Association of bcl-1 Rearrangements With Lymphocytic Lymphoma of Intermediate Differentiation

By L. Jeffrey Medeiros, Johan H. Van Krieken, Elaine S. Jaffe, and Mark Raffeld

Previous studies using classical cytogenetics have demonstrated the presence of the t(11;14) (q13;q32) chromosomal translocation in some cases of lymphocytic lymphoma of intermediate differentiation (IDL), a distinct type of low grade B-cell lymphoma. This finding suggested that the bcl-1 region (located at band q13 of chromosome 11) might be involved in this neoplasm. Using a genomic probe from the major breakpoint area of the bcl-1 locus, we identified rearrangements of the bcl-1 region in 10 of 19 cases, 2 of which comigrated with a rearranged allele of the immunoglobulin heavy chain gene joining region. In contrast, bcl-1 rearrangements were not found in other types of low grade B-cell lymphoma, specifically in 36 cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and 27 cases of follicular lymphoma (FL). To further assess the molecular pathology of IDL, we analyzed these cases for rearrangements of the bcl-2 proto-oncogene, which is associated primarily with follicular lymphomas. None of the 19 cases of IDL had rearrangements. Furthermore, none of the 36 cases of CLL/SLL showed bcl-2 rearrangements, whereas, as expected, 21 of 27 cases of FL had rearrangements of the bcl-2 locus. Our findings demonstrate an association between a rearranged bcl-1 region with approximately 50% of IDLs and suggest that abnormalities of this locus may be important in the pathogenesis of IDL.

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Materials and Methods

Nineteen cases of IDL with available frozen cell suspensions or tissue blocks were analyzed. Each case was classified without knowledge of the molecular results using histologic criteria previously described. Other well characterized low grade B-cell lymphomas including 36 cases of CLL/SLL and 27 cases of FL (8 small cleaved cell type and 19 mixed small cleaved and large cell type) were also selected as control groups with which to compare the prevalence of bcl-1 rearrangements in IDL. The prevalence of bcl-2 rearrangements in each subtype of low grade lymphoma was also compared.

Immunophenotypic methods. Eighteen of the 19 IDLs were immunophenotyped as a part of their initial diagnostic workup by various methods previously described including frozen section immunohistochemistry (13 cases) and cell suspensions studied by flow cytometry (12 cases). Although the panel of antibodies varied from case to case, in all lesions the following panel of reagents was used: immunoglobulin heavy (mu, delta, gamma, alpha) and light (kappa, lambda) chains (either Bethesda Research Laboratories, Bethesda, MD, or Tago Laboratories, Burlingame, CA), B1 (CD20), B4 (CD19), CALLA (CD10), T11 (CD2) (Coulter Monoclonal Antibodies, Hialeah, FL), Leu1 (CD5), and Leu4 (CD3) (Becton-Dickinson, Mountain View, CA).

Molecular techniques. Frozen tissue or cell suspensions were

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thawed and high molecular weight DNA was obtained by standard proteinase K/RNase phenol-chloroform extraction. Following purification, 15 to 20 micrograms of DNA were completely digested with the EcoRI, HindIII, and BamHI restriction enzymes according to the conditions recommended by the supplier (Bethesda Research Laboratories, Bethesda, MD). The digested DNA was electrophoresed through 0.7% agarose gels, depurinated with 0.25N HCl, and denatured and transferred in 0.4N NaOH onto a nylon membrane (GeneScreen Plus, New England Nuclear Research Products, Boston, MA). The membranes were hybridized and washed in high stringency conditions as described previously.26 Cloned genomic DNA probes were radiolabeled with P3' using the random primer method.27 The probes used have been previously described.3,26 The immunoglobulin heavy chain gene was assessed with a BglII-BglII, 3.8 kb fragment cloned from the joining (Jh) region (courtesy of Dr P. Leder). The bcl-1 region of chromosome 11 was studied using a SstI-SstI, 2.0 kb fragment (probe "b" in reference 6) (gift of Dr Y. Tsujimoto). The bcl-2 region of chromosome 18 was analyzed with three probes: a HindIII-EcoRI, 2.8 kb fragment cloned from the major breakpoint region (mbr), an EcoRI-EcoRI, 4.0 kb fragment encompassing the minor breakpoint cluster region (mcr) (probe "pft-2" of reference 4), and a BamHI-BamHI, 0.4 kb fragment 12 kb 3' to the major breakpoint region (near, but not identical with the probe "c" in reference 3). (The major and minor breakpoint region probes were gifts from Dr Y. Tsujimoto and Dr M. Cleary, respectively.)

RESULTS

Clinical data. The clinical features of patients with IDL have been previously reported.14 In this study, the median age of the patients was 59 years (range, 32 to 78). There were 14 men and 5 women. All patients presented with clinical stage III (6 patients) or IV (13 patients) disease. Twelve received various chemotherapy regimens, six were observed without therapy, and in one case the details of treatment are unknown. Clinical follow-up was available for 16 patients (median, 13 months). Six patients have died of disease.

Immunophenotypic findings. All 18 cases immunophenotyped demonstrated a monoclonal B-cell population. All cases expressed IgM (10 lambda, 8 kappa) and 11 lesions were also IgD positive. All cases expressed at least one pan-B-cell antigen (CD19 and/or CD20), 16 of 18 were CD5 positive, 6 of 15 expressed CD10 antigen, and all were negative for the pan-T-cell antigens CD2 and CD3.

Genotypic results. In all IDLs the immunoglobulin heavy chain gene was rearranged demonstrating the presence of tumor DNA in each sample of extracted DNA. In 16 cases two rearrangements and in 3 cases one rearrangement were identified with the JH probe.

Ten of 19 IDLs had a single rearrangement of the bcl-1 region; nine of 18 IDLs using the described bcl-1 probe and one additional case, detected previously and reported separately6 (Table 1). In 8 of the 10 cases with bcl-1 rearrangements, the rearrangements were detected with more than one restriction enzyme (BamHI, EcoRI, and/or Hind II) (Fig 1A). In 2 additional IDLs a bcl-1 rearrangement was detected with only one restriction enzyme, that enzyme being BamHI in each case (Fig 1B). This finding suggests that the breakpoint on chromosome 11 is likely to occur within the 3.0 kb germline HindIII fragment (Fig 1C). In the two cases which showed bcl-1 rearrangements with only BamHI-digested DNA, the results suggest that the breakpoints occurred between the 5' BamHI and HindIII restriction enzyme sites. Although the rearrangement pattern is compatible with a break between the 3' EcoRI and BamHI restriction enzyme sites, in both cases the sizes of the rearranged fragments (16 to 17 kb) are smaller than the smallest theoretical size expected from a rearrangement occurring in this region. (The distance from the 5' BamHI site to the 3' EcoRI site is 19 kb.) Thus, we infer that the breakpoints are 5'. In addition, the rearranged fragments in each case were of similar size (Fig 1B) suggesting that the breakpoints on chromosome 11 (as well as on chromosome 14) are clustered.

In each case with a bcl-1 rearrangement, two rearrangements of the immunoglobulin heavy chain gene were identified. In two cases, the rearranged bcl-1 fragment (present in all DNA digests) comigrated with one of the rearranged fragments detected with the Jh probe indicative of the t(11;14) (q13;q32) translocation.

Using the bcl-1 probe no rearrangements were detected in 36 cases of CLL/SLL or in 27 cases of FL (data not shown).

Eighteen IDLs were also analyzed with three probes derived from the bcl-2 region of chromosome 18. Rearrangements of the bcl-2 region were not detected in any case of IDL. Similarly, bcl-2 rearrangements were not found in the 36 cases of CLL/SLL. In contrast, 21 of the 27 cases of FL had unequivocal rearrangements of the bcl-2 region, the majority of which comigrated with rearrangements of the immunoglobulin heavy chain gene, showing evidence of the t(14;18) (q32;q21) translocation (data not shown).

Lack of correlation of bcl-1 results with clinical and immunophenotypic data. There were no clinical differences between patients with bcl-1 rearranged lymphomas

Table 1. Summary of Genotypic Results

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Abbreviations: R, rearranged; G, germline; ND, not done; R+, bcl-1 rearrangements present in DNA digested with two or three restriction enzymes; R++, bcl-1 rearrangements present only with BamHI-digested DNA.

*Results with all bcl-2 probes.
Fig 1. (A) Two cases (Nos. 1 and 5) of IDL demonstrating bcl-1 rearrangements with all three restriction enzyme digests. The suggested breakpoint in these cases is within the 3.0 kb HindIII germline fragment (dark arrow in C). (B) Two cases (Nos. 9 and 13) of IDL in which the bcl-1 rearrangement was detected only with BamHI-digested DNA. The breakpoint in these cases is likely to be 5' to the 3.0 kb HindIII germline fragment (light arrow in C). In both A and B, placental (P) DNA was used as the control. (C) Map of the bcl-1 region indicating the relevant restriction enzyme sites and the location of the bcl-1 probe.
and unrearranged lymphomas. In particular, the percentage of patients treated was approximately the same, and response to therapy and survival were similar. Four of 9 patients (1 lost to follow-up) whose tumors had bcl-1 rearrangements died of disease compared with two of seven (two lost to follow-up) cases without bcl-1 rearrangements. Similarly, there were no significant immunophenotypic differences between the neoplasms with bcl-1 rearrangements and the IDLs without bcl-1 rearrangements (data not shown).

**DISCUSSION**

These results demonstrate the presence of bcl-1 rearrangements in 10 of 19 (53%) cases of IDL using a probe cloned from the bcl-1 region of chromosome 11. In contrast, rearrangements of the bcl-1 locus were not found in 36 cases of CLL/SLL or 27 cases of FL. These findings suggest a role for the bcl-1 region of chromosome 11 in the pathogenesis of IDL.

We speculate that, similar to the mechanisms of activation of both bcl-2 and c-myc in the t(14;18) (q32;q21) and t(8;14) (q24;q32) translocations, respectively, translocation of the bcl-1 region to the immunoglobulin gene locus activates a postulated proto-oncogene leading to unregulated cell growth. Alternatively, rearrangement of the bcl-1 region may induce lymphomagenesis by other, as yet unknown, mechanisms.

The 53% prevalence of bcl-1 rearrangements in IDLs reported here may, in fact, underestimate the true incidence. Our own data suggest the presence of at least two breakpoint clusters detected with the bcl-1 probe; one breakpoint within the 3.0 kb HindIII germline fragment and one breakpoint farther 5', between the 5' BamHI site and the 3.0 kb HindIII germline fragment. Other breakpoints farther 5' in the bcl-1 region have been reported, and these breakpoints would not be detected with the probe used. Furthermore, additional breakpoints, as yet unreported, may exist.

We did not find bcl-1 rearrangements in 36 cases of CLL/SLL in this study. Although bcl-1 rearrangement was first detected in a case reported as CLL, our review of the literature indicates that bcl-1 rearrangements are uncommon in cases of CLL/SLL. Combining our own data with those of large survey studies, bcl-1 rearrangements have been identified in only 6 of 163 unselected cases (3%) of CLL/SLL. There are 4 additional cases of bcl-1 rearrangement in CLL/SLL, but these were specifically selected for the t(11;14) chromosomal translocation, and to include them in prevalence reporting would bias the true prevalence. Also, occasional cases reported in the literature as CLL/SLL may, in fact, be examples of IDL. For example, Ince et al describe one of their two cases of SSL as being a mantle-zone variant. The mantle-zone pattern is a morphologic feature that is characteristic of IDL. In addition, in the leukemic phase IDL may be difficult to distinguish from CLL/SLL without a lymph node biopsy.

There have not been other studies that have specifically looked for bcl-1 involvement in a series of IDL. However, of the 9 reported cases of leukemias and lymphomas other than CLL/SLL with bcl-1 rearrangements, it is possible that 4 cases may be IDLs. Athan et al have found bcl-1 rearrangements in 2 cases of diffuse small cleaved cell lymphoma. IDL is usually best classified as diffuse small cleaved cell lymphoma in the Working Formulation. In addition, Koduru et al described bcl-1 rearrangements in 2 cases of “nodular poorly differentiated lymphocytic lymphoma.” Bcl-1 rearrangements have not been described in other studies of follicular lymphomas, including this report, and occasional cases of IDL may exhibit a vaguely nodular growth pattern and resemble follicular small cleaved cell lymphoma.

The absence of bcl-2 rearrangements in all cases of IDL studied is of interest. Since 80% to 90% of FLs have bcl-2 rearrangements as a result of the t(14;18) (q32;q21) chromosomal translocation, the absence of bcl-2 rearrangements in IDL suggests that IDL is not related to FL and that the molecular mechanisms involved in the pathogenesis of IDL and FL are distinct and unrelated.

In summary, we have identified bcl-1 rearrangements in a high percentage of IDLs, which suggests that this locus may be involved in the pathogenesis of IDL.

**ACKNOWLEDGMENT**

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