Oncogene Rearrangements in Chronic B-Cell Leukemia


Forty-four B-chronic lymphocytic leukemias (CLL) were studied by Southern blot analysis using probes for the Ig genes and bcl-1, bcl-2 (major, minor and 5’ breakpoint region), bcl-3, c-myc, and retinoblastoma (Rb) loci. Eight cases had three or more rearranged JH bands, indicating oligoclonality, clonal evolution, or chromosomal translocation. One case had a rearrangement of the bcl-1 locus and three of the bcl-2 locus. In the first case, co-migration of the rearranged bcl-1 and JH sequences indicated a t(11;14)(q13;q32) translocation, which, in contrast to previously described cases, seems to be completely reciprocal. One case with a bcl-2 rearrangement showed co-migration of the bcl-2 major breakpoint region and a rearranged JH band. This indicates a t(14;18) (q32;q21). The two other cases showed rearrangements of the bcl-2 5’ breakpoint region without apparent co-migration. No rearrangements were detected of c-myc and bcl-3, located at chromosome 19, nor was a deletion of Rb found. All but three cases had CDS expression. The exceptions included the t(11;14) and the t(14;18) cases. Our results confirm recent data on rearrangements at the 5’ site of bcl-2 in CLL. Additionally, they corroborate the presumption that CD5-negative chronic B-cell leukemias should be considered apart from classical CLL.

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B-CHRONIC lymphocytic leukemia (B-CLL) is a leukemia of low-grade malignancy that occasionally changes to a more aggressive subtype. Several cases of CLL with a chromosomal translocation have been described, but the incidence and importance of the involved oncogenes is unknown. Moreover, the distinction between CLL and leukemic non-Hodgkin’s lymphoma (NHL) may sometimes be difficult.1,4 We investigated the use of Southern blot analysis of leukemic cells to identify rearrangements of oncogenes and other loci known to be involved in primary lymphomagenesis and/or tumor progression.

The t(11;14)(q13;q32) occurs nonrandomly in various B-cell malignancies, and involves the bcl-1 locus at chromosome 11.3,4 The t(11;14) is present in at least 30% of lymphocytic lymphomas of intermediate differentiation.9,10 Similarly, the bcl-2 gene is involved in the t(14;18)(q32;q21), present in many follicular lymphoma and in a minor portion of diffuse large cell lymphomas. The bcl-2 breakpoints cluster in several regions called the major, the minor, and the 5’ end breakpoint regions.11,13 Recently a bcl-3 gene has been identified on chromosome 19 by cloning the chromosome translocation breakpoint junction of the (14;19)(q32;q13.1) in a case of CLL.6,17 The c-myc oncogene is involved in the translocations in Burkitt’s lymphomas, but has also been associated with tumor progression.18,21 Inactivation (deletion) of the retinoblastoma (Rb) tumor suppressor oncogene has also been identified in a variety of human lymphomas and leukemias.22,23 (A. Ginsberg, personal communication, June 1990). Thus far, the involvement of these loci has not been systematically analyzed in a large series of patients with B-CLL.

The Ig gene, bcl-1, bcl-2 (major, minor, and the 5’ end regions), bcl-3, c-myc, and Rb loci were studied in multiple blood, bone marrow, and lymph node samples obtained from 44 patients with morphologically defined B-CLL.24 We report that the combined analysis of immunophenotype, especially CD5 expression, and genotype may be helpful in discriminating true B-CLL from leukemic NHL.

MATERIALS AND METHODS

Patients. Forty-four patients, clinically and morphologically diagnosed as having B-CLL, collected at the department of Hematology of the Leiden University Hospital (Leiden, The Netherlands) were studied. The morphology was defined as described previously and each case was also studied for cell surface markers using standard techniques.25 In 11 cases two or more successive samples were available. The series included 60 peripheral blood, three bone marrow, and four lymph node samples.

Methods. Peripheral blood lymphocytes were separated from most of the blood samples by Ficoll-Hypaque centrifugation. In a few cases with high numbers of tumor cells no cell separation was performed. High molecular weight DNA was isolated using standard methods.26 The DNAs were digested to completion with various restriction enzymes, size fractionated in 0.7% agarose gels, and transferred to Genescreen (NEN) nylon filters. Blots were hybridized with random primer 32P-labeled probes, washed in 0.3 x SSC/0.1% sodium dodecyl sulfate (SDS) at 65°C, and autoradiographed at ~70°C. The filters were re-used with different probes after removal of the previous signal by washing in 0.1 x SSC/0.1% SDS at 100°C. EcoRI, BamHI, HindIII, and BclI digestions were performed in all cases. If enough material was available additional digestions were performed.

Probes. IgH gene rearrangements were detected with a 2.4-kb Sau3AI Ig JH probe and a 1.2-kb EcoRI-EcoRI Ig Cl probe. Rearrangements of Ig k genes were studied with a 1.8-kb SacI-SacI Jk-probe in EcoRI- and HindIII-digested DNA, and λ gene rearrangements were studied with a 3.5-kb EcoRI-HindIII Ca probe using EcoRI-digested DNA (the Ig probes were kindly provided by P. Leder, Boston, MA). A partial restriction map of the bcl-1 locus, identifying the probes bcl-1a29 (provided by T. Rabbits, MRC, Cambridge, UK) and bcl-1b (provided by Y. Tsujimoto, Philadelphia, PA), is presented in Fig 1 (B. Rearrange-
Fig 1. (A) Southern blot analysis of case 35. DNA from a blood sample was digested with the restriction enzymes indicated, and hybridized with the bcl-1a, JH, and bcl-1b probes. Arrowheads indicate the germline bands and dashes indicate the rearranged fragments. Comigrating fragments are indicated by double dashes between the lanes. The rearranged bcl-1a and bcl-1b fragments each comigrate with a different rearranged JH band. No germline JH band is present, indicating a sample with pure tumor cells. (B) Deduced partial restriction maps of normal chromosomes 11 and 14 and of the presumptive 14q- and 11q- chromosomes in case 35. Filled and open boxes show chromosomes 11 and 14, respectively. Restriction sites are indicated as: E, EcoRI; H, HindIII; S, SacI; B, BamHI. The used bcl-1a (a), bcl-1b (b), JH, and Cm probes are indicated. The star represents the breakpoint region for case 35. With the bcl-1a probe a rearranged band is found in the HindIII and SacI digests, but not in the EcoRI digest. After stripping and hybridizing with the bcl-1b probe a rearranged band was found in the HindIII and EcoRI digests, but not in the SacI digest. Therefore, the breakpoint falls within the 1.6-kb EcoRI-SacI fragment of bcl-1. The joining between bcl-1 and JH (hatched region) is schematically drawn.

ments of the bcl-2 gene were studied by hybridizing the filters with a 2.8-kb EcoRI-HindIII fragment of the bcl-2 gene containing the major breakpoint area (mbr)14 (provided by Y. Tsujimoto), a 4.0-kb EcoRI-HindIII fragment containing the minor breakpoint region (pFI-2)14 (provided by M.L. Cleary, Stanford, CA), and a 1.6-kb EcoRI-EcoRI fragment containing the 5' end breakpoint region14 (provided by Y. Tsujimoto). The bcl-3 rearrangements were studied by using a 1.4 PstI-PstI fragment containing the breakpoint of bcl-3 in a case of B-CLL16,17 provided by T.W. McKeithan, Chicago, IL). The c-myc gene was studied with a 1.4-kb EcoRI-ClaI fragment containing the breakpoint of c-myc. The deletion of the Rb gene was studied by using cDNA probes (provided by S.H. Friend, Boston, MA) designated “Rb0.9” and “Rb3.8” and representing 5' and 3' portions of the Rb cDNA, respectively.

RESULTS

All 44 cases showed Ig heavy chain gene rearrangements, which demonstrated that each sample contained an adequate number of malignant cells to detect a DNA rearrangement.28 Eight cases had three or more rearranged JH bands, indicating oligoclonality,9,26 the occurrence of additional alterations within the IgH genes,31,32 or chromosomal translocations. One case showed a rearrangement of bcl-1 and
three cases showed a rearrangement of \( bcl-2 \). No \( bcl-2 \) minor breakpoint region, \( bcl-3 \), \( c-myc \), or \( Rb \) mutations were detected (Table 1).

All cases demonstrated pan-B cell marker (CD19 and CD20) expression. All cases showed Ig expression, except for case 2 (both the blood samples). One CLL with multiple IgH and IgL chain gene rearrangements had bytropic expression of IgL chains (case 44, data not shown further). All leukemias had expression of CD5, except for three cases, including cases 2 and 35, which had, respectively, a t(14;18) and t(11;14) (see below). Case 2 also showed expression of FMC-7 (Table 1). During follow-up, case 35 developed prolymphocytic transformation. Leukemic cells were CD5-negative from the onset of disease in this patient.

Hybridization experiments with JH, \( bcl-1a \), and \( bcl-1b \) probes in HindIII, EcoRI, and Sacl digestions of DNA from case 35 showed comigration of rearranged \( bcl-1 \) and JH fragments, indicating a t(11;14) (Fig 1A). In contrast with previously described cases, the rearranged \( bcl-1a \) and \( bcl-1b \) fragments each comigrated with a different rearranged JH fragment (Fig 1A). Both comigrations were seen in the HindIII, BamHI, BglII, and EcoRV digests, which exclude the possibility of coincidence. The third rearranged JH band in the HindIII digest presumably represents the functionally rearranged JH allele. Additionally, two rearranged \( C_m \) bands were present in the BamHI digestion, and one of these bands comigrated with the rearranged \( bcl-1a \) fragment but not with the rearranged \( bcl-1b \) fragment (bands not shown). The other rearranged \( C_m \) fragment presumably represents a functionally rearranged \( C_m \) allele. These data indicate a reciprocal t(11;14) with a breakpoint in between two JH gene sequences without deletion of sequences flanking any of the rearrangements (Fig 1B).

Case 2 showed a rearrangement of the \( bcl-2 \) gene within the mbr. Comigration with a rearranged JH fragment indicated a t(14;18). The only rearranged JH fragment was involved in the t(14;18) translocation, explaining the defect in heavy chain expression44 (Fig 2).

Table 1. Immunogenotype and Immunophenotype of CLL

<table>
<thead>
<tr>
<th>Cases</th>
<th>Ig</th>
<th>CD5*</th>
<th>FMC-7†</th>
<th>JH</th>
<th>Jκ</th>
<th>λκ</th>
<th>bcl-1</th>
<th>bcl-2</th>
<th>mbr 5'</th>
</tr>
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<tbody>
<tr>
<td>2a/b</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2R</td>
<td>R</td>
<td>G</td>
<td>G</td>
<td>R*</td>
<td>G</td>
</tr>
<tr>
<td>16</td>
<td>MDAG/L</td>
<td>+</td>
<td>–</td>
<td>3R</td>
<td>R</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>R</td>
</tr>
<tr>
<td>19</td>
<td>M/K</td>
<td>+</td>
<td>–</td>
<td>2R</td>
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<td>–</td>
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<td>R</td>
<td>ND</td>
<td>R*</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

Abbreviations: a/b, two successive blood samples were available; –, negative; +, positive; R, rearrangement; G, germline; ND, not done; K, κ chain gene expression; λ, λ chain gene expression; A, D, G, M, heavy-chain gene expression.

*Comigration with JH.

†No λ rearrangement was detected although the cells had cytoplasmic expression of λ.

‡Positive for > 80% of the cells and negative for < 25% of the cells.

DISCUSSION

We investigated the incidence of oncogene involvement in CLL to assess whether Southern analysis could assist in discriminating a leukemic phase of different types of B-cell NHL from classical B-CLL. Most loci studied are involved in specific chromosomal translocations in B-cell malignancies.22-23 Some of these translocations (ie, those involving \( bcl-2 \) and \( c-myc \)) probably involve errors in the recombination of the Ig regions in the pre-B cell stage of differentiation. These are thought to be early oncogenic events.35-38 Other translocations and rearrangements may occur later on and have been associated with tumor progression.16,20,21 Deletion of the Rb oncogene has recently been associated with some lymphocytic malignancies22-23 (A. Ginsberg, personal communication, June 1990).

Most of the leukemias lacked rearrangements for the loci mentioned. No \( bcl-3 \) or \( c-myc \) rearrangements and no mutations of the Rb gene were found in any of the studied cases; thus, involvement of these genes in the development of B-CLL must occur rarely, if at all. Another study of 38 patients with CLL showed one patient with a \( c-myc \) rearrangement but no \( bcl-1 \) and \( bcl-2 \) rearrangements.40 A recent cytogenetic analysis of 40 patients with B-CLL showed three patients with a t(14;19) and one patient with
Cloning of the chromosome breakpoint of neoplastic B cells with only a fraction of the breakpoints within the bcl-3 gene and the bcl-1 gene at 14q32. We found 1 of 44 patients with a t(11;14). The breakpoint of our patient's DNA maps to the same region as the breakpoints of the two previously described CLL cases (Fig 1), the bcl-1 major translocation cluster (MTC).

Further investigation of this breakpoint is in progress. Clinically, our (11;14) case showed no CDS expression from the start of disease, was FMC-7 positive, and developed prolymphocytic progression. These data are at variance with the typical CLL phenotype. It should be noted that almost all cases of CLL with the t(11;14) are described as "aggressive" or "prolymphocytic" CLL.

We found 1 of 44 patients with a t(14;18)(q32;q21) in the mbr. The possibility was considered that this represented a leukemic phase of follicular lymphoma rather than a B-CLL, especially in light of the absence of CD5 expression. However, the patient presented with very mild inguinal lymphadenopathy and did not develop further lymphadenopathy or organomegaly (stage I CLL). Furthermore, no typical "notched" or "buttock" cells, characteristic of a leukemic phase of follicular lymphoma, were found.

Strikingly, and in contrast to the previously mentioned cases, two CDS-positive cases had a rearrangement within the 5' end breakpoint region of the bcl-2. Recently, 3 of 32 B-CLL cases with a rearrangement of the first exon of the bcl-2 gene have been reported. These cases might represent a subtype of CLL in which bcl-2 is translocated to IgL chain genes. This translocation may occur during light-chain rearrangement and, therefore, would be fundamentally different from the t(14;18) translocation in follicular lymphomas. The absence of comigration with Ig gene sequences in our two cases does not exclude such a translocation.

In conclusion, both the bcl-1 and bcl-2 loci are frequently involved in chronic B-cell leukemias. Our data on the CD5-positive cases with a rearrangement of bcl-2 at the 5' site corroborate recent suggestions that this breakpoint is regularly but infrequently found in true CLL. In contrast, the association of t(11;14) or t(14;18), with the absence of CD5 expression observed in 3 of 44 cases, may suggest that these leukemias represent a leukemic NHL, rather than true CLL.

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