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Rapid Communication

Absence of t(14;18) Major and Minor Breakpoints and of *Bcl*-2 Protein Overproduction in Reed-Sternberg Cells of Hodgkin's Disease

Diane C. Louie, Jeffrey A. Kant, John J. Brooks, and John C. Reed
From the Department of Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

The t(14;18) translocation, found in most human follicular non-Hodgkin's lymphomas (NHLs), juxtaposes the* Bcl-2 *oncogene at 18q21 with the immunoglobulin heavy chain locus at 14q32. As a result, the* Bcl-2 *protein is markedly overproduced. Most of the breakpoints on chromosome 18 cluster at one of two sites, the major breakpoint region (mbr) and the minor cluster region (mcr). Recently, others used the polymerase chain reaction (PCR) to detect the t(14;18) mbr in 32% of specimens diagnosed as Hodgkin's disease (HD). In an attempt to confirm and extend those observations the authors used PCR to assay for both the mbr and mcr in HD specimens diagnosed at their institution and examined the specimens for* Bcl-2 *overproduction. The authors subjected the DNAs from 28 well-characterized HD tumors of 26 patients to PCR analyses using primers specific for the t(14;18) mbr and mcr breakpoints. Based on various PCR controls, the authors ascertained that 26 of the 28 specimens contained amplifiable template DNA. Southern blotting of the amplification products showed that none of the 26 HD DNAs had detectable t(14;18) mbr or mcr breakpoints. By admixing small amounts of t(14;18)-bearing NHL DNA with HD DNA samples, the authors directly demonstrated that the sensitivity of the PCR assays was adequate for the molecular detection of t(14;18)-bearing cells at a frequency comparable to that of Reed–Sternberg cells and their variants in HD. Immunohistochemical studies employing a highly specific anti-*Bcl-2 *antiserum under conditions optimized to detect t(14;18)-mediated overexpression of the* Bcl-2 *gene showed that the Reed–Sternberg cells and variants in all 19 HD tumors examined were negative for* Bcl-2 *immunostaining. In conclusion, the PCR and immunohistochemical data provided evidence that the t(14;18) translocation was not involved in the pathogenesis of the HD cases. (Am J Pathol 1991, 139:1231–1237)

Chromosomal translocations that activate the *Bcl*-2 (*B-cell leukemia/lymphoma-2*) oncogene appear to play a pathogenetic role in a large group of B-cell tumors.[1,2] For example, approximately 90% of follicular and 30% of diffuse B-cell non-Hodgkin's lymphomas (NHLs) possess the t(14;18) translocation[1] which juxtaposes *bcl*-2 at 18q21 with the immunoglobulin heavy chain (Ig_H) locus at 14q32.[3] The *Bcl*-2-Ig_H fusion gene is deregulated in its expression, resulting in high levels of the *Bcl*-2 mRNA and of the *Bcl*-2 protein.[4–6] Approximately 85% of the breakpoints on chromosome 18 cluster at one of two sites, the major breakpoint region (mbr), located in the 3′ untranslated region of the *Bcl*-2 gene, and the minor cluster region (mcr), located 3′ of *Bcl*-2.[7–10]

Hodgkin's disease (HD) is an entity with clinical and histologic features distinct from follicular NHL. The histogenesis of the putative malignant cells of HD, the Reed–Sternberg cell and its variants, is controversial. Virtually every cell of the hematolymphoid system has been im-

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Address reprint requests to Dr. Reed, Department of Pathology & Laboratory Medicine, University of Pennsylvania, 36th St. & Hamilton Walk, 231 John Morgan Building, Philadelphia, PA 19104-6082.

plicated.[11] The Reed–Sternberg cells typically constitute a small minority (approximately 0.1–1%) of cells in the tumor.[12] The remaining, presumably reactive cells, are comprised primarily of lymphocytes, with intermixed plasma cells and eosinophils. Histologically, the differential diagnosis of HD includes T- and B-cell NHLs. In one large series, the percentage of lymphoma cases misdiagnosed as HD was greater than 10%.[13] To maximize the accuracy of the pathologic diagnoses, our institution routinely applies a multiparameter approach (including histologic review, immunohistochemistry, flow cytometry, and antigen receptor gene rearrangement analysis) to lymphoma specimens. In this way, we hope to avoid misdiagnosing T-cell and B-cell NHLs as HD. HD patients diagnosed at our institution typically have Reed–Sternberg cells with a Leu-M1$^+$, LCA$^-$, UCHL-1$^-$ immunophenotype by immunohistochemistry, absence of light-chain restriction by flow cytometry, and absence of immunoglobulin and T-cell antigen receptor gene rearrangements by Southern blotting.

Others used the polymerase chain reaction (PCR) to detect the t(14;18) mbr in 32% of specimens diagnosed as HD.[14] They proposed that activation of *Bcl-2* through the t(14;18) translocation may play an important role in the pathogenesis of a substantial number of HD patients and that follicular NHL is in some way related to HD. In that study, an assay for the mcr was not performed. Using PCR methods, other laboratories did not detect the t(14;18) translocation in HD.[15–17] Although the reason for the discrepant findings is unknown, one possibility is that the PCR assays used in the latter studies lacked the sensitivity necessary to detect t(14;18)-bearing cells at a frequency comparable to that of Reed–Sternberg cells and variants in HD.

To help resolve the controversy, we used PCR methods under conditions of defined sensitivity to assay for both the mbr and mcr in 28 well-characterized HD specimens diagnosed at our institution. In addition, we used a highly specific anti-*Bcl-2* antiserum to test for *Bcl-2* overproduction immunohistochemically.

Materials and Methods

Patient Material

DNA was obtained from viable cell suspensions prepared from 28 HD tumor specimens derived from 26 patients: 18 nodular sclerosing [NS]; 4 syncytial NS; and 6 mixed cellularity [MC] subtypes (Table 1). Eight of the tumors were from eight patients who had received chemotherapy and/or irradiation for HD. All cases had been examined by at least four hematopathologists, all of whom concurred with the diagnoses. In almost all cases (n = 27), support for the diagnosis of HD was obtained from at least two (n = 6), but usually three (n = 21), of the following modalities: immunohistochemistry, flow cytometry, and gene rearrangement analysis. In 25/26 of the specimens examined immunohistochemically, the Reed–Sternberg cells were Leu-M1$^+$, LCA$^-$, UCHL-1$^-$. (In one case, the Reed–Sternberg cells were Leu-M1$^-$, LCA$^-$, UCHL-1$^-$. We presume the lack of Leu-M1 positivity was due to loss of antigenicity by the fixation process, since the Reed–Sternberg cells were Leu-M1$^+$ in a previous biopsy of this patient's HD which had similar histology.) None of the specimens studied by flow cytometry (0/25) or gene rearrangement of the immunoglobulin heavy chain (0/25) showed evidence of clonal B-lymphocyte populations. None of the specimens studied for gene rearrangement analysis of the T-cell receptor beta chain (0/25) showed evidence of clonal T-lymphocyte populations.

Control DNAs

For a positive control for the mbr, DNA was extracted from a mbr-bearing lymphoma cell line, RS11846.[18] DNA (provided by M. Cleary) from a patient's mcr-bearing NHL served as a positive control for the mcr.

Table 1. *Summary of Results for Detection of t(14;18) and* Bcl-2 *Protein in Hodgkin's Disease*

			t(14;18) PCR*			PCR controls			
Histologic subtype	No. DNA specimens	No. patients	mbr + mcr	mbr	mcr	Eth staining†	Admix DNA‡	5′ *Bcl-2* PCR	*Bcl-2* protein‖
NS	18	16	0/16	0/15	0/13	17/18	6/7	6/7	0/11
Syncytial NS	4	4	0/3	0/1	0/1	3/4	1/1	1/1	0/4
MC	6	6	0/6	0/6	0/6	6/6	6/6	1/1	0/4
Total	28	26	0/25	0/22	0/20	26/28	13/14	8/9	0/19

* Data do not include the 2 specimens whose DNAs were determined to be inadequate for PCR (see PCR controls).

† Positive specimens were those with PCR generated bands that were easily visible on ethidium staining. Of the 2 negative specimens, one was weakly positive and the other was negative in the 5′-*Bcl-2* PCR; the latter was also negative in the admix DNA PCR assay.

‡ 1 ng of mbr-bearing DNA or 0.1–1 ng of mcr-bearing DNA was admixed with 1 μg of HD-derived DNA.

‖ Refers to the immunostaining of Reed-Sternberg cells and variants. Data include matched pairs of untreated and treated HD specimens from 3 patients (2 NS, 1 MC).

Polymerase Chain Reactions

For detection of the t(14;18) mbr and mcr breakpoints, 1 ug of DNA from each of the HD samples was subjected to 40 cycles (95°C × 1 min, 55°C × 2 min, 72°C × 3 min) of amplification (Thermocycler, Perkin-Elmer/Cetus) using 0.6 ug each of *Bcl*-2 forward and J_H reverse primers, as described previously[19,20] and 2.5 U of Taq polymerase (Perkin-Elmer/Cetus) in a reaction volume of 100 uls.[20] To directly evaluate the sensitivity of the PCR methods for detecting the t(14;18) translocations, 0.1 to 1 ng of positive control DNA was added to 1 ug aliquots of the HD DNAs.

The control PCR primers targeted at the 5′ (unrearranged) end of the *Bcl*-2 gene ("5′-*Bcl*-2") were: (forward) 5′-AGAGGTACCGTTGGCCCCCGTTGC-3′ and (reverse) 5′-GTCTGCAGCGGCGAGGTCCT-3′.[21] These were used to verify the presence of PCR-amplifiable DNA in particular HD samples. For the 5′ *Bcl*-2 control reactions, 500 ng of DNA was subjected to 40 cycles (95°C × 1 min, 55°C × 2 min, 72°C × 2 min) of amplification using 0.12 ug each of the forward and reverse primers in the same reaction conditions as described earlier, but with the addition of 10% (vol:vol) dimethyl sulfoxide (DMSO) to improve specificity.[22] All experiments included a "no DNA" control reaction designed to detect contaminating DNA, as well as a positive control [containing t(14;18) DNA] reaction.

The PCR products (20 ul) were electrophoresed in 1.5% agarose gels, stained with ethidium, and blotted to nylon filters. The filters were hybridized to ^{32}P-labeled oligonucleotide or genomic probes, washed under high-stringency conditions, and exposed to X-ray film. The mbr and mcr oligonucleotide and plasmid probes have been described elsewhere.[10,19,20] The sequence of the 5′ *Bcl*-2 oligonucleotide probe was: 5′-CGCTCGAGGCCATCTTCTCCTCCCAGC-3′.

Anti-Bcl-2 Immunohistochemistry

The anti-*Bcl*-2 antiserum used for these studies is described in detail elsewhere.[27] Briefly, the antiserum (Ab 9718-4) was produced by immunizing a New Zealand white rabbit with a synthetic peptide corresponding to amino acids 61 to 76 of the human *Bcl*-2 protein.[23] The peptide had the sequence ASRDPVARTSPLQTPA[C] and was coupled to maleimide-activated KLH. The antiserum was highly specific for p26 *Bcl*-2, as demonstrated by Western blot, immunoprecipitation, and peptide competition analyses (not shown).

Immunohistochemistry was performed on Bouin's fixed, paraffin-embedded tissue using the avidin-biotin-peroxidase complex (ABC) technique.[24] The anti-*Bcl*-2 antiserum was used at a 1:600 dilution, which resulted in little to no background staining. Normal rabbit serum was used as a primary antibody control. Nineteen HD tumors from 16 patients in our panel were evaluated with the anti-*Bcl*-2 antiserum. Sixteen of the tumors had provided DNA for the PCR analyses. The three additional tumors represented untreated HD from three patients whose post-treatment HD specimens had provided DNA for the PCR analyses. Support for the diagnosis of HD in the three additional cases was obtained immunohistochemically (Reed–Sternberg cells Leu M1$^+$, LCA$^-$, UCHL-1$^-$). A total of 14 of the 19 tumors represented untreated HD. NHLs that were shown by cytogenetic analysis to bear the t(14;18) translocation served as positive controls for *Bcl*-2 overproduction. Under the conditions of this immunohistochemical assay, use of this anti-*Bcl*-2 antisera accurately predicted the presence of a t(14;18) translocation in 23/24 cases of NHL for which cytogenetic data were available (D. Louie, P. Nowell, J. Brooks, J. Reed, manuscript in preparation).

Results

The DNAs of 28 HD specimens were obtained from 26 patients whose diagnoses were corroborated by immunohistochemistry, flow cytometry, and antigen receptor gene rearrangement analysis. Most of the specimens represented untreated disease. Initially, 25 HD DNA samples were subjected to simultaneous PCR amplification of the t(14;18) mbr and mcr breakpoints using both sets of *bcl*-2/J_H amplification primers. The resulting products were analyzed by Southern blotting with probes specific for the mbr and the mcr. None of the specimens (0/25) (Table 1) had detectable t(14;18) mbr or mcr breakpoints. Titration experiments wherein small amounts of a t(14;18)-containing DNA sample were admixed with 1 ug of normal DNA showed that this PCR method (which simultaneously employed both the mbr and mcr primers) produced unequivocal strong signals at a sensitivity of at least 10^{-2} during a 1 to 2-day film exposure (not shown). Although this is comparable to the frequency of Reed–Sternberg cells and variants in HD tumors, we were able to markedly improve the sensitivity by employing independent PCR amplifications for each breakpoint.

When HD DNA samples were subjected to PCR amplification with primers for the mbr ($n = 22$) and the mcr ($n = 20$), respectively, none were positive for either breakpoint region (Table 1 and Figure 1). The minimal sensitivity of these PCR methods for detecting t(14;18) translocations within the HD DNA samples was directly determined by admixing 1 ng or 0.1 ng of t(14;18)-bearing NHL DNA with 1 ug of DNA from 14 of the HD

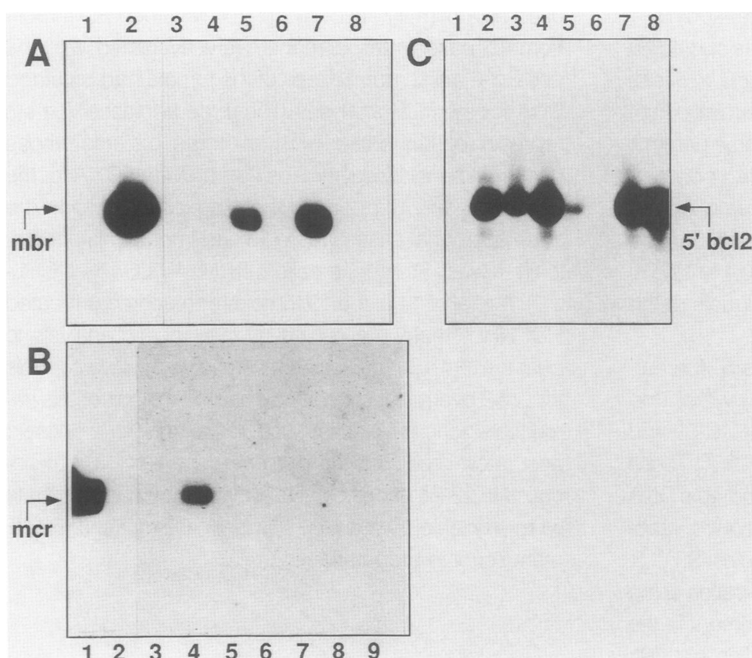

Figure 1. *Southern-blot analysis of PCR-amplified sequences.* **A**: *t(14;18) major breakpoint (mbr).* **B**: *Minor cluster region (mcr) PCR. One microgram of DNA from HD tumors (A3,4,6,8; and B3,5–9); admixtures of 1 ng of mbr-bearing control DNA (A5,7) or 0.1 ng of mcr-bearing control DNA (B4) with 1 μg of HD DNA; 1 ng of positive control mbr-bearing (A2) DNA; 0.1 ng of positive control mcr-bearing DNA (B1); and no DNA (A1 and B2) were PCR-amplified with primers specific for the mbr (A) or mcr (B). After gel/electrophoresis and transfer to filters, blots were hybridized with the appropriate* Bcl-2 *^{32}P-oligonucleotide probes. None of the reactions containing HD template DNA alone showed positive signals on the autoradiograms. The sensitivity of the assays was at least 10^{-3} for the mbr and 10^{-4} for the mcr, as revealed by the positive signals in the reactions containing admixtures of t(14;18)-bearing positive control and HD DNA.* **C**: *5'-*Bcl-2 *PCR. To verify the presence of PCR-amplifiable DNA, 500 ng of t(14;18) mbr-bearing (2) or mcr-bearing (3) control DNA; HD DNA (4–8); and no DNA (1) were PCR-amplified with primers targeted at the 5' (unrearranged) end of the* Bcl-2 *gene, and the products were hybridized with the corresponding ^{32}P-oligonucleotide probe. The two HD specimens that yielded weak (5) or no signal (6) were excluded from the t(14;18) PCR data, since the absence of efficient amplification could result in a false negative.*

samples. We detected strong unequivocal bands by hybridization of *Bcl*-2-specific ^{32}P-probes to these PCR products for 13 of the 14 reactions in which admixing was performed, thereby establishing a detection sensitivity of at least 10^{-3} for the mbr and at least 10^{-4} for the mcr assays. Like other investigators,[19,20] we found by titration experiments (not shown) that the actual level of sensitivity of these PCR assays for detection of the *Bcl*-2 mbr and mcr was much higher, but at high dilutions the hybridization signals produced as a result of t(14;18) breakpoint amplifications were faint and thus unreliable for clinical diagnostic purposes. Nevertheless, PCR assay sensitivities in the 10^{-3} to 10^{-4} range are clearly sufficient for detection of t(14;18)-bearing cells at a frequency comparable to that of RS cells and their variants.[12]

Of the 28 HD DNA samples, 26 appeared to contain amplifiable DNA when the mbr and mcr PCR reaction products were examined on ethidium-stained agarose gels (not shown) before Southern blotting. We and others[16] have noted that nonspecific bands are produced during PCR with the mbr and mcr amplification primers. Although these bands are not amplified t(14;18) junctions (they do not hybridize with the specific probes), their presence and intensity provide evidence that adequate amplifiable template DNA was present. We correlated the nonspecific bands with the ability to amplify individual DNA samples by subjecting nine HD samples (including the two samples that did not easily yield visible nonspecific bands in the t(14;18) PCR assays) to amplification with primers targeted to the 5′ (unrearranged) region of *Bcl*-2, a region distant from those involved by the mbr and mcr. All of the seven samples that were expected by ethidium staining to contain adequate amplifiable template DNA yielded the anticipated 261 bp control amplification product, and the specificity of this product was confirmed by Southern blotting (Figure 1c). In contrast, little or no product was detected for the two samples suspected of poor performance by ethidium staining. Since the low amplification efficiency for these two samples could result in false negative results, these samples were excluded from the t(14;18) PCR data. (For at least one of the two samples that did not efficiently amplify with the 5′ *Bcl*-2 control primers, we attribute the source of the problem to a nonspecific inhibitor of the PCR amplification since the mixture of 1 ng of mcr-bearing positive control NHL DNA with this HD sample also did not result in a detectable mcr amplification product in Southern blots.) Thus, 26 of 28 HD-DNA specimens yielded PCR-generated ethidium-detectable bands. Of these 26 specimens, 18 were further verified to contain DNA adequate for PCR by the admixing of t(14;18) positive control DNAs, by the 5′-*Bcl*-2 control PCR assay, or both. Of these 26 HD DNA samples judged adequate for PCR, none had detectable t(14;18) mbr or mcr breakpoints even with long x-ray film exposures (summarized in Table 1).

To further explore the possible role of the t(14;18) translocation in HD, we obtained 19 HD specimens from

16 of the patients in our panel and examined the Reed–Sternberg and variant cells immunohistochemically for overproduction of the *Bcl*-2 protein. The conditions of this immunohistochemical assay were optimized for detection of *Bcl*-2 protein overproduction as a result of the t(14;18) translocation. In a separate study, we demonstrated the ability of this assay to accurately predict the presence of a t(14;18) translocation in 23/24 cases of NHL for which cytogenetic data were available (manuscript in preparation). In all of the 19 HD tumor specimens examined, the Reed–Sternberg cells and variants exhibited no specific staining for *Bcl*-2 (Figure 2 and Table 1) indicating that these cells do not overproduce the *Bcl*-2-protein. In some HD specimens, reactive lymphocytes exhibited weak cytoplasmic staining with the anti-*Bcl*-2 antiserum, as reported by others.[26] The absence of detectable *Bcl*-2-protein in the RS cells of these HD cases could not be explained by fixation problems since all of these cases exhibited strong reactivity with the Leu M1 antibody (not shown). From these immunohistochemical data, we conclude that the *Bcl*-2 protein is not overproduced in the Reed–Sternberg cells of our HD cases. In contrast, positive control cases of t(14;18)-bearing NHLs analyzed in parallel with these HD samples all exhibited intense cytoplasmic staining of the malignant cells with the anti-*Bcl*-2 antiserum.

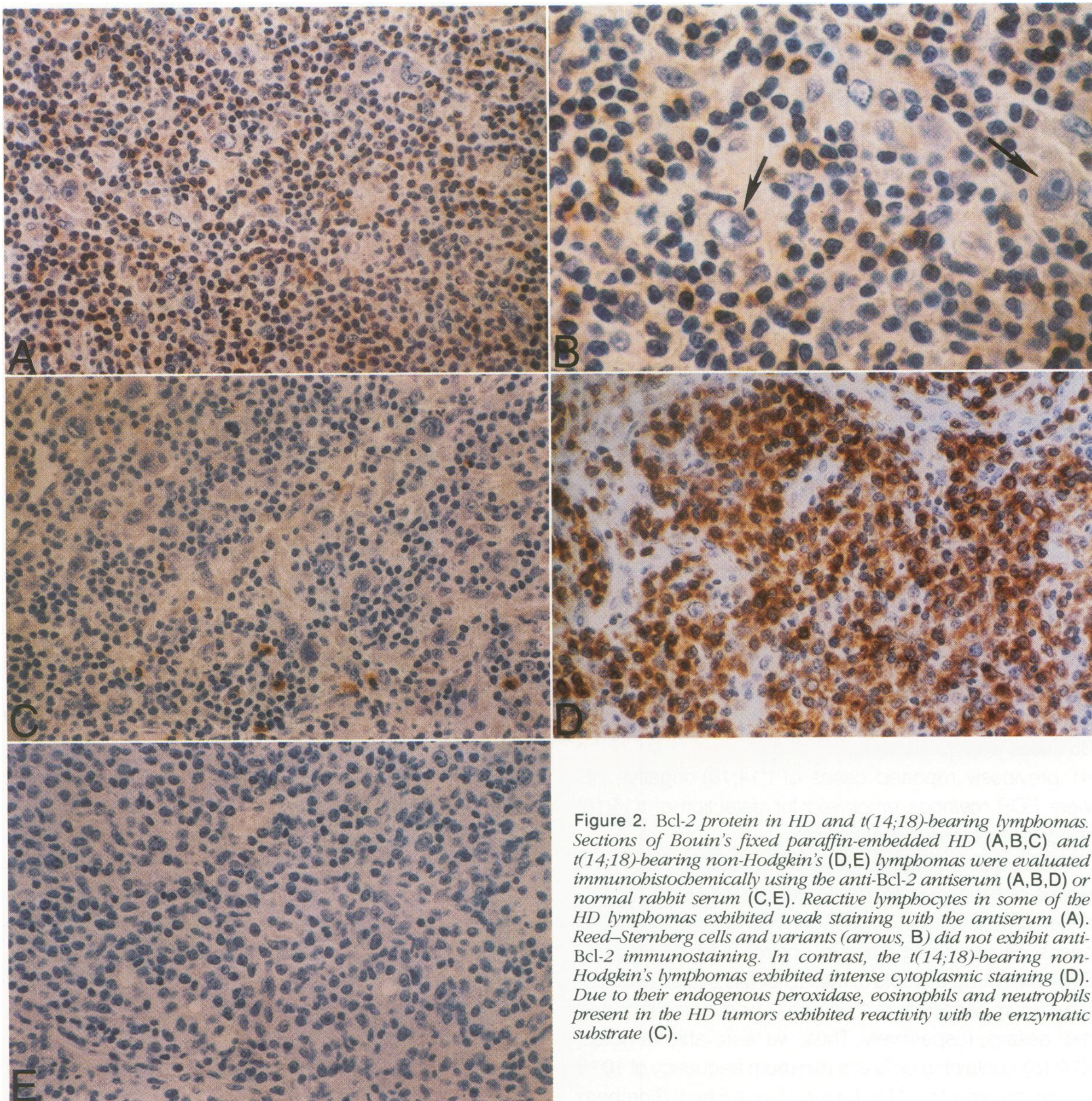

Figure 2. *Bcl-2 protein in HD and t(14;18)-bearing lymphomas. Sections of Bouin's fixed paraffin-embedded HD* (A,B,C) *and t(14;18)-bearing non-Hodgkin's* (D,E) *lymphomas were evaluated immunohistochemically using the anti-Bcl-2 antiserum* (A,B,D) *or normal rabbit serum* (C,E). *Reactive lymphocytes in some of the HD lymphomas exhibited weak staining with the antiserum* (A). *Reed–Sternberg cells and variants (arrows,* B*) did not exhibit anti-Bcl-2 immunostaining. In contrast, the t(14;18)-bearing non-Hodgkin's lymphomas exhibited intense cytoplasmic staining* (D). *Due to their endogenous peroxidase, eosinophils and neutrophils present in the HD tumors exhibited reactivity with the enzymatic substrate* (C).

Discussion

Controversy has emerged over the question of whether Hodgkin's lymphomas frequently contain the t(14;18) translocation that activates the *Bcl*-2 gene in most follicular NHLs. In a study from the National Cancer Institute, 32% of specimens diagnosed as HD were found by PCR assays to contain t(14;18) major breakpoint translocations.[14] No attempt was made to detect minor cluster region breakpoints. The authors did not mention what laboratory methods, other than microscopic examination, were used to establish the diagnosis of HD in their specimens.

Three other investigations, involving a total of 41 HD cases, did not detect t(14;18) translocations by PCR.[15–17] However, the ability to interpret each of the three latter studies is compromised by one or more of the following reasons: 1) inadequate documentation of the sensitivity level of the PCR assays; 2) assay for the t(14;18) mbr only, without assay for the mcr; 3) failure to document adequate template DNA; and 4) limited documentation that the lymphomas represented HD, not NHL. For example, for 35 of the 41 previously published cases of HD,[15,17] which were negative for the t(14;18) by PCR, the sensitivity of the assay was reported as 10 ng (equivalent to $>10^3$ cells, assuming a DNA content of 7 pg per diploid human cell[25]) and was determined without mixing the t(14;18)-containing control DNA with 1 ug of normal DNA to simulate the conditions of HD specimens, which contain a large proportion of normal DNA from reactive cells. We consistently find that the sensitivity of the t(14;18) mbr and mcr PCR assays decreases markedly when 1 ug of normal human DNA is included in the reactions. Also, no positive-control PCR was performed for these 35 HD cases, which would have assured that the HD specimens contained amplifiable DNA. Moreover, the PCR products were analyzed by a dot-blot method, rather than by gel-electrophoresis in which ethidium staining can assist in ascertaining whether adequate template DNA was present in every specimen. Thus, the possibility of false negative results among the 35 cases was not excluded. Furthermore, in only 5 of the 41 previously reported cases of t(14;18)-negative HD were PCR methods employed for detection of t(14;18) minor cluster region breakpoints.

For those reasons, we used PCR methods under conditions of defined sensitivity to detect t(14;18) major and minor breakpoints in a panel of well-characterized HD cases. By admixing positive control [t(14;18)-bearing] DNAs with 1 ug aliquots of the HD DNAs, we established a minimum sensitivity of 1 ng and 0.1 ng for the mbr and mcr assays, respectively. Thus, we were able to detect t(14;18)-containing cells at a minimum frequency of 10^{-3} for the mbr and 10^{-4} for the mcr. Since Reed–Sternberg cells and their variants represent 0.1 to 1% of the cells in HD,[12] our PCR assays should have detected t(14;18) translocations if they occurred at either the mbr or mcr in the Reed–Sternberg cells of our HD cases.

Our failure to detect the t(14;18) translocation in the HD specimens cannot be attributed to inadequate DNA preparations. For 26 of 28 HD DNA specimens, ethidium-stained nonspecific PCR products were easily visible in agarose gels. Eighteen of 26 specimens were further confirmed to be satisfactory by admixing low amounts of t(14;18)-containing DNA and/or by performing a control PCR using 5′ *Bcl*-2 specific amplification primers. Six of the remaining eight cases, as well as the two whose t(14;18) PCR data were excluded because of poor PCR efficiency, were among the 19 cases demonstrated immunohistochemically to lack *Bcl*-2 overproduction in their Reed–Sternberg cells. Thus, we conclude that t(14;18) translocations involving either the mbr or mcr were not present in the 26 cases of HD we successfully analyzed by PCR.

Although our PCR results do not exclude the possibility that our HD cases contain t(14;18) translocations whose breakpoints fall outside the mbr or mcr, our anti-*Bcl*-2 immunohistochemistry data argue strongly against it. Under conditions in which the anti-*Bcl*-2 antiserum resulted in intense cytoplasmic staining of t(14;18)-bearing lymphoma cells, we found no detectable immunostaining of Reed–Sternberg cells and their variants in all 19 of the HD tissue specimens examined.

It is unclear why our data conflict with the NCI study.[14] The t(14;18) translocations may occasionally occur in the setting of HD, but their absence in the 28 cases studied here may reflect differences among the patient populations seen by various institutions. For example, most of our HD specimens (20/28) were obtained from patients at initial presentation, before the institution of therapy. The proportion of patients who received therapy was not mentioned in the other PCR studies of the t(14;18) abnormality in HD.[14–17] Certain referral centers may be more likely to see patients post-therapy for their HD. Irradiation and chemotherapy may set the stage for the emergence of small numbers of t(14;18)-containing cells, most of which never advance to frank NHL.[28] Alternatively, the absence of the t(14;18) translocation in our panel of HD cases may reflect differences in the criteria used to diagnose HD. Our institution routinely applies a multiparameter approach (including immunohistochemistry, flow cytometry, and gene rearrangement analysis) to diagnose lymphomas. We employ this strategy in hopes of maximizing the accuracy of our diagnoses, and in particular, to avoid misdiagnosing NHLs as HD. Thus, we believe that the cases presented in this study are unequivocal examples of HD.

Resolution of the controversy over whether the t(14;18) translocation is frequently present in HD is of critical importance. Confirmation that this translocation is

found in a significant number of HD cases would imply that follicular NHL and HD are somehow related. It might also affect lymphoma patient diagnosis, staging, and monitoring, since the t(14;18) translocation can serve as a molecular marker. Interinstitutional studies are warranted that employ well-characterized cases of HD, sensitive and appropriately controlled PCR methods, and highly specific anti-*Bcl*-2 antibodies.

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