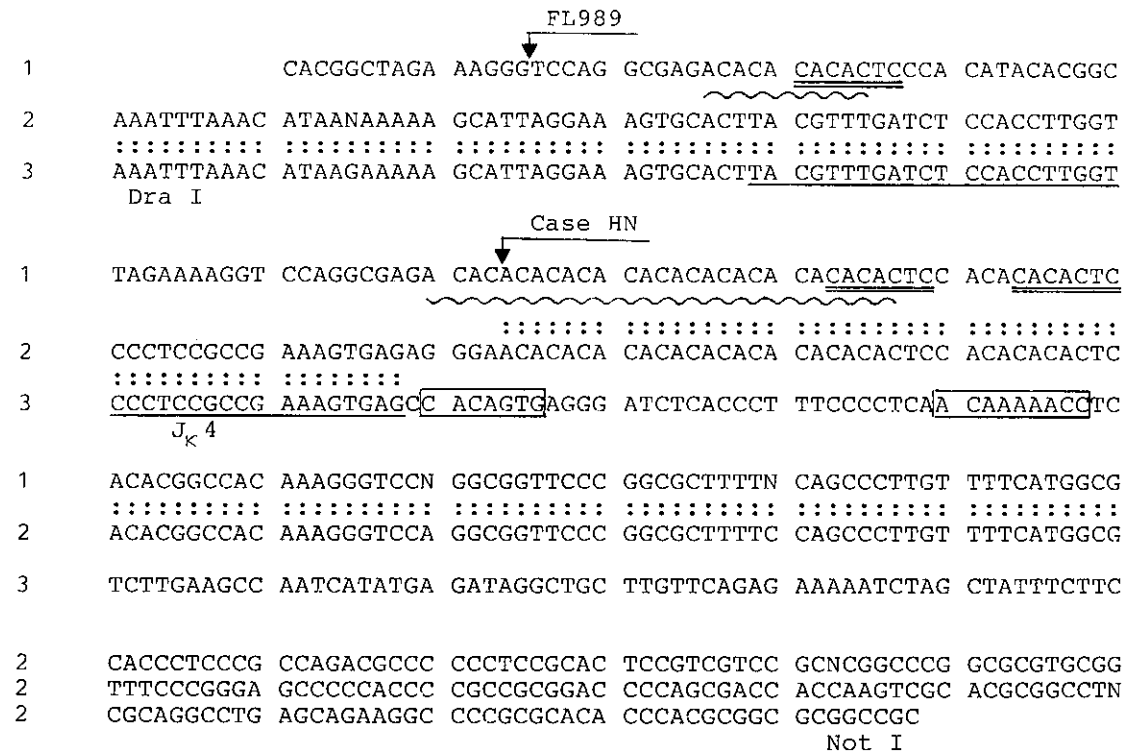

Fig. 3. Nucleotide sequence of the breakpoint region in Case HN. The sequence of the breakpoint region is shown (lane 2) with the sequence of the 5′ region of germline *bcl*-2[12] (lane 1) and the complementary sequence of germline J_κ region[13] (lane 3). Arrows indicate the breakpoints of Case HN and FL989.[17] Colons (:) indicate identical sequences among these lanes. The coding region of $J_\kappa 4$ is underlined. Boxed nucleotides denote RSS of $J_\kappa 4$. Three sets of heptamer-like sequences are double-underlined. Poly (CA) family sequences are noted 3′ to the breakpoints of both cases, and are represented by wiggly lines.

lymphoma (0%) in Japan.[16] The incidence of *bcl*-2 rearrangement in these lymphomas in this study is lower than that in the United States, but it is correlated rather well with that of t(14;18).

The *bcl*-2 rearrangement was also observed in 3 of 13 cases (23%) with diffuse small cleaved cell lymphoma, showing a similar incidence to that in follicular lymphoma. In the United States, the frequency of *bcl*-2 rearrangement in this type of lymphoma was studied by Weiss *et al.* (0%, 0/2)[5] and Aisenberg *et al.* (21%, 3/14).[6] The number of cases studied, however, is relatively small. Therefore, it is difficult to conclude whether the incidence of *bcl*-2 rearrangement in this type of lymphoma in Japan is different from that in the United States.

Out of 10 cases with *bcl*-2 rearrangement, two did not show comigration with J_H fragment. One had translocation with Ig κ in head-to-head orientation, which has never been reported before. The translocation with J_κ suggested that the translocation event had occurred at a more differentiated stage of B cell development than that with Ig H, because Ig κ gene rearrangement occurs later than Ig H in normal B cell differentiation.

According to the organization of *bcl*-2 and Ig genes on the respective chromosomes, translocation of *bcl*-2 gene with Ig light chain gene should break on the telomeric side (5′ side) of *bcl*-2, placing Ig light chain and *bcl*-2 genes in head-to-head orientation on der 18 chromosome, in contrast to head-to-tail orientation of the Ig H and *bcl*-2 translocation. The head-to-tail configuration places Ig H enhancer about 370 kb away from *bcl*-2 promoter.[12] In Case HN, however, Ig κ enhancer is placed in close proximity to *bcl*-2 promoter, and it is conceivable that *bcl*-2 gene is transcriptionally activated by Ig κ enhancer, although somatic mutation within *bcl*-2 gene may also contribute to deregulation. A preliminary study with Northern blotting of this case demonstrated the up-regulated steady state of *bcl*-2 mRNA. Cytogenetic analysis of Case HN has been attempted, but no dividing cell was available.

Nucleotide sequence analysis defined the breakpoint within the 5′ upstream region of the *bcl*-2 gene.

Tsujimoto *et al.* reported case FL989 with *bcl*-2-J_H rearrangement at the 5′ region of *bcl*-2.[17] The breakpoint of this case is about 60 bp upstream from that of Case HN. There are three sets of the RSS heptamer-like sequence CACACTC near the breakpoints of both cases. However, the significance of these sequences for the recombination needs to be studied. Poly (CA) family sequence[14] is noted 3′ to the breakpoint of Case HN and a short one also appears 3′ to the breakpoint of FL989. Since this sequence is able to form Z-DNA conformation, it might be possible that this conformational change is related to the target site for cleavage 5′ to the *bcl*-2 gene.[14]

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