The *bcl*-2 Gene Is Rearranged in Many Diffuse B-Cell Lymphomas

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Southern blotting was used to detect rearrangement of the *bcl*-2 gene in 104 cases of non-Hodgkin's lymphoma subclassified by the Working Formulation, 24 cases of B cell chronic lymphocytic leukemia (B-CLL) and 14 cases of T cell malignancy. Earlier workers reported rearrangement of this gene (located on chromosome 18) in a major fraction of follicular lymphomas, lymphomas in which a 14;18 chromosome translocation is frequently observed. In the present study, *bcl*-2 was rearranged in 30% (11 of 37) of follicular lymphomas and 19% (11 of 58) of diffuse lymphomas of follicle center cell lineage. In 18 of 19 samples studied, the rearranged *bcl*-2 fragment also hybridized with a probe for the joining region of the immunoglobulin heavy chain gene located on chromosome 14, indicating a 14;18 translocation. In lymphomas not derived from follicle center cells, ie, diffuse lymphomas of small B lymphocytes, B-CLL and T cell neoplasms, the *bcl*-2 gene was always in germline configuration. The frequent rearrangement of *bcl*-2 in a variety of B cell lymphomas of diffuse morphology (small cleaved cell, large cell, small noncleaved cell and immunoblastic) is noteworthy.

THE BREAKPOINT of the 14;18 chromosome translocation frequently observed in follicular lymphoma[1-5] was first cloned in 1984 by Tsujimoto et al.[6] This translocation involves a gene termed *bcl*-2 on chromosome 18 and the immunoglobulin (Ig) heavy chain gene on chromosome 14: rearrangement of *bcl*-2 was reported in ~60% of follicular lymphomas.[7] The 14;18 breakpoint has since been cloned in other laboratories,[8,9] and partial analysis of the structure, transcripts, and protein products of *bcl*-2 have been reported recently.[10] DNA sequence analysis suggests that the translocation results from mistakes in joining the VD and J segments of the Ig heavy chain gene.[11] In the present study, we examined the spectrum of B cell neoplasms for *bcl*-2 rearrangement and found the gene frequently rearranged in both follicular and diffuse lymphomas of follicular center cell lineage.

MATERIALS AND METHODS

Patients and surface marker analysis. In an earlier publication,[12] surface markers, and Ig and T cell receptor genes were analyzed in adult patients with non-Hodgkin's lymphoma or B cell chronic lymphocytic leukemia (B-CLL). The present study is based on the 120 earlier specimens and eight additional consecutive samples. Biopsy specimens were subclassified according to a modified Working Formulation for non-Hodgkin's lymphoma,[13] and the diagnosis of B-CLL was based on conventional morphological criteria. Lymphoblastic lymphoma and leukemias of T cell lineage were specifically excluded from this series. An additional group of 14 cases of T cell malignancy, comprised of 4 patients with T cell acute lymphoblastic leukemia, 4 with adult T cell leukemia/lymphoma, 3 with cutaneous T cell lymphoma, and 3 with T cell chronic lymphocytic leukemia served as the T cell control group.

Lymphocyte surface markers were determined in all cases, either by immunofluorescence on suspensions prepared from fresh biopsy material (84 samples of lymphoma) or from Ficoll-Hypaque gradient-purified mononuclear blood cells (24 samples of B-CLL), or on frozen material with the immunoperoxidase method (20 samples of lymphoma). Suspension surface marker studies were performed on aliquots of lymphocytes (10^6 cells in 0.05 mL Medium 199) incubated with 0.05 mL each of the following monoclonal antibodies: common acute leukemia antigen (CALLA), OKT1 (peripheral and thymic T cells), OKT4 (inducer-helper T cells), OKT8 (cytotoxic-suppressor T cells), OKT11 (sheep cell receptor), HLA-DR (HLA-DR antigen), and B1 (B cells). Nonfluoresceinated monoclonal mouse antibodies were obtained from the Ortho Pharmacutical Corporation (Raritan, NJ), with the exception of the CALLA and B1 (Coulter Immunology, Hialeah, FL) and HLA-DR (Becton Dickinson, Sunnyvale, CA) antisera. The lymphocytes were washed after incubation with the monoclonal antibody and then reincubated with a fluorescein-conjugated $F(ab')_2$ fraction of goat anti-mouse γ-globulin antibody (Tago, Burlingame, CA). After being washed again, the cells were suspended in buffered glycerin and examined with a Zeiss ultraviolet microscope equipped with an Osram HBO 200 mercury arc lamp and a fluorescein isothiocyanate 485-nm excitation primary filter. A minimum of 200 cells was examined after staining with each antibody. Surface immunoglobulin (SIg) was assessed with fluorescein-conjugated heteroantisera specific for the human IgM and IgG heavy chains, and the κ and λ light chains (Meloy Laboratories, Springfield, VA). Details of the suspension surface marker techniques and the immunoperoxidase method used are available in the earlier publication.[12]

Southern blot analysis of genomic DNA. High-mol-wt DNA was prepared from each of the immunotyped specimens, and 6 to 10 μg was digested with the restriction enzymes *Pst*I, *Bam*HI, *Sac*I and, in selected instances, *Hin*dIII (all obtained from New England Biolabs, Beverly, MA), and subjected to electrophoresis on 0.8% agarose gel slabs. After denaturation and neutralization, the DNA was transferred to nitrocellulose paper by the technique of Southern.[14] Hybridization was carried out at 68°C in a solution of 5× SET (1× SET: 15 mmol/L NaCl; 1 mmol/L EDTA; 30 mmol/L Tris HCl, pH 8.0); 1× Denhardt's solution; 0.5% sodium dodecyl sulfate (SDS); 10% dextran sulfate; 20 mmol/L Na_2HPO_4 (pH 7.0); 40 μg/mL salmon sperm DNA; and 0.025 to 0.10 μg nick-translated or oligonucleotide (Pharmacia, Piscataway, NJ) ^{32}P-labeled (1 to 3 × 10^7 dpm) DNA probe. A 2.1-kilobase (Kb) fragment of *bcl*-2 was used (probe b in reference 7). Filters were prehybridized for 2 hours at 68°C in prehybridization buffer and hybridized for 15 to 20 hours at the same temperature. Following hybridization, filters were washed successively for 1-hr periods at 68°C with 2× SET containing 0.5% SDS, 1× SET with 0.25% SDS, and twice with 0.5× SET containing 0.125% SDS. Autoradiography of the washed filters was carried out for 1 to 7 days at −70°C with two intensifying screens. Selected filters were rehybridized with a probe J_H for the immuno-

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Table 1. Rearrangement of *bcl*-2 Gene in non-Hodgkin's Lymphomas

Case No./Cell Type	Restriction Enzyme			
	*Pst*I*	*Bam*HI	*Sac*I	*Hin*dIII
Follicular lymphomas				
1. Small cleaved	R*	R	R	
2. Small cleaved	R (ND)	R	R	
3. Mixed small and large	R*	R	R	
4. Mixed small and large	R	G	R	G
5. Mixed small and large	R*	R	G	
6. Mixed small and large	R*	R	R	
7. Mixed small and large	R*	R	R	
8. Mixed small and large	R*	R	R	
9. Mixed small and large	R*	R	R	R
10. Mixed small and large	R*	R	R	
11. Large	R*	R	R	
Diffuse lymphomas				
12. Small cleaved	R*	R	R	
13. Small cleaved	R*	R	R	G
14. Small cleaved			R*	
15. Large	R (ND)	G	G	R
16. Large	R*	R	R	R
17. Large	R*	R	R	
18. Large	R*	R	R	
19. Small non-cleaved	R*	R	R	
20. Small non-cleaved			R	R
21. Small non-cleaved	R*	R	R	R
22. Immunoblastic	R*	R	R	

R, rearranged; G, germline; ND, not rehybridized.

*Rearranged *bcl*-2 fragment that rehybridized with the immunoglobulin heavy chain joining region probe J_H. Rearranged J_H fragments were not detected in case 4. Details given in text.

globulin heavy chain joining region[15]: the previously hybridized probe was removed by a 2-hour incubation at 68°C in 5× Denhardt's solution. Details of the standard methods are available.[16]

RESULTS

Table 1 lists the 22 lymphomas in which one or more rearranged *bcl*-2 gene segments were identified and indicates the findings in DNA digested with the various restriction enzymes. Rearrangements observed after *Pst*I digestion are illustrated in Fig 1. In 18 samples, the *Pst*I-digested DNA was rehybridized with a probe for the joining region of the immunoglobulin heavy chain, and a non-germline *bcl*-2 fragment hybridized with J_H in 17 (representative cases are illustrated in Fig 2): the single exception (Table 1, Case 4) was unusual among B cell lymphomas in that rearrangements of J_H were not identified.[16] Except for case 14, *bcl*-2 rearrangements could be identified after digestion with at least two restriction enzymes, eliminating the possibility of polymorphism: in case 14, sequential hybridization with the J_H probe excluded *bcl*-2 polymorphism by establishing the presence of genetic material from both chromosomes 14 and 18 on the fragment.

Table 2 summarizes the observed incidence of rearranged *bcl*-2 genes in various non-Hodgkin's lymphomas subclassified by a modified Working Formulation.[13] Rearranged *bcl*-2 genes were detected in all major subtypes of non-Hodgkin's lymophma of follicular center cell lineage[13,17] although the overall incidence of rearrangements was higher in follicular tumors (30%) than in those with diffuse morphology (19%). When phenotype was correlated with *bcl*-2 gene status, a somewhat higher incidence of CALLA positivity was noted in non-Hodgkin's lymphomas with rearranged *bcl*-2 genes: 59% of specimens with rearranged *bcl*-2 were CALLA-positive whereas only 36% of samples with germline *bcl*-2 were CALLA-positive. It is noteworthy that *bcl*-2 was

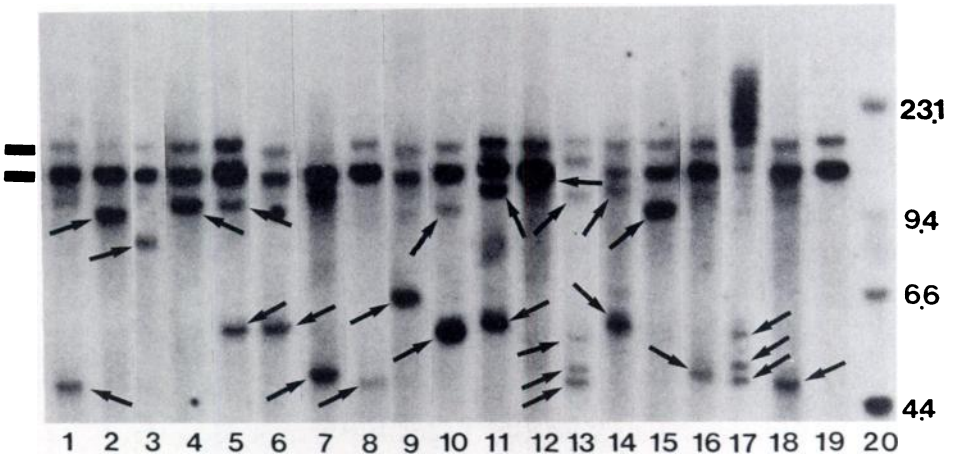

Fig 1. Rearrangement of *bcl*-2 in non-Hodgkin's lymphomas of follicular and diffuse morphology. After digestion with the restriction enzyme *Pst*I, genomic DNA was hybridized with a probe specific for *bcl*-2. The germline configuration is indicated by the solid bars (left), and rearrangements are indicated by arrows. The DNA in lanes 1 through 10 is derived from follicular lymphomas (cases 1, and 3 through 11, respectively from Table 1), lanes 11 and 12 from diffuse small cleaved cell lymphomas (cases 12 and 13), lanes 13 through 15 from diffuse large cell lymphomas (cases 16 through 18), lanes 16 and 17 from diffuse small non-cleaved cell lymphomas (cases 19 and 21), and lane 18 from an immunoblastic lymphoma (case 22). Lane 19 demonstrates the germline configuration in DNA from a patient with chronic myelogenous leukemia, and lane 20 contains a DNA size marker expressed in kilobases.

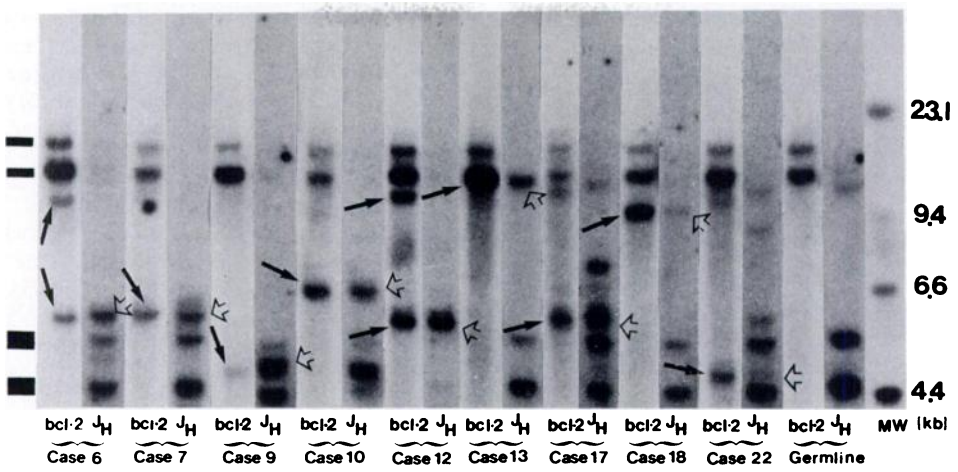

Fig 2. Rehybridization of rearranged *bcl*-2 fragments with a probe (J_H) for the immunoglobulin heavy chain joining region. Solid arrows indicate rearranged *bcl*-2 fragments; open arrows in the next lane indicate the same band after removal of the *bcl*-2 probe and rehybridization with J_H. DNA was digested with *Pst*I, and case numbers refer to Table 1. Germline DNA was derived from a patient with chronic myelogenous leukemia: thin bars (left) indicate germline position of *bcl*-2; thick bars indicate germline position of J_H. Additional details given in text.

always in germline configuration in neoplasms not of follicular cell lineage, ie, small lymphocytic lymphoma, B-CLL and T cell malignancies.

DISCUSSION

Earlier studies of Tsujimoto et al detected 12 rearrangements of *bcl*-2 in 18 specimens (67%) of follicular lymphoma.[17,18] Separate probes detected a major breakpoint cluster (nine rearrangements) in the 3′ untranslated region of the gene, and a minor cluster (two rearrangements) just 3′ to *bcl*-2.[7] More recently, with a third probe,[18] an additional rearrangement was identified 5′ to the gene in the same series of cases.

Table 2. Observed Incidence of *bcl*-2 Gene Rearrangement and Reported Incidence* of 14;18 Chromosomal Translocation in non-Hodgkin's Lymphoma

Lymphoma Subtype	*bcl*-2 Rearrangement	14;18 Translocation
Follicular		
Small cleaved cell	2/18 (11%)	44/83 (53%)
Mixed	8/17 (47%)	15/31 (48%)
Large cell	1/2 (50%)	8/32 (25%)
All follicular	11/37 (30%)	67/146 (46%)
Diffuse		
Small cleaved cell	3/14 (21%)	2/3 (67%)
Mixed	0/4 (0%)	0/27 (0%)
Large cell	4/27 (15%)	14/85 (16%)
Small non-cleaved cell	3/9 (33%)	0/4 (0%)
Immunoblastic	1/4 (25%)	1/13 (8%)
All diffuse above	11/58 (19%)	17/132 (13%)
Small lymphocytic	0/33† (0%)	0/26 (0%)
Lymphoblastic	— —	0/5 (0%)
T cell leukemias and lymphomas	0/14 (0%)	— —

*References 1-5.

†Includes 24 cases of B cell chronic lymphocytic leukemia.

We evaluated the usefulness of *bcl*-2 gene rearrangements in classifying non-Hodgkin's lymphomas employing a *bcl*-2 probe that detects the major breakpoint cluster.[7] One hundred twenty-eight specimens of non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemias were examined. Eleven rearrangements were identified among 35 nodular lymphomas (30%), a somewhat lower incidence than the 50% figure (9 of 18) reported by earlier workers with the same reagent. Our lower figure probably does not reflect technical problems, since closely similar results were obtained with three different restriction enzymes (*Pst*I, *Bam*HI, and *Sac*I). More likely this lower incidence is attributable to the chance distribution in different clinical material. The incidence of 14;18 chromosome translocations in follicular lymphomas obtained by averaging recent karyotype analyses is only 46% (Table 2),[5] although one investigation reported an incidence of 85%.[1] Although cytogenetic analysis was not performed in the present study, the finding that almost all rearranged *bcl*-2 fragments hybridize with a probe for the joining region of the immunoglobulin heavy chain gene located on chromosome 14 strongly supports the presence of 14;18 translocations in our tumors.

The present findings in diffuse lymphomas extend the observations of earlier investigators. Thus, our observed incidence of *bcl*-2 rearrangement of 19% in diffuse non-Hodgkin's lymphoma is approximately three-fifths of the incidence we detected in follicular tumors. This finding indicates that *bcl*-2 rearrangements occur commonly in all non-Hodgkin's lymphomas of follicle center cell lineage,[18] be they diffuse or follicular in morphology, a conclusion consistent with the cytogenetic data of others for 14;18 translocations summarized in Table 1. Both *bcl*-2 rearrangement and 14;18 chromosome translocation also evidently occur very infrequently in lymphoid neoplasms not of follicular cell lineage, ie, diffuse lymphomas of small B lymphocytes, B-CLL and T cell neoplasms. Quite recently, other investiga-

tors have also reported[19] *bcl*-2 rearrangement in diffuse non-Hodgkin's lymphomas.

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