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Short Communication

Detection of the t(14;18) at Similar Frequencies in Hyperplastic Lymphoid Tissues from American and Japanese Patients

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Follicular lymphoma shows a wide geographic variation in incidence, occurring more frequently in the U.S. than in Japan. A translocation involving the* bcl-*2 gene on chromosome 18 and the immunoglobulin heavy chain gene on chromosome 14 is frequently found in follicular lymphomas and is believed to play a critical role in the pathogenesis of these tumors. Recently,* bcl-*2/IgH rearrangements have been detected in reactive lymphoid tissue obtained from European patients, indicating that such rearrangements occur at some low but measurable background rate. In non-malignant tissues, the polymerase chain reaction was used to study the frequency of* bcl-*2/IgH rearrangements in reactive lymphoid tissue obtained from American and Japanese patients to find out whether geographic variation in the incidence of follicular lymphoma was caused by differences in sporadic occurrence of the t(14;18). We found such rearrangements in 5 of 15 American hyperplastic tonsils and lymph nodes and 5 of 10 Japanese tonsils, an incidence close to that previously seen in European patients. These data suggest that the background incidence of such rearrangements is similar in all populations, regardless of the incidence of follicular lymphoma. (Am J Pathol 1992, 141:291–299)

The 14;18 translocation is frequently associated with B-cell neoplasia, being found in up to 90% of follicular lymphomas and about 30% of large cell lymphomas (LCLs) of B-cell type in the United States.[1–3] This translocation leads to the juxtaposition of the *bcl*-2 gene on chromosome 18 band q21.3 and J_H or D_H segments of the IgH gene on chromosome 14 band q32.3.[4–6] Junctional sequences found at *bcl*-2/IgH rearrangement sites resemble those produced during normal antigen receptor gene rearrangement, suggesting that these events occur during attempted V(D)J_H recombination in pre-B cells.[7] B-cell tumors bearing the t(14;18) express a *bcl*-2/IgH fusion transcript[8] and demonstrate increased *bcl*-2 protein production.[9] In normal tissues that undergo rapid cell turnover, *bcl*-2 protein may abrogate programmed cell death (apoptosis).[10–13] Inappropriate expression of *bcl*-2 in tumor cells bearing the t(14;18) appears to confer a survival advantage, possibly through this anti-apoptosis mechanism, thereby contributing to the pathogenesis of lymphomas associated with the translocation.

There are a variety of data that indicate that, although the t(14;18) may be necessary for the development of certain types of lymphoid neoplasia, it is not by itself sufficient for malignant transformation. Introduction of *bcl*-2 into human B- and T-cell lines results in increased growth *in vitro*, but does not lead to tumorigenicity unless other proto-oncogenes, such as *myc*, are co-introduced.[14,15] Transgenic mice carrying a *bcl*-2/IgH fusion gene develop persistent polyclonal follicular hyperplasia, but only about 10% ultimately progress to a monoclonal lymphoma, typically of large-cell type.[16,17] Moreover, one European study has reported detection of *bcl*-2/IgH rearrangements using the polymerase chain reaction (PCR) in lymphoid tissue undergoing follicular hyperplasia.[18] In this study, *bcl*-2/IgH rearrangements were found among reactive lymph node and tonsillar follicles and

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were undetectable in normal bone marrow and lymph nodes displaying interfollicular hyperplasia. These results suggest that cells containing the t(14;18) arise in pre-B cells in bone marrow of normal individuals at some low background rate below the level of the sensitivity of the PCR, but then migrate to peripheral lymphoid tissues in which a growth advantage in the specific setting of follicular hyperplasia enables detection. The ability to detect *bcl-2*/IgH rearrangements in follicular hyperplasias thus provides an indirect measure of the rate of occurrence of the t(14;18) in B-cell progenitors.

One interesting and unexplained characteristic of follicular lymphoma is its wide geographic variation in incidence. Although common in the U.S.,[19] it is substantially less frequent in parts of Asia, such as Hong Kong,[20] Taiwan,[21,22] China,[23,24] and Japan,[25,26] where it occurs with an absolute incidence close to an order of magnitude lower than that seen in the U.S.[25] Further, several studies suggest that when follicular lymphoma does occur in Asia, the tumor is less likely to contain the t(14;18) than are follicular lymphomas in the United States. Southern blot analysis of American follicular lymphomas has shown rearrangements in the major breakpoint region (MBR) of *bcl-2*, a 150 bp stretch of nucleotides located several kilobases 3′ of the coding sequence, in about two-thirds of cases, or in a more distant 3′ site, the minor breakpoint cluster region (MCR), in an additional 25% of cases.[3] In contrast, similar analyses performed on Japanese and Chinese follicular lymphomas have shown rearrangements involving one or the other of these two sites in 25% to 57% of tumors.[27–31] Although these differences could simply reflect preferential breakage of *bcl-2* in Asian tumors at sites outside of the MBR and MCR, available cytogenetic data also show that the t(14;18) is uncommon in Asian B-cell lymphomas.[32]

In view of the aforementioned findings, a reasonable hypothesis is that the observed geographic variation in the incidence of follicular lymphoma could be due to differences in the sporadic occurrence of the t(14;18) across populations. To test this hypothesis, we have investigated the incidence of the t(14;18) in lymphoid tissues with follicular hyperplasia obtained from Japanese and American patients.

Materials and Methods

Tissues

Tonsils were obtained from Japanese and American patients undergoing routine tonsillectomy. Lymph nodes were selected after histologic, immunohistologic, and flow cytometric evaluation revealed no evidence of neoplasia. Histologic examination revealed follicular hyperplasia in all lymphoid specimens studied. American tissues were snapfrozen and stored at −70°C until use or, alternatively, fixed immediately after receipt in 95% ethanol and stored at room temperature. Japanese tissues were initially snapfrozen and stored at −70°C, and were then placed in 95% ethanol for shipping to the U.S. Nonlymphoid control tissues were obtained from surgical specimens and processed immediately.

DNA Preparation

Tissue was minced with razor blades in disposable plastic dishes and subjected to proteinase K digestion, phenol/chloroform extraction, and RNase digestion according to a standard method.[33] DNA was stored in 10 mmol/l Tris, 1 mmol/l EDTA, pH 8.0, at 4°C.

Amplification of bcl-2/IgH Rearrangements

Oligonucleotide primers were synthesized on an Applied Biosystems DNA Synthesizer (Model 381A, Foster City, CA). All reactions were carried out in 50 μl of 10 mmol/l Tris, pH 8.3, in the presence of 50 mmol/l KCl, 1.5 mmol/l MgCl2, 0.001% gelatin, 1.25 U of thermostable DNA polymerase (AmpliTaq, Perkin-Elmer Cetus, Emeryville, CA), and 100 ng of each oligonucleotide primer. Two identical rounds of 30 amplification cycles were performed in an automated thermal cycler (Perkin-Elmer Cetus, Emeryville, CA). DNA was denatured at 94°C for 3 minutes in the first cycle and for 1 minute in subsequent cycles, and extension was carried out at 72°C for 2 minutes in the first 29 cycles and for 8 minutes in the last cycle. Primer annealing was carried out for 1 minute at 58°C in all cycles. Two μg of template DNA was initially amplified using a universal J_H primer (5′-ATGAATTCCTGAGGAGACGGTGACCAGGGT-3′) and a primer lying 5′ of the major breakpoint cluster region of *bcl-2*, MBR-E (5′-CCAGATGGCAAATGACCAG-3′); here and elsewhere, underlined primer sequences designate nucleotides that are noncomplementary to the template DNA and have been added to create restriction sites useful in cloning of PCR products. Two microliters of initial reaction mixture were then reamplified with the same J_H primer and an internal major breakpoint region primer, MBR-I (5′-TATCTAGAGAGAGTTGCTTTACGTG-3′). Reactions were stored at −20°C.

PCR Controls

The ability of the PCR primers to amplify *bona fide bcl-2*/IgH rearrangements was initially tested using DNA from a

follicular lymphoma control specimen, designated T4, as template; a second positive control, COL178, was subsequently used in some amplifications. Dilution of T4 DNA into nonlymphoid DNA prepared from normal kidney showed that the method was capable of detecting one tumor cell in 2×10^5 cells (not shown), the theoretical limit for the system since the standard amount of template DNA added to each reaction, 2 μg, represents approximately 2×10^5 cell equivalents. Before attempting to detect *bcl-2*/IgH rearrangements in reactive tissue, the quality of DNA from all specimens was assessed by amplifying for 30 cycles with the MBR-I primer and a primer lying 370 basepairs 3′, MBR-3′(5′-GAGAATTCTAAAGCAGCTTGGAGGATC-3′), using the conditions described earlier. Reaction mixtures were then analyzed by electrophoresis on agarose gels. The presence of an ethidium bromide-stained band of the appropriate size was taken as evidence that a given DNA preparation was amplifiable. Ethanol fixation, used for storage of some samples, did not affect DNA amplification in control reactions. Subsequently, attempted amplification of *bcl-2*/IgH rearrangements was performed in parallel with negative controls consisting of reactions with no added DNA and with non-lymphoid DNA isolated from normal tissue. To avoid contamination with previously amplified products, PCRs were prepared in a dedicated laminar flow hood, reaction mixtures were treated with ultraviolet light (254 nm) for 10 minutes in a Fotodyne (New Berlin, WI) UV box before addition of Taq polymerase and DNA template,[34] and pipet tips with aerosol filters were employed.

Analysis of PCR Products

PCR products were electrophoresed in 1.8% agarose gels, stained with ethidium bromide, and transferred to nylon membranes (Plasco, Inc., Woburn, MA) by Southern blot hybridization. Membranes were prehybridized for 1 hour in a solution containing 6x sodium chloride/sodium citrate (SSC) 5x Denhardt's solution, 2% formamide, 0.2% sodium pyrophosphate, and 0.5 mg/ml sonicated salmon sperm DNA, then hybridized in 6x SSC, 5x Denhardt's solution, 2% formamide, 0.2% sodium pyrophosphate, 0.5 mg/ml salmon sperm DNA, and 2.5% dextran sulfate at 42°C for 5 hours with 100 ng of an oligonucleotide probe which had been end-labeled with γ-^{32}P-ATP (New England Nuclear, Boston, MA) using T4 kinase (Gibco BRL, Gaithersburg, MD). The probe used, MBR-IHP (5′-GCCTGTTTCAACACAGACCC-3′), lies just 3′ to the MBR-I primer. Membranes were then washed twice for 15 minutes in 6x SSC- 0.1% sodium dodecyl sulfate (SDS) at 52°C and autoradiograms were prepared.

DNA Sequencing

PCR products identified as possible *bcl-2*/IgH rearrangements on Southern blots were excised from a second agarose gel and DNA was isolated with GlassMilk (GeneClean II, La Jolla, CA). The purified DNA was cut with *Xba*1 and *Eco*R1 and ligated into M13mp18 or M13mp19 RF DNA; enzymes and vectors were obtained from Gibco BRL (Gaithersburg, MD). Transformation of competent *E. coli* strain JM109 with ligation mixtures using a heat shock method, identification of *lac*$^-$ recombinant phage, and purification of single-stranded template DNA were carried out using standard procedures.[35] Inserts were sequenced with a kit (United States Biochemical, Cleveland, OH) using the dideoxy method according to provided instructions.

Results

Previous work indicated that sequences consistent with MBR/IgH and MCR/IgH rearrangements could be detected in about 50% of reactive tonsils obtained from Western European patients and that the prevalence of cells bearing the rearrangements was in the range of 1 in 10^5 to 10^6 cells.[18] Based on these data, we studied 15 tonsils and lymph nodes from 14 American patients and 10 tonsils from Japanese patients, all of which showed reactive follicular hyperplasia, to assess the possible sporadic occurrence of *bcl-2*/IgH rearrangements in patients from these two populations. Except for lymphoid hyperplasia, all patients were otherwise well at the time of the surgical procedure, and none have subsequently developed lymphoid neoplasia. Because the prevalence of the t(14;18) in reactive tissues appears to be near the detection limit of the PCR-based method, all samples were subjected to amplification in at least 10 separate reactions. The search was limited to MBR/IgH rearrangements, since a substantial proportion of *bcl-2*/IgH rearrangements are of this type.

This approach lead to detection of hybridizing bands consistent with *bcl-2*/IgH rearrangements in 6 of 15 American specimens (5 of 14 patients) and 5 of 10 Japanese samples (5 of 10 patients); the data are summarized in Table 1. Positive signals were seen with tissues obtained from American and Japanese patients as young as 4 and 5 years of age, respectively. Tonsillar DNA from one 8-year-old Japanese male, JB, generated three distinct positive bands, and tonsillar DNA from two other Japanese patients, JD, a 40-year-old male, and JI, a 7-year-old female, generated two. Two lymph-node specimens, AM1 and AM2, obtained from a single American patient 3 years apart, produced bands of differing

Table 1. *Detection of* bcl-2/IgH *Rearrangements in Reactive Lymphoid Tissue from American and Japanese Patients*

	Age/Sex	Tissue	*Bcl-2*/IgH products (size in basepairs)	Times detected Times amplified	Estimated prevalence (per 10^6 cells)
American patients					
AA	4M	T	A4.1 (176 bp)	1/12	0.4
AB	2M	T	None	0/12	
AC	7M	T	AX.1 (106 bp)	1/12	0.4
AD	3M	T	None	0/12	
AE	15F	T	None	0/10	
AF	3M	T	None	0/10	
AG	6F	T	None	0/12	
AH	5M	T	None	0/10	
AI	9F	T	None	0/10	
AJ	32M	T	A6.1 (297 bp)	6/16	2.4
AK	23F	T	None	0/12	
AL	28M	T	None	0/12	
AM1	25M	LN	A1.1 (226 bp)	6/10	4.6
AM2	28M	LN	A4.2 (178 bp)	5/10	3.5
AN	40F	LN	None	0/10	
AO	64F	LN	AR.1 (74 bp)	1/10	0.5
Japanese patients					
JA	40M	T	None	0/14	
JB	8F	T	J4.2 (126 bp)	8/17	3.2
			J6.1 (191 bp)	1/17	0.3
			J4.4 (222 bp)	1/17	0.3
JC	5M	T	None	0/14	
JD	40M	T	J4.1 (135 bp)	3/14	1.2
			J4.5 (235 bp)	5/14	2.2
JE	5M	T	J4.3 (239 bp)	1/14	0.4
JF	3M	T	None	0/12	
JG	5M	T	None	0/12	
JH	5M	T	None	0/12	
JI	7F	T	J6.2 (197 bp)	12/12	60.2*
			J6.3 (145 bp)	12/12	11.2*
JJ	9M	T	J6.4 (98 bp)	3/12	1.4

* The prevalence of J6.2 and J6.3 was determined by further dilution of template DNA, as explained under Results.

LN = lymph node; T = tonsil. AM1 and AM2 represent two lymph node biopsies obtained from the same patient three years apart. Age is given in years. Histologically, all specimens showed follicular hyperplasia. The size of PCR products that hybridized to an internal oligonucleotide probe, MBR-IHP, which is complementary to *bcl-2*, are given in basepairs. Cell equivalents are calculated assuming 2×10^5 cells per 2 μg DNA. The prevalence of cells bearing *bcl-2*/IgH rearrangements was estimated assuming a Poisson distribution at limiting dilution.

size. Each unique product was amplified from only one tissue specimen, and while some specimens yielded a particular product only once, nine products were generated from specific specimens in multiple separate amplifications. The two products from JI were exceptional in this regard, since they were seen in all ten amplifications containing 2 μg of DNA. Further PCRs performed with 0.2 μg of DNA revealed amplification of one of these two products, J6.2 (197 bp in size), in seven of ten reactions, the other, J6.3 (145 bp in size), in two of ten (Figure 1). Using the Poisson equation, one can roughly estimate that the prevalence of cells bearing particular *bcl-2*/IgH rearrangements involving the MBR ranges among the cases from 0.4 to 60 per 10^6 cells. In contrast to results seen with reactive lymphoid tissues, no hybridizing bands were observed in 100 control reactions, representing 2×10^7 cell equivalents, using DNA prepared from normal kidney.

All hybridizing bands were subsequently cloned and sequenced on at least one occasion; those of identical size that appeared in more than one amplification were sequenced at least twice to confirm their identity. All 15 hybridizing PCR products were distinct and 14 had structures resembling those of t(14;18) breakpoints isolated and sequenced previously from B-cell lymphomas (Figure 2). Each of these 14 sequences consisted of a 5′ MBR sequence and a 3′ J_H segment separated by varying numbers of interposed nucleotides. Among J_H segments, J_H4 and J_H6 were most frequently represented, although one rearrangement involved J_H1 and one, designated AX.1, had an ambiguous J_H sequence derived from J_H1 or J_H2. Two rearrangements, A6.1 and J4.4, contained junctional sequences homologous to D_H segments. From 6 to 25 nonhomologous nucleotides, consistent with N insertions, were interposed in 13 of these 14 sequences between *bcl-2* and each J_H or D_H sequence, all of which showed evidence of endonucleolytic digestion. The structure of these sequences is therefore consistent with a rearrangement event occurring between the MBR of *bcl-2* and IgH during attempted D_H to J_H or V_H to D_HJ_H joining. To rule out the possibility of contamination of PCRs with products amplified from two follicular

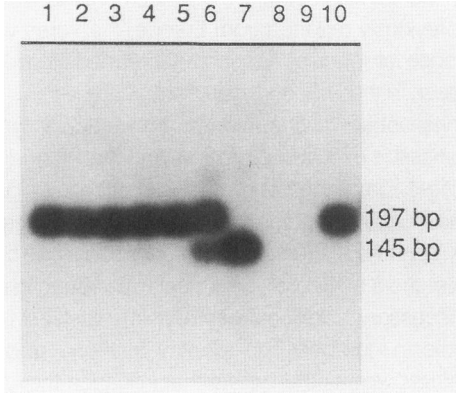

Figure 1. *Detection of PCR products consistent with* bcl-*2/IgH rearrangements. Two hundred ng of DNA prepared from a tonsil obtained from a Japanese patient, JI, was amplified in 10 separate PCRs using* bcl-*2 and J_H specific primers (lanes 1–10). An aliquot of each reaction mixture was electrophoresed on an agarose gel, transferred to a nylon membrane, and hybridized to MBR-IHP, an internal oligonucleotide specific for* bcl-*2, which was end-labeled with ^{32}P. The resultant autoradiogram is shown.*

lymphoma controls, these products were also sequenced and were found to be unique from the rearrangements detected in reactive lymphoid tissue (Figure 2).

Two types of artifactual sequences were also detected and sequenced. The first was produced in an initial series of control amplifications performed with a slightly different J_H primer and an annealing temperature of 55°C. Under these conditions, a hybridizing band of variable intensity, 510 bp in size, was frequently observed with lymphoid and nonlymphoid templates (not shown). Sequence analysis revealed that this band was the product of nonspecific priming from a *bcl*-2 sequence which lies 467 bp 3′ of the MBR-I primer and is homologous to the last six basepairs of the J_H primer used. Priming and amplification from this site was prevented by addition of five bases to the 3′ end of the J_H primer and increasing the annealing temperature to 58°C. The second probable artifact, designated AR.1, was produced during amplification of DNA prepared from a hyperplastic lymph node obtained from a 64-year-old female. It consisted of *bcl*-2 sequence joined directly to a J_H1 or J_H2 segment at the site of a five basepair overlap sequence common to both genes (Figure 2). This "rearrangement," which was seen only once in more than 300 amplifications, may have been created *in vitro* by partial extension from one primer into the short region of homology, followed by annealing and extension from common sequences in the other gene.

This second type of artifact raised the possibility that infrequent probability priming events involving partially extended PCR products could give rise to composite sequences resembling *bona fide bcl*-2/IgH rearrangements occurring *in vivo*. However, an additional charac-

	Bcl-2 Breakpoint (bp)	N-(D)-N	J_H	Endonucleolytic Digestion (bp)
American Rearrangements				
A1.1	3161	GGGGTTCTCCGG	1	10
A4.1	3114	AATTTCTC	4	4
A4.2	3113	GGCGATGG	4	11
A6.1	3163	ATGCAAGGCCAGACCTAGTAGTGGTTATTATCGACGCACGTACAGTG	6	15
AX.1	3114	CATTGCCCTCCCTGCCTCAGCC	1 or 2	~25
Japanese Rearrangements				
J4.1	3060	AAGGTAGCCCGAGACC	4	7
J4.2	3053	AGGGTAG	4	3
J4.3	3164	GAAGTTGTCCT	4	5
J4.4	3110	GGCGCCAATGTAGAGAGGCGATATTGTAGTGGTGGTAGCTGCTACCCACCGGGGG	4	2
J4.5	3164	GGCCCGGAGCCTTTTTGGGG	4	7
J6.1	3111	CCAGGTTTTTC	6	27
J6.2	3121	GGGGCG	6	10
J6.3	3057	CCATCAGCACTGAGAGGT	6	8
J6.4	3041		6	22
Control Lymphomas				
T4	3113	GTAAAAGGGTATCGGGAGCAGGGGT	6	4
COL178	3113	AGTAGCT	4	11
Probable Artifact		*Bcl*-2 / **CACCC** / J_H		
AR.1	3035	TTCAACACAGACC **CACCC** *TGGTCACCGTCTCCTCCAGG*		

Figure 2. *Sequence of* bcl-*2/IgH junctions detected in reactive tonsils and lymph nodes and in follicular lymphoma controls. The positions of the breakpoints in* bcl-*2 are based on a published* bcl-*2 cDNA sequence.[8] Underlined nucleotides are homologous to human D_H sequences; the sequences in A6.1 are homologous to D_{HXP4} and D_{HXP5}, while those in J4.4 are homologous to D_{HLR2}. Bold sequences present in AR.1 are common to both* bcl-*2 and J_H1 and J_H2, rather than N insertions; the italicized bases correspond to the sequence of the J_H primer.*

teristic shared by *bcl-2*/IgH rearrangements previously cloned and sequenced from follicular lymphomas and the rearrangements which we found in reactive lymphoid tissue argues strongly against this explanation. The position of breakpoints within the MBR in follicular lymphomas tend to fall into three subclusters occurring about 44, 100, and 150 bp 3′ of the MBR-I primer.[36] The distribution of the breakpoints in *bcl-2* detected in reactive lymph nodes and tonsils mirrors the distribution of breakpoints seen in follicular lymphoma (Figure 3), as would be expected if such rearrangements occurred *in vivo*. In contrast, the position of the break in AR.1, a probable artifact, lies outside of the subclusters. We conclude that small numbers of cells bearing *bcl-2*/IgH rearrangements are frequently present in a substantial proportion of biopsies of hyperplastic lymphoid tissue obtained from both American and Japanese patients.

Discussion

Geographic heterogeneity in the incidence of certain tumors is a well known but poorly understood aspect of human neoplasia. Some of these tumors, of which follicular lymphoma is an example, are associated with characteristic chromosomal rearrangements.[37] Assuming that such rearrangements play a critical role in tumorigenesis, variation in the sporadic occurrence of such rearrangements could account for the observed geographic differences in tumor incidence.

Our data, however, suggest that the background incidence of the (14;18) translocation, a rearrangement associated with up to 90% of American cases of follicular lymphoma, is similar in populations with relatively high and low rates of follicular lymphoma. Using a sensitive PCR-based detection method, rearrangements between the MBR of *bcl-2* and IgH resembling those seen in follicular lymphoma were detected in 5 of 15 American and 5 of 10 Japanese reactive tonsils and lymph nodes. The fraction of reactive specimens from these two locales which contain detectable rearrangements is similar to that previously reported from Europe,[18] an area with an incidence of follicular lymphoma intermediate between that seen in the U.S. and Asia.[38,39]

These observations indicate that follicular lymphomagenesis is a multistep process and that, in addition to *bcl-2*/IgH rearrangement, other genetic or epigenetic events must occur before a tumor develops. Since follicular lymphoma is a tumor primarily of middle age or later, and yet the t(14;18) can be detected in young children, the necessary additional alteration(s) must be rare or multiple in number. The identity of these additional events, as well as the factors that influence their occurrence, remain unknown. Epidemiologic studies have linked some types of non-Hodgkin's lymphomas in the U.S. to pesticide use,[40,41] and the degree of exposure to certain environmental agents may explain differing incidences of follicular lymphoma among populations. The incidence of follicular lymphoma in Japanese immigrants living in Hawaii approaches that of native-born Americans, supporting this possibility.[42] The surprisingly frequent detection of *bcl-2*/IgH rearrangements in normal individuals from all populations thus far examined argues that acquisition of this genetic lesion is unlikely to be a rate-limiting event in follicular lymphomagenesis, suggesting that environmental factors are more likely to act by promoting or inducing additional genetic alterations distinct from those involving *bcl-2*. Nonetheless, it would be interesting to investigate the level of background *bcl-2*/IgH rearrangement in non-neoplastic lymphoid cells of individuals with newly diagnosed follicular lymphoma to look for evidence of increased chromosomal rearrangement.

Little is known about the biology of cells bearing the t(14;18) which arise sporadically outside of malignancy. Selective expansion and survival of clones with the rearrangement in hyperplastic follicles would appear to require expression of *bcl-2*/IgH fusion transcripts and overproduction of *bcl-2* protein, neither of which have yet been demonstrated in reactive tissue. Potentially, stimu-

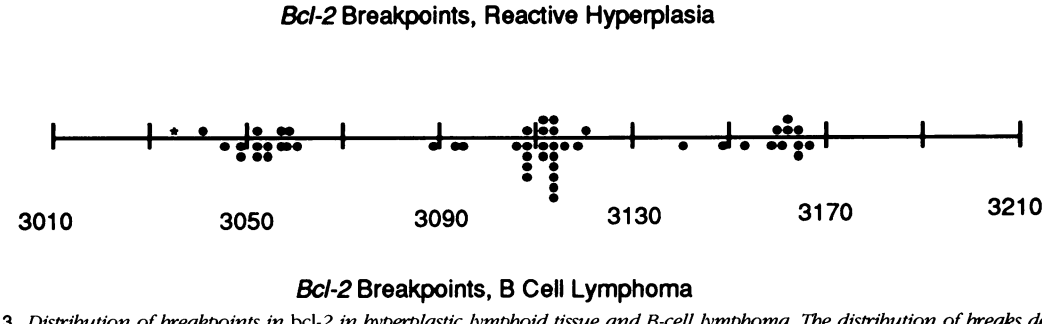

Figure 3. *Distribution of breakpoints in* bcl-*2 in hyperplastic lymphoid tissue and B-cell lymphoma. The distribution of breaks detected in hyperplastic lymphoid tissue are displayed above the line, while those detected in B-cell lymphomas in this study and in previous studies[5–7,43,46–49] are shown below. The positions of the breakpoints are based on a published* bcl-*2 cDNA sequence.[8] The break in AR.1, a probable artifact is designated by *.*

lation of cells containing the t(14;18) by chance interaction with specific antigen may also play a role in initial expansion of such cells and could influence their ultimate fate. Most of these cells never give rise to malignant clones, but at least a subset may persist, thus constituting a pool in which further genetic events could lead to lymphomagenesis, akin to what has been observed in transgenic mice.[16,17]

The sporadic occurrence of the t(14;18) has a number of potentially important clinical ramifications. Although the prevalence of the t(14;18) in reactive tissues appears to be below the level at which rearrangements can be detected with 30 cycles of PCR amplification, on occasion, tissues involved by follicular hyperplasia may contain a high enough proportion of cells to produce a positive result in PCR tests for the t(14;18). For this reason, a positive result in a diagnostic setting must be correlated with morphology and other tests aimed at assessing the clonality of the proliferation. Sporadic *bcl-2*/IgH rearrangement could also confound interpretation of more sensitive assays designed to assess the presence of minimal residual disease. This possibility is supported by occasional detection of *bcl-2*/IgH rearrangements in the peripheral lymphocytes of patients with treated follicular lymphoma that differ from tumor-specific *bcl-2*/IgH rearrangements detected at the time of diagnosis,[43] an observation that can be explained by additional intercurrent rearrangements occurring in lymphocytes that are not a part of the malignant clone. How much of a problem this will present remains to be seen; given that positive selection for "benign" cells containing the t(14;18) occurs at the level of the follicular center, the tissue under investigation (e.g., bone marrow versus lymph node) may be a critical issue. Even if background rearrangements do prove to be a complicating factor in clinical specimens, it should be possible to distinguish them from persistent disease through the use of junctional sequence-specific probes or primers. A more difficult issue is the potential detection of precursor cells containing the tumor-specific *bcl-2*/IgH rearrangement but lacking at least one other mutation essential for malignancy.[18]

Recognition that the t(14;18) can be found in reactive tissue may also help resolve some recent controversy regarding the possible occurrence of this rearrangement in Hodgkin's disease. Using 60 cycles of PCR amplification, one group has recently reported that 32% of cases of Hodgkin's disease have detectable *bcl-2*/IgH rearrangements.[44] In contrast, several other groups using less sensitive PCR-based assays have failed to find rearrangements,[45,46] suggesting that the prevalence of the t(14;18) in tissues involved by Hodgkin's disease must be low. This discrepancy could be resolved if the rearrangements are present at low frequency in background reactive lymphocytes, rather than in Reed–Sternberg cells and variants which, while a minor population, usually comprise 1% or so of the total cellularity. The apparent absence of *bcl-2* protein overexpression in Reed–Sternberg cells and variants[46] would seem to favor the former explanation. Precise localization of the t(14;18) in tissues involved by Hodgkin's disease will probably be necessary to definitively resolve this controversy.

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