Oncogene Rearrangements in Chronic B-Cell Leukemia


Forty-four B-chronic lymphocytic leukemias (B-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 CDS-negative chronic B-cell leukemias should be considered apart from classical CLL.

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. 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. Similarly, the bcl-2 gene is involved in the t(14;18)(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. Recently a bcl-3 gene has been identified on chromosome 19 by cloning the chromosome translocation breakpoint junction of the t(14;19)(q32;q13.1) in a case of CLL. The c-myc oncogene is involved in the translocations in Burkitt's lymphomas, but has also been associated with tumor progression. Inactivation (deletion) of the retinoblastoma (Rb) tumor suppressor oncogene has also been identified in a variety of human lymphomas and leukaemias (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. 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. 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 techniques. The DNA were digested with 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 SauIIIa 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-1αβ (provided by T. Rabbits, MRC, Cambridge, UK) and bcl-1β (provided by Y. Tsujimoto, Philadelphia, PA), is presented in Fig 1B. Rearrange-
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) (provided by Y. Tsujimoto), a 4.0-kb EcoRI-HindIII fragment containing the minor breakpoint region (pFl-2) (provided by M.L. Cleary, Stanford, CA), and a 1.6-kb EcoRI-EcoRI fragment containing the 5′ end breakpoint region (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-CLL provided by T.W. McKeithan, Chicago, IL). The c-myc gene was studied with a 1.4-kb EcoRI-ClaI fragment containing the third exon 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. Eight cases had three or more rearranged JH bands, indicating oligoclonality, 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 bitypic 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 IgL chain gene expression; A, D, G, M, heavy-chain gene expression.

Hybridization experiments with JH, bcl-la, and bcl-lb 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-la and bcl-lb 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 Cμ bands were present in the BamHI digestion, and one of these bands comigrated with the rearranged bcl-la fragment but not with the rearranged bcl-lb fragment (bands not shown). The other rearranged Cμ fragment presumably represents a functionally rearranged Cμ 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).

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).

### 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. 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. Other translocations and rearrangements may occur later on and have been associated with tumor progression. Deletion of the Rb oncogene has recently been associated with some lymphocytic malignancies (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. A recent cytogenetic analysis of 40 patients with B-CLL showed three patients with a t(14;19) and one patient with no.


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