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, comigration 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 comigration 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 comigration. 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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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. 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 procedures. The DNAs were digested to completion with various restriction enzymes, size fractionated in 0.7% agarose gels, and analyzed by Southern blot technique. The DNAs were hybridized with random primer 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 2.2-kb Sau3A I Ig JH probe and a 1.2-kb EcoRI-EcoRI I Ig CI probe. Rearrangements of Ig genes were studied with a 1.8-kb Sac1-Sac1 J-probe in EcoRI- and HindIII-digested DNA, and a 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-1a (provided by T. Rabbits, MRC, Cambridge, UK) and bcl-1b (provided by Y. Tsudojima, Philadelphia, PA), is presented in Fig 1.

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ONCOGENES IN CHRONIC B-CELL LEUKEMIA

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 Cµ 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-Sacl fragment of bcl-1. The joining between bcl-1 and JH (hatched region) is schematically drawn.

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. Eight cases had three or more rearranged JH bands, indicating oligoclonality, or the occurrence of additional alterations within the IgH genes 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 bitopic 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 SacI digestions of DNA from case 35 showed comigration of rearranged bcl-1 and JH fragments, indicating a t(11;14) (Fig 1). 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, BglIII, 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\(_{\mu}\) 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\(_{\mu}\) fragment presumably represents a functionally rearranged C\(_{\mu}\) 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 expression\(^*\) (Fig 2).

Cases 16 and 19 showed a rearrangement of the bcl-2 gene within the 5' end breakpoint region. Both cases showed a distinct rearranged fragment with the BamHI and HindIII restriction enzymes. No comigration with rearranged Ig gene sequences was found (Table 1).

### 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>Jx</th>
<th>Cl</th>
<th>bcl-1</th>
<th>bcl-2</th>
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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>
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<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>
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<td>M/K</td>
<td>+</td>
<td>-</td>
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<td>R</td>
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<td>R*</td>
<td>G</td>
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</tr>
</tbody>
</table>

Abbreviations: a/b, two successive blood samples were available; -, negative; +, positive; R, rearrangement; G, germline; ND, not done; K, \(\kappa\) chain gene expression; L, \(\lambda\) chain gene expression; A, D, G, M, heavy-chain gene expression.

\*Comigration with JH.

\*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.\(^5\)\(^-\)\(^2\) 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.\(^3\)\(^-\)\(^9\) Other translocations and rearrangements may occur later on and have been associated with tumor progression.\(^1\)\(^0\)\(^-\)\(^2\)\(^1\) Deletion of the \(Rb\) oncogene has recently been associated with some lymphocytic malignancies.\(^2\)\(^2\)\(^-\)\(^2\)\(^3\) (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.\(^4\) A recent cytogenetic analysis of 40 patients with B-CLL showed three patients with a t(14;19) and one patient with...
a (t(11;14)). It is likely that the bcl-3 probe we used detects only a fraction of the breakpoints within the bcl-3 gene at chromosome 19.

Tsujimoto et al.\(^{35}\) described two CLL patients with a t(11;14)(q13;q32). 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).\(^{35}\) However, more recent studies have documented breakpoints outside the MTC.\(^{26,37-44}\) Therefore, the frequency of bcl-1 rearrangements in our series could be underestimated. We note that the t(11;14) found here seems to be reciprocal, without the normally concomitant deletion of JH regions. It will be of interest to learn if extra nucleotides (N-regions), commonly appearing at the recombination site of the t(11;14), are present. Their presence would suggest a mistake in the recombination of Ig genes.\(^{37}\)

Further investigation of this breakpoint is in progress. Clinically, our t(11;14) case showed no CD5 expression from the start of disease, was FMC-7 positive, and developed prolymphocytic progression. These data are at variance with the typical CLL phenotype.\(^{3,42}\) It should be noted that almost all cases of CLL with the t(11;14) are described as “aggressive” or “prolymphocytic” CLL.\(^ {3,41,44}\)

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.\(^{24}\)

Strikingly, and in contrast to the previously mentioned cases, two CD5-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.\(^ {46}\) 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 infrequently 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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