Cytogenetic and Interphase Cytogenetic Characterization of Atypical Chronic Lymphocytic Leukemia Carrying BCL1 Translocation

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ABSTRACT

Conventional chromosome analysis (CCA) and fluorescent in situ hybridization (FISH) studies, using a 390-kb yeast artificial chromosome probe spanning the area of multiple breakpoints of the BCL1 locus at 11q13, were performed on 57 patients fulfilling the French-American-British criteria for the diagnosis of atypical B-cell chronic lymphocytic leukemia (CLL). To better define the incidence of 13q deletions and trisomy 12, FISH analysis was also performed using a cosmid probe that recognized a DNA sequence between the Rb gene and the D13S25 locus at band 13q14 and a chromosome 12-specific pericentromeric probe. All patients were characterized by cytogenomic and hematological studies. Fourteen displayed three fluorescent signals in 41-98% interphase cells when hybridized to the BCL1 yeast artificial chromosome probe, documenting the presence of BCL1 translocation (BCL1-positive cases). The presence of t(11;14) was ascertainment in 12 cases using CCA and by dual color interphase FISH using the BCL1 probe and a 14q telomere probe in 2 karyotypically normal cases. The remaining 43 cases had two signals in more than 95% interphase cells (BCL1-negative) and did not have the t(11;14) at CCA.

Although 13q14 deletions were seen by means of CCA in only 5 of 14 BCL1-positive cases, hemizygous or homozygous deletions at band 13q14 were detected by FISH in 11 of 14 BCL1-positive cases, as compared with 17 of 43 BCL1-negative cases (P = 0.01). A subclone with trisomy 12 in addition to BCL1 translocation and del(13q14) was present in four BCL1-positive cases.

We arrived at the following conclusions: (a) FISH with this BCL1 YAC probe is an efficient method for the detection of the t(11;14) and of the corresponding involvement of the BCL1 locus in this lymphoproliferative disorder; (b) the majority of BCL1-positive atypical PLLs by French-American-British criteria may carry 13q14 deletions; (c) the recognition of this cytogenetic subset of atypical CLL, sharing some immunological and cytogenetic features with mantle cell lymphoma, may be important, because these patients usually present isolated peripheral blood and marrow lymphocytosis, with or without mild to moderate spleen involvement, and may require early cytotoxic treatment.

INTRODUCTION

The translocation t(11;14)(q13;32) juxtaposes the BCL1 locus and the immunoglobulin heavy chain locus (1), determining the overexpression of the PRAD1/cyclin D1 gene, normally located on chromosome 11, at band q13 (2-5).

The association of the t(11;14)(q13;32) with lymphomas of follicle mantle line was unequivocally documented in a large series (6). Although the presence of the t(11;14) in B-CLL3 was questioned by some investigators, several recent reports indicated that some patients with so-called “atypical CLL” may carry this balanced translocation (7-12).

In this form of leukemia, two factors may limit the detection of the t(11;14) and of its molecular counterpart (the rearrangement of the BCL1 locus): (a) the low mitotic index of PB B-lymphocytes; and (b) the variable location of translocation breakpoints in the BCL1 locus, probably spanning an area of more than 200 kb (13, 14). The use of FISH using probes of adequate size may overcome these limitations, allowing interphase cells to be studied and permitting the detection of breakpoints located within the cDNA region (15).

To better define the cytogenetic features of atypical B-CLL with t(11;14)/BCL1 rearrangement, we studied 57 patients by CCA and by FISH using a YAC, containing a 390-kb insert covering the BCLI breakpoint region on 11q13. Because del(13q) and trisomy 12 represent the most frequent abnormalities in B-CLL and related disorders, all patients were further characterized by FISH, using a cosmid probe derived from chromosome 13q14 and a chromosome 12-specific pericentromeric probe (16).

Results of this combined cytogenetic/FISH approach for the detection of BCL1 translocation are presented and discussed together with hematological features of atypical CLL with BCL1 involvement.

MATERIALS AND METHODS

Patient Selection. Fifty-seven patients with a diagnosis of atypical B-CLL were selected on the basis of the presence of more than 10% large lymphocytes and/or prolymphocytes (see below) among approximately 260 CD5+ B-CLLs, seen at the Institute of Hematology, Ferrara, since 1988.

Diagnoses were made in all cases according to standard clinical, cytological, and immunological criteria (17); BM aspiration or biopsy were performed in all cases. Patients with histologically documented MCL, with de novo PLL, or other B-cell chronic lymphoproliferative disorders were excluded from this analysis. Staging procedures according to Rai’s classification included a physical examination, a routine laboratory profile, a chest X-ray film, and abdomen ultrasonography.

Morphological, Histological, and Immunological Studies. Cytological diagnoses were made in all CD5+ B-CLLs on PB and BM smears. The percentage of small lymphocytes, LLS, and PLs was recorded, and the patients were classified according to the French-American-British criteria (18) as CLL in the presence of less than 10% LLS and PLs, as atypical CLL when LLS and PLs comprised between 10 and 55% of all lymphocytes, and as PLL when more than 55% PLs were present on PB smears.

Cytocentrifuge study of PB cells, gating primarily on lymphocytes, was performed on a FACScan analyzer. Commercially available reagents detecting the following surface markers were used: B-cell line- age: CD10, CD19, CD22, an CD23; T-cell lineage: CD2, CD3, and CD5. Double labeling with monoclonal antibodies detecting the CD19 and CD5 antigens was performed. Expression of the CD11c and CD11b integrin-asso-

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3 The abbreviations used are: B-CLL, B-cell chronic lymphocytic leukemia; PB, peripheral blood; FISH, fluorescence in situ hybridization; CCA, conventional chromosome analysis; YAC, yeast artificial chromosome; BM, bone marrow; MCL, mantle cell lymphoma; PLL, prolymphocytic leukemia; L.L, large lymphocyte; PL, prolymphocyte; slg, surface immunoglobulin; MTC, major transplantation cluster.
At the bottom, a diagram of the YAC214D1 is shown. The translocation cluster, located about 110 kb telomeric to MTC and just upstream of the CCND1 gene. The distance between CCND1, FGF4, and FGF3 is indicated (150 and 35 kb). Breakpoints associated with the t(ll;l4Xql3;q32) in chronic B-cell lymphoma/leukemias. mTC1 minor translocation cluster 1 located about 23 kb telomeric to the MTC; mTC2 minor translocation cluster 2, located about 110 kb telomeric to MTC and just upstream of the CCND1 gene. The distance between CCND1, FGF4, and FGF3 is indicated (150 and 35 kb).

Associated markers as well as reactivity with the monoclonal antibody FMCC7 (Silenius Lab, Hawthorn, Australia) were assessed. The cutoff point for positivity was set at 30% cells showing fluorescence above controls. slgs were detected using rabbit antihuman antibodies against immunoglobulin heavy and light chain. slg expression of the leukemic cells was interpreted as "weak" if the mean intensity of fluorescence was <256 and "bright" if >256 (logarithmic acquisition, 0–1024 channel range).

**Cyto genetic Studies.** Cyto genetic techniques used in our laboratory were described previously (18). The following mitogens were used: lipopolysaccharide (0.1 mg/ml); phytohemagglutinin (0.1 mg/ml); pokeweed mitogen (0.1 mg/ml); and phorbol myristate acetate (50 ng/ml).

Karyotypic analyses were performed within the first two years of diagnosis in all patients; in those patients with no mitoses or normal karyotype, cytogenetic studies were repeated on at least one occasion. Whenever possible, at least 10 mitoses were karyotyped, and chromosome anomalies were described according to the ISCN (19).

**FISH Studies.** Cells for FISH studies were taken from the same samples that were used for the first cyto genetic analysis. In all cases, samples with more than 90% clonal CD5+/CD19+ cells were obtained by separation over a Ficoll gradient. Hybridization of the BCLI YAC probe and of the chromosome 12-pericentromeric probe with normal PB cells (10 control cases) yielded two signals in more than 98% interphase cells, with three signals present in 0.8 and 0.7% interphase cells, respectively (mean value). In these controls, more than 95% normal interphase cells had two signals when hybridized with the 13q14 C21 cosmid, whereas 3.8 and 3.1% interphase cells (mean values) had 0 or 1 signal, respectively. The cutoff point in patient samples for BCLI translocation and trisomy 12 was set at >5% interphase cells with three signals. Deletion 13q14 was identified in the presence of >10% cells with 0 or 1 signal.

**Detection of BCLI Translocation.** The YAC clone (214D11), specific to the MTC at the bcl-1 locus (20) was obtained from a library constructed at the Centre pour l'Etude des Polymorphismes Humains in Paris, France (Fig. 1). The slides were incubated for 60 min with RNase (100 µg/ml; Boehringer Mannheim), washed twice in 2× SSC, dehydrated in ethanol alcohol series (75, 85, and 100%), and air dried. The denaturation was performed by immersion of the slides in a 70% formamide/2× SSC solution at 70°C for 2 min and dehydrated in the same alcohol series. The YAC clone DNA was biotinylated with a BioNick labeling system kit (Life Technologies, Inc.) according to the manufacturer’s instructions and precipitated with human Cot-1 DNA (Life Technologies, Inc.) to suppress nonspecific signals due to repetitive sequences. Two µg of the YAC DNA were resuspended in a hybridization mixture (240 µl total volume) with 50% formamide, 10% dextran Sulfate in 12.5× SSPE, and 5× Denhard’s solution. Sixty µl of the mixture were added to each slide and incubated overnight. Posthybridization washes included 50% formamide/2× SSC, 1× SSC, and 0.1× SSC baths at 45°C each, with intermittent agitation. Detection was performed with alternating layers of fluoresceinated avidin and biotinylated goat anti-avidin (5 ng/µl; Vector Laboratories, Burlingame CA), until two layers of avidin were applied; each treatment was followed by 2× SSC washes 2 min each. A 4′,6-diamidino-2-phenylindole/propidium iodide fluorescent antifade solution was applied onto the slides as counterstain. The evaluation was performed on more than 200 interphase cells on a Leitz Wetzlar fluorescence equipped microscope; images for illustration purposes were captured using a cooled charge-coupled device camera (Princeton Instruments) and IP-Lab Spectrum software (Signal Analytics). To prevent misinterpretation of data deriving from signal screening in interphase analysis, all patients were assessed for the presence of trisomy or monosomy 11, using a chromosome 11-specific pericentromeric probe (Oncor, Gaithersburg, MD). In addition, dual color interphase FISH was performed using the bcl-1 probe and a 14q telomere probe (Oncor) in two karyotypically normal cases with evidence of BCLI involvement.

**Detection of del(13q) and Trisomy 12.** Deletions involving the 13q14 region in interphase cells were studied with the biotin-labeled C21 cosmid. The C21 cosmid clone was isolated by PCR screening, with GCTJ6C05 microsatellite-specific primers, of a cosmid library constructed from CEPH B YAC clone 745E3. The GCTJ6C05 marker is located between Rb and the D13S25 marker and is homozgyously or hemizygozously lost in more than 40% of B-CLL (21, 22). To prevent false-positive results, dual color hybridization was performed by adding a digoxigenin-labeled probe (control probe), recognizing DNA sequences at the telomeres of chromosome 13 (Oncor, Gaithersburg, MD). Signal screening was performed on those slides with high hybridization efficiency, indicated by the presence of more than 80% interphase cells with two 13q telomere signals. Hybridization conditions were the same as described above for the 214D11 YAC probe. Posthybridization washes at lower stringency included 45% formamide/2× SSC, 1× SSC baths at 42°C each, with intermittent agitation. The biotinylated 13q14 probe was detected as described above with fluoresceinated avidin (green), and the control probe was detected with rhodamine-conjugated monoclonal antibodies (red) using a commercially available detection kit (Oncor).

The presence of trisomy 12 was assessed by using a chromosome 12-specific pericentromeric probe (Oncor). Experimental conditions, according to the manufacturer’s instructions, were used as described previously (23).

**RESULTS**

CCA and FISH Studies. Recurrent chromosome changes and the outcome of FISH studies in 57 patients with atypical CLL are summarized in Table 1. BCLI translocation was detected by FISH in 14 cases, displaying three signals (one deriving from the normal BCLI allele plus two deriving from the split allele involved in the translocation) in 41–98% of interphase cells. These 14 patients, here referred to as BCLI+ cases, did not have trisomy or monosomy 11 (>95% interphase cells with two signals with the chromosome 11-specific pericentromeric probe). The remaining 43 patients did not have the
BCLI + ATYPICAL CLL

Table 1 Overview of recurrent cytogenetic/interphase cytogenetic abnormalities in 57 patients with atypical B-CLL

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>CCA</th>
<th>Interphase FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal 13q</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>12b</td>
<td>14 + 12</td>
</tr>
<tr>
<td>del(10)(q)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Abnormal 11q</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Abnormal 6q</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Abnormal 1q</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Abnormal 17p</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>del(7)(q32)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Abnormal 3q</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

* Some patients had multiple abnormalities in the same clone.
+ Including four patients with normal karyotype or no mitoses on occasion of the first chromosome analysis.
, Including two cases with partial trisomy 12q.
, not done.


Table 2 Karyotypes and interphase cytogenetic findings in 14 patients with atypical CLL and BCLI translocation

<table>
<thead>
<tr>
<th>Case</th>
<th>Karyotypes [no. of metaphases]</th>
<th>Abnormal/Total</th>
<th>bcl-1+</th>
<th>13q−</th>
<th>+12</th>
</tr>
</thead>
<tbody>
<tr>
<td>15F</td>
<td>46,XX, t(11;14)(q13;q32) [4]</td>
<td>4/15 (48%) Yes</td>
<td>13/13</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>2BD</td>
<td>46,XY,t(9;22q22), add(9)(q21), t(11;14)(q13;q32), del(13)(q14q21) [9] / 46, idem, del(11)(q23q32) [4]</td>
<td>13/13</td>
<td>13/13</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>3PM</td>
<td>46,XX, del(7)(q22), t(11;14)(q13;q32), del(13)(q14q22) [7] / 46, idem, add(12)(q?) [5]</td>
<td>12/12</td>
<td>12/12</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>4TE</td>
<td>46,XY, del(10)(q24), del(11)(p11), der(14)(q13;q2), del(13)(q14q21) [2] / 47, idem, +12 [10]</td>
<td>12/12</td>
<td>12/12</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>5DT</td>
<td>46,XX, del(10)(q21q22), t(11;14)(q13q22), del(13)(q14q21) [8] / 46, idem, add(1)(p32) [4]</td>
<td>12/12</td>
<td>12/12</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>7NS</td>
<td>46,XX, del(7)(q21), t(11;14)(q13q32), del(13)(q14q21) [10]</td>
<td>10/12</td>
<td>10/12</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>8BR</td>
<td>46-47,XY, del(6)(q23), +7, del(8)(p21), t(11;14)(q13q32)</td>
<td>15/15</td>
<td>15/15</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>9MG</td>
<td>46,XY, t(11;14)(q13q32) [4]</td>
<td>4/12</td>
<td>4/12</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>10FA</td>
<td>46,XY, t(11;14)(q13q32) [4]</td>
<td>4/20</td>
<td>4/20</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>11OG</td>
<td>46,XY, add(10)(p36), +2, add(12)(p21), del(4)(q28), add(5)(p12), del(6)(q23), +7, t(11;14)(q13q32), del(13)(q14q21) [14]</td>
<td>14/14</td>
<td>14/14</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>12GM</td>
<td>46,XY, t(11;14)(q13q32) [13]</td>
<td>13/13</td>
<td>13/13</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>13BL</td>
<td>46,XX [20]</td>
<td>0/20</td>
<td>0/20</td>
<td>13(1%)</td>
<td></td>
</tr>
<tr>
<td>14CT</td>
<td>46,XY [18]</td>
<td>0/18</td>
<td>0/18</td>
<td>13(1%)</td>
<td></td>
</tr>
</tbody>
</table>

* "Yes" indicates the presence of the BCLI translocation, 13q14 deletion, or trisomy 12 in interphase cells. The percentage of interphase nuclei with three signals (BCLI +), with zero of one signal (13q14 deletion), and with three signals (+12) is shown in parentheses (200 cells observed).
+ Presence of two clones with monoallelic and biallelic deletion of 13q14.
, Normal karyotype or no mitosis in one to two previous cytogenetic studies.

FISH detected a subclone with +12 (26—44% interphase cells) in four BCLI + cases with concomitant 13q14 deletion. Additional recurrent karyotype aberrations in 14 BCLI + cases included partial trisomy 3q and structural abnormalities at 6q23, seen in 2 cases each.

Hematological Features. By definition, all patients had more than 10% LLs plus PLs in PB smears. Lymphoid cells usually displayed round nuclei without indentations and clefts in PB smears and BM biopsy sections (Fig. 4). The median percentage of PLs in 14 BCLI + cases was 15% (range, 10—50%), as compared with 3% (range, 0—50%) in 43 BCLI− cases (P = 0.001). Due to a progressive increase of PLs, 3 of 14 BCLI + patients (nos. 4, 5, and 6) developed PLL over a 2—5-year period. Patients nos. 2, 4—6, and 8 were included in two previous studies (9, 11).

Immunological findings in the 57 cases are summarized in Table 3. A CD5+/CD19+, CD10− phenotype was found in all cases. Immunological profiles in 14 BCLI + cases are detailed in Table 4. Median age in 14 BCLI + patients was 74 years (range, 60—85), with a 1:1 male:female ratio. PB lymphocytes at presentation ranged between 14
of the green signal colocalizes with 14q telomere sequences (arrow), documenting that a cytogenetically undetected t(11;14)(q13;q32) has occurred. The isolated pink spots derive from the normal 14q telomere. Right, hemizygous 13q14 deletion by FISH using the C21 cosmid (see text for details) is shown (one green signal) with two normal pink signals deriving in the BCLJ region covered by the YAC probe, one signal deriving from the normal BCLJ allele plus two deriving from the split allele.

and 80 × 10⁹/liter (median, 37.5), with a 65–89% B-lymphocyte BM infiltrate.

The clinical history in these 14 patients was as follows. Ten patients (nos. 1, 5–10, and 12–14) presented with isolated peripheral and BM lymphocytosis (Rai stage 0). Lymph node involvement was not observed throughout the history of the disease in these 10 cases, 8 of whom are alive and well at 1–8 years (median follow-up, 5 years). One patient (no. 5) developed prominent splenomegaly and progressed to Rai stage IV over a 4-year period with death occurring at 5 years. A CLL unrelated death (lung cancer) was recorded 1 year after diagnosis in one patient (no. 10).

Four patients presented with mild splenomegaly (less than 5 cm below costal margin; nos. 3, 4, and 11) or with marked splenomegaly (no. 2; Rai stage II). Progressive spleen enlargement, without lymphadenopathy, developed in these four cases, who progressed to Rai stage III/IV in a 2–3-year period. Three patients are alive; patient no. 2 developed Richter’s syndrome and died at 5 years. All 14 patients required cytotoxic treatment at <1–32 months from diagnosis (median, 16 months).

DISCUSSION

Little information is presently available on the cytogenetic profile of atypical CLL as defined by the French-American-British group (17). This form of CLL accounted for approximately 20% of all CLLs seen at our Institution, a figure comparable with that reported in previous investigations (24, 25). Our data and those published in the literature suggest that trisomy 12 is one of the most frequent chromosome abnormalities in atypical CLL and the 11;14 translocation was described recently, as shown in Table 5. Because FISH is not dependent on the precise location of the breakpoint within a locus, it is becoming an important tool for the detection of lymphoma-specific translocations, such as t(8;14)(q24;q32) and t(11;14)(q13;q32) (27–32).

Although the separation of our cases from leukemic MCL proved to be difficult (6, 17, 33), we felt that the diagnosis of atypical CLL was appropriate in our BCLI+ patients for two reasons: (a) unlike MCL in leukemic phase (9, 34–37), cells in PB smears and BM sections (see Fig. 4 for description) did not display irregularity of nuclear outline, nuclear indentations, and clefts; (b) hematological features at presentation were isolated PB and marrow lymphocytosis, with splenomegaly in a minority of patients. Significant lymph node or visceral involvement, typically observed in MCL (38), did not occur throughout the course of the disease, suggesting that our cases may have a different clinical course with respect to the entity that was originally referred to as “leukemic MCL” by Pombo de Oliveira et al. (37). Clearly, our BCLI+ patients shared with MCL some cytogenetic and immunological features, and interestingly, some authors recently proposed the term “mantle cell leukemia” for similar patients presenting with CD5+ B-cell leukemia with t(11;14) and the clinical picture of lymphocytosis without lymphadenopathy (39). It is reasonable to assume that the transformation of B lymphocytes of the follicle mantle may give rise to a spectrum of lymphoid neoplasias, ranging from the classical form of MCL to a “de novo” leukemic condition with the clinical and morphological features of atypical CLL. This scenario may be reminiscent of the existing relationship between small lymphocytic lymphoma and typical CLL, which represent different clinical manifestations, possibly deriving from the same transformed cell.

BCLI+ atypical CLLs differed on immunological grounds from typical CLLs in that they frequently showed bright slg expression (9 of 14 cases), CD23 negativity (7 of 14 cases), and FMC7 positivity (9 of 12 cases tested). Also, a different immunological profile was found in BCLI+ cases, as compared with BCLI− cases (Table 3). In addition, most BCLI+ cases were CD11c negative, suggesting that this cytogenetic subset of atypical CLL is distinct from those cases of atypical CLL having intermediate morphological features between CLL, PLL, and hairy cell leukemia (40, 41).

In this study, we were able to show that: (a) a fraction (24.5%) of atypical B-CLL may carry the t(11;14) translocation, with the resultant BCLI rearrangement; (b) all cases with the 11q− and the 14q+ chromosomes were associated with BCLI involvement in the area covered by the 214D11 YAC probe; and (c) FISH with this BCLI YAC probe can be considered a reliable study method for the t(11;14) in interphase cells. In addition, FISH may be more sensitive than CCA, because evidence of BCLI translocation in interphase cells was obtained in two patients with normal karyotype and in four additional cases having normal karyotype or no mitoses at the first cytogenetic study.

The variable incidence of t(11;14) that was observed in our study and in previous investigations of atypical CLL (Table 5) may be accounted for by differences in classification criteria adopted at different institutions as well as by technical factors (i.e., use of different B-cell mitogens, use of molecular genetic techniques).
Table 3 Immunological findings in 57 atypical CLLs: BCLI+ and BCLI− cases

<table>
<thead>
<tr>
<th>Antigen</th>
<th>BCLI+</th>
<th>BCLI−</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>CD23</td>
<td>7/14</td>
<td>30/38</td>
<td>0.04</td>
</tr>
<tr>
<td>CD22</td>
<td>14/14</td>
<td>36/39</td>
<td>NS</td>
</tr>
<tr>
<td>Bright slg</td>
<td>9/14</td>
<td>15/42</td>
<td>NS</td>
</tr>
<tr>
<td>FMC7</td>
<td>9/12</td>
<td>13/27</td>
<td>NS</td>
</tr>
<tr>
<td>CD11c</td>
<td>1/11</td>
<td>20/35</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*All cases were CD5/CD19 positive by definition. NS, not significant.

In line with previous findings in MCL and chronic B-cell leukemias with t(11;14) (7, 42), a minority of our BCLI+ cases (5 of 14) had karyotypic evidence of concomitant 13q deletions. However, the actual incidence of genetic loss at band 13q14 was largely underestimated by CCA, because 11 of 14 bcl-1+ cases in this series had hemizygous (8 cases) or homozygous deletion (3 cases) of DNA sequences located between the Rb gene and the D13S25 locus. In two recent studies, approximately 40% of unselected B-CLLs were found to carry DNA loss in this region (21, 22); analysis of more cases will be required to confirm our data showing a 78% incidence of concomitant BCLI translocation and 13q14 deletions in atypical CLL with

Fig. 4. Morphological and histological features in atypical CLL with t(11;14). Upper, two different magnifications showing small lymphocytes and some PLs in PB smears. Lower, a BM nodule is shown, consisting of small lymphocytes with clumped chromatin, intermingled with larger lymphoid cells with less condensed nuclei and visible nucleoli.
4, 6, and 10) also had a subclone with monoallelic deletion, suggesting targeting the 13q14 region. In this respect, it is worth noting that 3 of that genetic damage in these patients occurred through discrete events in our BCLJ — cases.

A 39.5% incidence of 13q14 deletion was detected by FISH evidence of BCLJ translocation by FISH (Table 3), whereas the large percentage of abnormal cells by FISH may be difficult in the absence of automated image analysis, allowing for the assessment of adequate numbers of cells (43). It should also be noted that, when using YAC hybridization conditions for the simultaneous use of the YAC probe and the cosmid probe targeting 11q13 and 13q14, respectively, which would allow for the direct visualization of the primary anomaly in the same patient. Conflicting results were described recently by two single patient. Conflicting results were described recently by two

As in previous studies (23, 46), trisomy 12 involved only a fraction of caseswith 13q14 deletion was detected by FISH in our BCLJ — cases.

Interestingly, all three cases with biallelic deletions at 13q14 (nos. 4, 6, and 10) also had a subclone with monoallelic deletion, suggesting that genetic damage in these patients occurred through discrete events targeting the 13q14 region. In this respect, it is worth noting that 3 of 14 BCLJ + cases (nos. 7, 9, and 10) had only 40—50% cells with del(13q14) (44, 45). A 39.5% incidence of 13q14 deletion was detected by FISH evidence of BCLJ translocation by FISH (Table 3), whereas the large percentage of abnormal cells by FISH may be difficult in the absence of automated image analysis, allowing for the assessment of adequate numbers of cells (43). It should also be noted that, when using YAC probes, there may be a possibility of a breakpoint located close to the cdNA region, which may originate two translocation signals displaying different intensity. This may determine failure of detecting the weaker signal in those nuclei having suboptimal hybridization efficiency. Studies are in progress aimed at defining similar hybridization conditions for the simultaneous use of the YAC probe and the cosmide probe targeting 11q13 and 13q14, respectively, which would allow for the direct visualization of the primary anomaly in the single patient. Conflicting results were described recently by two groups in relation to the possible association of so-called "primary chromosome changes" in the same CLL clone, i.e., +12 and del(13q14) (44, 45).

As in previous studies (23, 46), trisomy 12 involved only a fraction of the neoplastic cells in our cases, suggesting that this anomaly may not be the initiating transformation event. Other recurrent anomalies in our patients included partial trisomy 3q and structural abnormalities at band 6q23, each present in two cases. It is remarkable that these chromosome changes, usually regarded as possible primary anomalies in CLL and related disorders (47), may be present in various combinations in BCLJ + atypical CLL.

In conclusion, our data show that BCLJ + atypical CLL may represent a distinct clinicobiological entity, sharing some features with MCL. The sequential development of genetic changes involving chromosomes 11q13, 13q, and 12, and others may underlie leukemogenesis in this disorder and possibly determine the heterogeneity of disease evolution. The recognition of this disease is dependent on a complete morphological, immunological, and molecular cytogenetic assessment of B-cell chronic lymphoproliferative disorders and appears to be clinically important, because, as a rule, these patients may require early administration of cytotoxic therapy. The precise allocation of this disease in the nosology mature B-cell neoplasias awaits further studies.

**Note Added in Proof**

Five cases with atypical CLL or "mantle cell leukemia," carrying the t(11;14)(q13;q32) were found recently in 179 CLLs by Dascalescu et al. (Br. J. Haematol., 95: 572—573, 1996).

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Cytogenetic and Interphase Cytogenetic Characterization of Atypical Chronic Lymphocytic Leukemia Carrying BCL1 Translocation

Antonio Cuneo, Renato Bigoni, Massimo Negrini, et al.


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