Frequent p53 Gene Involvement in Splenic B-Cell Leukemia/Lymphomas of Possible Marginal Zone Origin

By Luca Baldini, Nicola Stefano Fracchiolla, Lilla Marcella Cro, Dino Trecca, Lorenza Romitti, Elio Polli, Anna Teresa Maiolo, and Antonino Neri

A phenotypic and molecular evaluation was made of 15 patients with mature B-cell leukemia/lymphomas showing exclusive spleen and bone marrow involvement. According to French-American-British criteria, these cases could not be classified as classical B-cell chronic lymphocytic leukemia, hairy cell leukemia and its variant forms, splenic lymphoma with villous lymphocytes, or leukemic phase non-Hodgkin’s lymphoma (NHL; follicular or intermediate type). The immunophenotype pattern (high surface Ig and CD25 expression, and little or no reactivity with CD5, CD23, and CD11c) and cytometric features of these neoplasms suggested an origin in the marginal zone of the spleen. Molecular analysis did not show any involvement of the dominantly acting oncogenes generally associated with lymphoid malignancies (c-myc, bcl-2, bcl-1, Ras), but mutations of the p53 tumor suppressor gene involving exons 5, 6, and 8 were found in 6 cases (6 of 15, 40%). In 4 cases, the p53 alterations consisted of a point mutation leading to amino acid substitution. In the remaining 2 cases, an insertion or deletion resulting in a frame-shift of the protein was observed. In all but 1 of the cases, the wild-type sequence at the mutation site was barely visible, implying the loss of the normal p53 allele in leukemic cells. All of the cases showed a clinical course compatible with that of low-grade NHL, regardless of the p53 loss/mutation. Overall, our data suggest the existence of a form of splenic B-cell leukemia/lymphoma of possible marginal zone origin in which p53 inactivation may play an important pathogenetic role.

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suggest the existence of a form of splenic B-cell leukemia/lymphoma of possible marginal zone origin in which alterations of the p53 tumor suppressor gene may play an important pathogenetic role.

MATERIALS AND METHODS

Patients. Of 350 consecutive cases of mature B-cell leukemia/lymphoma admitted to our clinical institution, 15 patients with splenomegaly and an absence of lymphadenopathy were selected because of our inability to classify them as B-CLL, PLL, HCL, HCL-V, or FL according to FAB criteria. At diagnosis, the suspicion was B-CLL in 6 cases, leukemia NHL in 4 cases, persistent lymphomacytosis in 3 cases, and HCL in 2 cases. Cell morphology was evaluated from peripheral blood and BM smears stained with May-Grunwald Giesma. For the cytochemical demonstration of alkaline phosphatase (ALP) activity, smears or cytospin preparations were fixed in cold paraformaldehyde-methanol solution (1:10 vol/vol) and then stained as previously described. For the immunologic and molecular analyses, mononuclear cell suspensions of greater than 95% viability were prepared by Ficoll-Hypaque gradient centrifugation.

Immunophenotype analysis. Direct or indirect immunofluorescence staining with monoclonal antibodies (MoAbs) and the characterization of surface IgS (SIgs) were performed as previously described. The MoAbs used were 15-fluorescein isothiocyanate conjugate (FITC) and (unclustered) PCA-1 for CD10 and plasma cells, respectively (Coulter Clone; Coulter Corp, Hialeah, FL); anti-Leu-1-FITC, anti-Leu-2-phycocerythrin (PE), and anti-Leu-20-PE for CD5, CD19 and CD23 (Becton Dickinson, Mountain View, CA); anti-IL-2R-FITC and (unclustered) anti-B-cells-2 for CD25 and FMC7 (Techno Genetics, Milan, Italy); IOT5c, IOT16, IOM1, IOM11c, and IOP49 for CD1c, CD11a, CD11b, CD11c, and 04- late antigens (VLA) (Immunotech SA, Marseilles, France); and A043 for a3- VLA (Telios Pharmaceuticals, Inc, San Diego, CA). For indirect immunofluorescence, the second-step reagent was (3901) TEC-F(ab)2 goat antimouse Ig-FITC (Techno Genetics, Milan, Italy). The cells were analyzed using a FACS-Scan Flow Cytometer (Becton Dickinson) with a filter set for FITC-PE two-color fluorescence and a log amplification scale. Dead cells were excluded by means of propidium-iodide staining. The controls for these experiments included irrelevant isotype-matched MoAbs and FITC or PE mouse Ig. Cytoplasmic Ig (CyIg) was studied using direct immunofluorescence on cytoplasmic preparations of peripheral blood as previously described. In all cases, the fraction of leukemic cells in the mononuclear suspensions was greater than 80%, as determined by immunocyto-morphologic analysis.

Cytogenetic analysis. Mononuclear cells were resuspended in RPMI 1640 with 20% fetal calf serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. The cells were cultured for 2 to 9 days in the presence of 100 mg/mL lipopolysaccharides from Escherichia coli (Sigma Chemical Co, Poole, UK) alone, or in combination with 25% vol/vol formalinized Staphylococcus aureus Cowan strain 1 (Calbiochem-Behring Diagnostics, La Jolla, CA). Colcemid (GIBCO-BRL, Uxbridge, UK) 0.05 mg/mL was added 4 to 12 hours before harvest. Karyotypic analyses were performed by means of quinacrine fluorescence binding technique, and the chromosomes were classified according to the International System for Human Cytogenetic Nomenclature. In 3 cases, no metaphases were observed; in case no. 13, clonal abnormalities could be shown even though fewer than 10 metaphases were observed. A clone was defined as two cells having the same structural rearrangement or a gain of the same chromosome or as three cells with the loss of the same chromosome.

DNA extraction and Southern blot analysis. DNA was purified by digestion with proteinase K, extraction with phenol/chloroform and precipitation by ethanol. For the Southern blot analyses, 10 μg of DNA was digested with the appropriate restriction enzyme, electrophoresed in a 0.7% agarose gel, and then denatured, neutralized, and transferred to nylon filters (Amersham International, Amersham, UK). The filters were hybridized to probes labeled by random priming according to the manufacturer’s specifications, washed in 0.5 × standard saline citrate/1% sodium dodecyl sulfate for 1 hour at 60°C, and then autoradiographed using intensifying screens at −80°C.

DNA probes. The rearrangements of the IgH locus were analyzed in EcoRII HindIII digests that were hybridized to the Iγ probe specific for the joining region of the IgH. The arrangement of the c-myc locus was investigated by hybridization of EcoRII HindIII, and BamHI-digested DNA to the human c-myc probe MC413RC, representative of the third exon of the c-myc gene. The bcl-2 locus was analyzed by hybridization of BamHI- and Sac I-digested DNA to the probe specific for the major breakpoint region (MBR), and by the hybridization of BamHI and EcoRII digests to the pFL2 probe, which is specific for the minor cluster region (MCR). Rearrangements of the bcl-1 locus were investigated by hybridization of BamHI and EcoRII digests to the probe specific for the major translocation cluster (MTC) and to probe p94, which detects rearrangements of the bcl-1 locus in centrolecytic lymphomas occurring about 24 kb upstream of the MTC.

Oligonucleotide primers. The polymerase chain reaction (PCR) amplification primers used in this study were purchased from Beckman Analytical (Milan, Italy). The primers derived from the c-myc locus sequence used to analyze the first exon-first intron boundary region of the c-myc gene (nucleotides 2716-3060) have been previously reported. The oligonucleotides used to amplify sequences of H-Ras and K-Ras across codons 12, 13, and 61, N-Ras codon 61, and N-Ras codons 12 and 13 have also been previously described, as have the primers used to amplify exons 5-9 of the p53 gene.

PCR—single-strand conformation polymorphism (SSCP) analysis. SSCP analysis was performed using a modified version of the method originally described by Orita et al. Briefly, PCR was performed using 100 ng of genomic DNA, 5 pmol of each primer, 2.5 mmol/L deoxynucleotide triphosphates (dNTPs), 1 μCi of [α-32P]deoxyctidine triphosphate (dCTP), 10 mmol/L TRIS-HCl (pH 8.8), 50 mmol/L KCl, 1 mmol/L MgCl2, 0.01% gelatin, and 0.5 U Taq polymerase (Boehringer Mannheim, Mannheim, Germany) in a final volume of 10 μL. Thirty cycles of denaturation (94°C), annealing (annealing temperatures being optimized for each pair of primers), and extension (72°C) were performed on an automated heat-block (Perkin Elmer-Cetus, Norwalk, CT). The reaction mixture (4 μL) was diluted 1:25 in 0.1% NaDodSO4-10 mmol/L EDTA and 1:1 mixed with a sequencing stop solution containing 20 mmol/L NaOH. The samples were heated at 95°C for 5 minutes, chilled on ice, and immediately loaded (4 μL) on a 6% acrylamide-TRIS-borate EDTA gel containing 10% glycerol. The gels were run at 8 W for 12 to 15 hours at room temperature, fixed in 10% acetic acid, and air-dried. Autoradiography was performed overnight at −80°C using an intensifying screen.

Direct sequencing of PCR-amplified fragments. PCR was performed using 1 μg of genomic DNA, 20 pmol of each primer, 200 μmol/L dNTPs, 10 mmol/L TRIS-HCl (pH 8.8), 50 mmol/L KCl, 1 mmol/L MgCl2, 0.01% gelatin, and 2.5 U of Taq polymerase in a final volume of 50 μL. The number and conditions of the amplification cycles were the same as described above for each pair of primers. For direct sequencing, one of the two primers used for DNA amplification was adopted. Direct DNA sequencing of the amplified fragment was performed as previously described.

RESULTS

Clinical and hematologic features. Details of the relevant clinical findings in our series of patients are reported
in Table 1. Their mean age at diagnosis was 60.7 years (range, 42 to 85); the male to female ratio was 8:7. In 8 patients, the diagnosis was made during routine blood examinations. In the remaining patients, the presenting symptoms examination only showed spleen enlargement extending caused by the enlarged spleen. In all of the patients, clinical were limited to mild weakness, fatigue, and discomfort

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The lymphoid cell count ranged from 10 to 71 x 10^9/L; the hemoglobin concentration was less than 12 g/dL in 3 cases, and the platelet count was less than 150 x 10^9/L in 6 cases. None of the patients showed serum and/or urine monoclonal component. BM histology showed a nodular pattern of lymphoid infiltration in 7, and a diffuse pattern in 8 cases.

Five patients were treated at diagnosis for the presence of conspicuous splenomegaly and/or high white blood cell count and/or signs of BM failure (3 with chlorambucil and prednisolone and 2 with the cyclophosphamide, epirubicin, oncovicin, prednisone [CEOP] regimen); 6 patients were treated with α-2a interferon after a period of indolent disease ranging from 1 to 3 years; and 4 patients did not receive any treatment. The survival of these patients ranged from 12 to 85 months with a median of 48 months. Only patient no. 8 died from acute peritonitis caused by the free perforation of the colonic diverticula.

Immunocytochemical features. The patients included in the study showed a certain cytomorphologic and immunophenotypic heterogeneity (Table 2). In 5 cases, the morphology was similar to typical B-CLL, although with more abundant cytoplasm. In 6 cases, the neoplastic cells appeared as medium-sized lymphocytes with abundant and pale cytoplasm, an often irregular nucleus, and the frequent presence of a single nucleolus. Four cases showed a certain polymorphism as a result of the simultaneous presence of small-, medium-, and large-sized lymphocytes with a variable degree of nuclear irregularities (see representative cases in Fig 1). ALP reactivity was evaluated in 8 patients. Strong positivity (>50% of leukemic cells) was detected in 5 cases (nos. 1, 3, 5, 6, and 9), whereas, in cases nos. 7, 12, and 13, the percentage of positive cells ranged from 10% to 30%. The B-cell lineage of the neoplastic cells was shown by a constantly high expression of CD19 and Slg, restricted for the light chain (κ/λ = 11/4; see Table 2). Slg were of class IgM in 14 of the 15 cases (associated with SlgD in 3 and with SlgG in 6 cases), and their intensity was moderate or high in all of the patients. The expression of FMC7 and CD11a was constant. None of the patients showed any CD10 reactivity or the presence of CyIg. CD1c was expressed in all of the cases except case no. 8. Reactivity to CD25 was detected in 12 of 15 cases, whereas CD11c, CD23, and CD5 expression was found only in some cases and to varying degrees. The expression of CD49d (α4-VLA) was demonstrable in all of the cases (high in 15 [>80% of cells] and intermediate in 1 [20% to 50% of cells]). α3-VLA antigen was only expressed in case no. 8 (57% of cells).

Cytogenetic findings. Of the 15 samples submitted to cytogenetic analysis, 12 (80%) had an adequate number of metaphase cells. A normal karyotype was found in seven cases. Clonal chromosome abnormalities were found in 5 cases, 3 of whom had single abnormalities, such as an unbalanced translocation involving chromosomes 15 and 17 (case no. 13); a trisomy 12 in case no. 15; and an apparently balanced translocation involving chromosomes 7 and 17 in case no. 10. In the remaining 2 cases, the karyotype was complex because of the presence of multiple structural abnormalities, ie, in case no. 5, 47,XY,+8,(t13;14)(q14;q32),del(15)(q21),i(17q) and in case no. 7, 46,XX,add(5)(p16),i(8q),t(9;21)(q11;q21),t(14;17)(q11;p11),add(20)(q13).

Molecular analysis. Southern blot analysis using the JH

<table>
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<th>Case No.</th>
<th>Morphology</th>
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<th>CD1</th>
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<tr>
<td>1</td>
<td>Sly</td>
<td>MGK+/+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Ily</td>
<td>MDK/++</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Ily</td>
<td>Ml/+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Ply</td>
<td>MK/++</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Sly</td>
<td>MDK/++</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
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<td>Gk/++</td>
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<td>MDI/++</td>
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<td>MGK/++</td>
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</tr>
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</tr>
<tr>
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<td>MK/++</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
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<td>MG/I/+</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Pley</td>
<td>MI/+</td>
<td>-</td>
</tr>
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<tr>
<td>15</td>
<td>Pley</td>
<td>MGK/++</td>
<td>-</td>
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</table>

* Morphology: Sly, small lymphocytes; Ily, intermediate lymphocytes; Pley, pleiomorphic lymphocytes.
† Sig Intensity: +, moderate; ++, strong.
‡ MoAb Reactivity: <20%, +/-, >20% and <50%; +, >50% of peripheral mononuclear cells.
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Fig 1. Cytomorphologic features of splenic marginal zone leukemia/lymphoma are shown. (A and B) Peripheral blood films from cases no. 1 and 6 showing CLL-like small lymphocytes, with slightly more abundant cytoplasm. (C) Peripheral blood films from cases no. 9 show a monotonous pattern characterized by slightly larger cells than in (A) and (B) with the frequent presence of a single evident nucleolus. (D and E) Peripheral blood films from case no. 14 show medium-sized lymphocytes with less condensed chromatine, abundant and pale cytoplasm, and a less evident nucleolus; occasionally, small lymphocytes (E) were observed. (F) Cellular pleomorphism of peripheral blood cells of case no. 4 show small-, medium-, and large-sized cells with nuclear irregularities and variable amount of cytoplasm; larger cells frequently showed an evident nucleolus.

probe to hybridize EcoRI- and HindIII-digested DNA showed a monoclonal pattern of IgH gene rearrangement in all cases (data not shown).

Analysis of c-myc activation was performed by Southern blot, using restriction enzymes cutting outside the locus (eg, EcoRI, BamHI, and HindIII) to detect gene rearrangement and PCR-SSCP to detect point mutations or small insertions/deletions in the region surrounding the first exon-first intron border.15 As previously described,20 under our experimental conditions, the PCR-SSCP method allows a monoallelic mutation to be detected in approximately 5% to 10% of a given cell population. In addition, this method is highly specific, as shown by its good concordance with PCR-direct sequencing analysis reported by us and other investigators.21,25-27 All of the NHL cases in our series showed a germline arrangement of the c-myc locus at Southern blot and a normal migrating pattern at SSCP analysis (data not shown).

Rearrangements of the bcl-2 gene were evaluated by hybridization of BamHI and Sac I digests to the probe specific for the MBR (which detects almost 80% of bcl-2 rearrangements) and by the hybridization of BamHI and EcoRI digests to the pFL2 probe specific for the MCR. No bcl-2 gene rearrangements were found in any of the 15 cases.

Analysis of the bcl-1 locus was performed by hybridization of the probe specific for the MTC region to BamHI and EcoRI digests. Further analyses were performed using the p94 probe, which is specific for a second breakpoint site located approximately 24 kb 5' of the MTC.19 No rearrangements of the bcl-1 locus were observed in any of the 15 cases.

The presence of activated Ras genes (N-, K-, and H-Ras) in our series of patients was investigated by in vitro amplification (PCR) of specific genomic regions across codons 12, 13, and 61 and by SSCP analysis. No alterations in the
Table 3. p53 Gene Mutations in Splenic B-Cell Leukemia/Lymphoma

<table>
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<tr>
<th>Case No.</th>
<th>Exon</th>
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<td>139-140</td>
<td>4-bp duplication</td>
<td>Frameshift</td>
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<td>8</td>
<td>270-277</td>
<td>22-bp deletion</td>
<td>Frameshift</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>193</td>
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<td>Hys → Arg</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>277</td>
<td>TGT → TTT</td>
<td>Cys → Phe</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>205</td>
<td>TAT → TGT</td>
<td>Tyr → Cys</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>176</td>
<td>TGC → TTC</td>
<td>Cys → Phe</td>
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</table>

normal migrating pattern were detected in any of the 15 cases, indicating the absence of point mutations (data not shown).

To investigate the presence of p53 alterations, DNAs were amplified for p53 exons 5 to 9 and analyzed by SSCP assay; mutations were detected in 6 of the 15 cases (40%). As shown in Fig 2, the mutations occurred at exon 5 in cases no. 4 and 14, at exon 6 in cases no. 7 and 13, and at exon 8 in cases no. 5 and 10. To confirm the PCR-SSCP data and determine the nature of the mutations in positive cases, we performed a direct sequencing analysis of the PCR products of the involved exons. The results are shown in Figs 3 and 4 and summarized in Table 3. In 4 cases (no. 7, 10, 13, and 14), p53 mutation was represented by a single nucleotide change with a missense mutation resulting in amino acid substitution. Two A to G transitions and two G to T transversions, not occurring at CpG dinucleotides, were detected. In case no. 4, there was a 4-bp duplication involving the third nucleotide of codon 139 and codon 140. In case no. 5, a deletion of 22 nucleotides involving the sequence between codons 270-277 was observed. In this last case, direct sequencing of only the mutated allele was possible because it was well separated in agarose gel electrophoresis from the normal one (data not shown). The percentage of the neoplastic population in the mononuclear cell suspension was 85% in this case, and this may suggest a p53 heterozygosity of tumor cells. In the remaining cases (no. 4, 7, 10, 13, and 14), p53 mutation in one allele was presumably accompanied by the loss of the other allele, as suggested by the presence of a faint wild-type sequence which may account for the normal cell population (<20%) present in all of the investigated samples. The mutations were located within the coding regions of the p53 gene displaying high homology among different species and have already been reported in different types of tumors.
**DISCUSSION**

Although splenic involvement frequently occurs in various forms of NHL, primary malignant lymphomas of the spleen are only rarely diagnosed. To contribute towards the better definition of these entities, we undertook a phenotypic and molecular investigation of a selected group of patients with mature B-cell leukemia/lymphoma characterized by an exclusive and prominent splenomegaly.

According to FAB criteria, the cases included in the present study could not be classified as CLL, PLL, HCL, SLVL, or FL. In particular, they could be distinguished from classical B-CLL because of the Slg class and intensity, the strong reactivity with FMC7, CD11a and CD1c in all cases, the low CD5 reactivity and the absence of the α3-VLA antigen.

Although some cases could be immunophenotypically defined as PLL (a high expression of Slg and FMC7, with a variable expression of CD5), such a diagnosis was incompatible with their cytomorphologic appearance and clinical course. A diagnosis of HCL could be ruled out in all of the cases because of the absence of the specific cytomorphology and peculiar BM infiltration and the presence of low CD11c reactivity. Furthermore, the diagnosis of SLVL could be excluded in all cases on the basis of the different morphology and the absence of monoclonal component. In particular, scanning electron microscopy analysis performed in 10 CD5-negative cases confirmed the absence of the villous aspect of the neoplastic cells (data not shown).

The negativity of CD10 expression, the absence of the chromosomal translocation t(14;18), as well as the rearrangements of the *bcl-2* gene make it possible to classify this pathologic form among the mature B-cell lymphomas originating from the mantle, marginal, or interfollicular...
dance with their normal counterparts. A number of pathologic entities originating from this area have been included in the recently revised Kiel classification: lymphocytic lymphoma, immunocytoma, intermediate and/or centrocytic lymphoma, monocytoid B-cell lymphoma, and mucosa-associated lymphoid tissue lymphoma. However, it is difficult to classify our series of cases as IDL, either in terms of immunophenotype (little or no reactivity to CD5) or genotype (the absence of bcl-1 rearrangement). Moreover, the low reactivity to CD5 and CD23 and the absence of Cy Ig, serum, and/or urine monoclonal components also make it difficult to classify them among the group of IC.

The immunocytomorphologic and clinicopathologic features of the cases reported here suggest their possible origin from the marginal zone of the spleen. In fact, in most cases, the phenotype of the leukemic cells was similar to that of the lymphocytes normally found in the splenic marginal zone (intermediate-sized cells frequently positive for ALP activity, expressing different classes of Slg [predominantly SlgM] and the CD25 antigen, and showing low reactivity to CD5 and CD23). Interestingly, other investigators reported that, among a series of small lymphocytic lymphomas, a specific immunophenotypic pattern similar to that described in our cases (low expression of CD5, CD11c, and CD23 antigen) was associated with a prevalent splenic involvement. More importantly, Schmid et al have recently reported four cases of low-grade splenic lymphoma characterized by BM infiltration without leukemic diffusion and occasional lymph node involvement with an immunophenotype similar to that found in the majority of cases described here. In those four cases, histologic analysis showed a constant involvement of the marginal zone of the spleen. In addition, no rearrangements of the bcl-1, bcl-2, and c-myc loci were detected. The investigators named this particular form of NHL splenic marginal zone lymphomas (SMZCL), and it is possible to speculate that our cases may represent the leukemic form of SMZCL. The phenotypic heterogeneity observed in some of our cases may reflect the presence of an immunophenotypic spectrum of SMZCL greater than that previously described in four cases. However, in the absence of histology a definitive characterization of these cases as SMZCL could not be made. In our series, histology was available only in 1 patient (case no. 4) who underwent therapeutic splenectomy and showed specific involvement of the splenic marginal zone (data not shown). Similar to the cases reported by Schmid et al, the patients described in the present study showed the clinical course of leukemic low-grade lymphomas. In particular, chemotherapy allowed good control of the clinico-hematologic parameters, even though no complete remissions were achieved. In 6 patients with slow disease progression, treatment with α2a-interferon (3 MU/d) was begun and, in 4 of these cases, a significant decrease in white blood cell count and spleen enlargement was observed.

With regard to the pathogenesis of the B-cell splenic lymphomas described here, the absence of involvement of the c-myc, bcl-2, and Ras oncogenes is not surprising. Our investigated cases did not show any morphologic or phenotypic features similar to the lymphoid malignancies generally associated with alterations of the c-myc or bcl-2 proto-oncogenes. In addition, our results are in agreement with the notion that activating Ras gene mutations represent a rare event in NHL, being frequently associated only with ALL, AIDS-NHL, and MM. An important aspect of our investigation was the absence of bcl-1 locus rearrangements documented by Southern blot analyses with probes detecting the
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majority of breakpoints. However, it remains possible that rearrangements of the PRAD1 gene may occur in these cases, as recently described in some cases of centrocytic lymphomas. Therefore, the absence of the chromosomal translocation t(11;14) in those cases investigated by cytogenetic analysis as well as the absence of bcl-1 rearrangement in all of the cases are important findings for further distinguishing these marginal splenic lymphomas from centrocytic (intermediate-cell, mantle-zone) lymphomas.

Inactivation of the p53 gene represented the only genetic lesion found in our series of splenic B-cell lymphomas. Alterations in the p53 gene have been found to be associated with various types of neoplasia, in a high proportion of cases with BL, and, less frequently, in B-CLL and MM. The appreciable frequency of p53 mutations in our patients (40%) suggests an important role of this tumor suppressor gene in the pathogenesis of these neoplasms. It remains possible that the high prevalence of p53 mutations in our group of patients may be related to the presence of prominent splenic involvement. However, this explanation should be considered unlikely on the basis of the low frequency of p53 mutations observed in a panel of 26 B-cell mature leukemia/lymphomas well-defined by FAB criteria (9 cases of CLL, 8 IDL, 4 IC, 5 SLVL) selected for the presence of prominent splenic involvement. Mutations were found in only 2 cases, at exon 7 in 1 SLVL patient and at exon 8 in 1 IC patient (data not shown).

In the majority of the cases reported here, the mutation in one p53 allele was associated with the probable loss of the other p53 allele, thus suggesting a recessive model of p53 gene inactivation. In our cases, cytogenetic analysis did not show any deletions in the short arm of chromosome 17, a chromosomal abnormality occasionally associated with NHL. However, the various abnormalities in chromosome 17 observed in the mutated cases may suggest the occurrence of small deletions involving the normal p53 allele as a possible consequence of chromatin instability.

As suggested for solid tumors, p53 mutations may also play an important role in the progression of lymphoid malignancies. In fact, inactivation of the p53 gene is more frequently associated with aggressive and advanced clinical forms, e.g., Richter's transformation of B-CLL, with the progression from follicular to diffuse NHL, and with acute/leukemic forms of MM. In our series, the patients with p53 mutations presented a clinical course similar to that of the negative patients, which was generally compatible with low-grade malignancy. Thus, it is possible to speculate that the inactivation of the p53 gene may represent an important molecular pathway in the pathogenesis of these forms, even if additional, and presently unknown genetic lesions may also be involved.

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