Background and Objectives. To improve the definition of the incidence and significance of chromosome lesions occurring in marginal zone B-cell lymphoma (MZBCL).

Design and methods. Fourteen cases of MZBCL diagnosed according to the REAL classification were studied by conventional chromosome analysis (CCA) and by interphase fluorescence in situ hybridization (FISH) using the following probes: 3q27/BCL6, 6q21, 7q31, 9p21/p16, 11q22/ATM, 13q14, 17p13, centromeres of #3, #7, #12. Pertinent clinical data were collected.

Results. Primary disease presentation consisted of histologically documented splenic MZBCL in 9 cases, nodal MZBCL in 3 cases and extra-nodal MZBCL in 2 cases. Four cases showed evolution into a high-grade lymphoma, due to the presence of a predominant large cell or blast cell component. Clonal karyotype anomalies were detected by CCA in 12 cases, 6 of which had a complex karyotype, including all 4 cases with high-grade histology. Interphase FISH confirmed cytogenetic data and revealed several cryptic chromosomal lesions. Overall, total/partial +12 was found in five cases; 13q14 and 17p13 deletion were found in four cases each; +3, 7q31 deletion and a BCL6 split signal were found in three cases; deletions at 6q21 and 11q22.3 in two cases each; +7 and a 9p21 deletion were found in one case each.

Interpretation and Conclusions. i) Besides +3 and 7q-, 13q14 deletion, total/partial +12, BCL6 rearrangement, and deletions at 6q21, 11q22-23, and 17p13.3 are relatively frequent events in MZBCL; ii) unlike in mantle cell lymphoma, 9p21 deletion occurred infrequently in MZBCL; iii) a switch into high grade histology is usually associated with complex chromosome defects, including 6q-, 11q-, +12, and 17p.

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Key words: non-Hodgkin's lymphoma; marginal zone B-cell lymphoma; chromosome deletions; BCL6 rearrangement

Marginal zone B-cell lymphoma (MZBCL) includes a primary extra-nodal form, namely lymphoma of the mucosa-associated lymphoid tissue (MALT), a nodal form, corresponding to monocy-toid B-cell lymphoma and a primary splenic form, with or without peripheral blood lymphocytosis, referred to as splenic marginal zone B-cell lymphoma, presenting overlapping features with splenic lymphoma with vil-lous lymphocytes. These three cliniopathologic forms share similar histologic and immunologic features, they usually run a relatively indolent clinical course, though a switch to high grade lymphomas may occur. Recent evidence was provided that, with the exception of MALT lymphomas carrying the t(11;18)(q21;q21) or the t(1;14) (p21;q32), MZBCL may display a similar pattern of recurrent chromosomal lesions, irrespective of the primary site of involvement. Recurring aberrations at disease presentation include total or partial trisomies of chromosomes 3, 18 and X in approximately 50%, 30% and 20% of the cases, structural aberrations of chromosome 1q21 and 1p34 in 20-30% of the cases, and partial or total deletions of chromosomes 17p and 9p in 10-20% of the cases.

Fluorescence in situ hybridization (FISH) allows detection of cryptic chromosomal lesions, especially deletions, in a substantial fraction of lymphoid neoplasias. For instance, the incidence of cytogenetic anomalies of chromosomes 13q, 17p and 12 in non-Hodgkin's lymphoma (NHL) was previously thought to be relatively low, whereas it turned out to approach 20% in some studies using FISH in interphase nuclei, with the 13q14 deletion reaching a surprisingly high incidence in some histologic subsets. Because limited information is presently available on the cliniopathologic profile of MZBCL, we elected to study 14 patients by conventional chromosome analysis (CCA) and FISH using a large panel of locus-specific probes. Results of this molecular-clinopathologic study are presented and discussed in correlation with the salient clinicopathologic features.
Design and Methods

Patients

Fourteen consecutive patients seen at our Institution over a ten-year period were included in this study. Inclusion criteria were the following: i) successful cytogenetic analysis on representative samples, ii) availability of a cytogenetic pellet for interphase FISH studies and, iii) histologically confirmed diagnosis of MZBCL according to the REAL classification. Immunohistochemical studies were performed in 13 out of 14 cases (case no. 8 could not be studied because no material was available) using commercially available monoclonal antibodies detecting the following antigens: CD5, CD20, CD79a, CD10, BCL1, BCL2, CD103. Those cases with splenic lymphoma with villous lymphocytes lacking histologic diagnosis on a spleen specimen were excluded. The patients included in this report do not represent the whole population of MZBCL seen at our center, because it is our policy not to perform cytogenetic analysis on extra-nodal material to ensure accuracy of histologic diagnosis. Staging procedures included physical examination, a routine laboratory profile, a bone biopsy, a chest X-ray film, abdominal ultrasonography, CT scan and, when indicated, gastroduodenal endoscopy. Peripheral blood (PB) involvement was studied by light microscopy examination of smears stained by the May-Grünwald-Giemsa method and by immunophenotyping using the following panel of commercially available monoclonal antibodies: anti CD2, CD3, CD5, CD19, CD22, CD23, CD10, CD11c, CD25 and FMC7. Double labeling with anti CD5/CD19 was performed and the expression of surface immunoglobulins (sIg) was studied using rabbit antihuman antibodies against the Ig heavy and light chains as previously reported.16

Clinical parameters were surveyed for all cases. Complete remission was defined as the resolution of all pathologic symptoms attributable to the lymphoma for at least 3 months after the completion of chemotherapy.

Conventional chromosome analysis (CCA)

Cytogenetic investigations were performed at diagnosis or before therapy was given, using lymph node samples in 3 cases, spleen samples in 2 cases and PB/BM samples in 8 cases. In one case (no. 1) cytogenetic studies were performed on occasion of a lymph node relapse of gastric MALT lymphoma, treated by gastrectomy plus splenectomy and multiagent chemotherapy (COP regimen, consisting of cyclophosphamide, vincristine and prednisone). Methods for preparation of single cell suspensions from spleen or lymph node samples have been previously described.18 These single cell suspensions and PB/BM samples were separated over a 1,077 density gradient and then cultured for 24-72 hours. The 72-hour cultures were stimulated by the following mitogens: phorbol myristate acetate (50 ng/mL) and lipopolysaccharide from E. coli (100 mg/mL). Whenever possible, 20 metaphases were studied and karyotypes described according to the ISCN (1995).

Interphase cytogenetics

Probe selection. We elected to use DNA probes targeting those chromosome bands found to be deleted and/or rearranged in at least two cases in our series plus a panel of probes recognizing chromosome regions which were previously shown to be involved in a significant fraction of B-cell NHL. Hence, probes recognizing DNA sequences at the following chromosome bands were used: the C21 cosmid, recognizing DNA sequences between the Rb gene and the D13S25 marker at 13q14;14 two lambda EM Bl3 clones, spanning an area of approximately 40 kb within the ATM gene at 11q22.3;19 a 6q21 probe prepared by B. Schlegelberger (Institute for Human Genetics, Kiel, Germany) as previously described;20 a commercially available 7q31 probe (Vysis); commercially available centromeric probes recognizing chromosomes 3, 7 and 12 (Oncor).

The following probes were also used which were made available by F. Birg (Institut de Cancérologie e d’Immunologie de Marseille, INSERM 119, Marseille, France) in the context of the Biomed I program, “E.U. concerted action for cytogenetic diagnosis of hematologic malignancies” (project leader: A. Hagemeijer, Centre for Human Genetic, K.U.L., Leuven, Belgium); a 17p13.3 cosmid recognizing p53 gene sequences isolated by H. Dohner (Ruprecht-Karls-Universitat, Medizinische Klinik und Poliklinik V, Heidelberg Germany); a 3q27 BCL6 probe prepared by Iwona Wlodarska (Centre for Human Genetics, K.U.L., Leuven, Belgium); a cosmid probe located at 9p21 band, recognizing p16 sequences, isolated by Bernt Beverloo (Centre for Human Genetics, Erasmus University, Rotterdam, The Netherlands). Hybridization conditions. FISH was performed using the same samples that had been submitted to cytogenetic analysis. Conditions for slide pre-treatment, probe preparation, hybridization, signal amplification and visualization, image capturing and screening have been described previously.15 Signal screening was performed on at least 200 interphase cells with well-delineated signals. Dual color hybridization using the test probe and an irrelevant control probe was performed to rule out inefficient hybridization, as previously reported.15 All probes were biotinylated, or digoxigenin-labeled and tested in 5 normal control samples: <1% cells showed 3 signals (false trisomy or false BCL6 rearrangement) and <3% cells showed 1 signal (false deletion) with each probe. The cut-off point for the recognition of trisomy or BCL6 rearrangement was set at >3% cells with three signals; >10% cells with 1 signal was a minimum requirement for a case to be classified as affected by deletion. To assess the efficiency of the BCL6 probe, one case with large cell lymphoma and a t(3;14) (q27;q32) was tested, producing three signals (one deriving from the normal BCL6 allele, two from the split allele) in 65% of the interphase cells.

Molecular cytogenetics of MZBCL
Results

Hematologic features and outcome

Clinicopathologic and hematologic features in our patients are outlined in Table 1. All cases had the following immunohistologic profile on representative biopsy samples: CD5+; CD10+; CD79a+; DBA44+; CD20+; BCL1+, BCL2+. Primary disease presentation was as follows: nine patients had splenic MZBCL, three had nodal MZBCL, 2 had a gastric MALT lymphoma. In most patients there was multi-organ involvement at presentation or at disease evolution; these data are detailed in Table 1, along with the outcome of induction therapy. PB involvement was detected in 10 cases and detailed immunologic findings on PB fresh cells in these patients are summarized in Table 2.

A predominant large and/or blast-like cell component suggestive of transformation into a high-grade NHL was observed in 4 patients: one (#14) had a primary gastric presentation with lymph node and spleen involvement, one (#13) suffered from nodal relapse of gastric MALT, one had splenic MZBCL (#6) and one had disseminated nodal and extra-nodal disease (#10).

Thirteen patients are alive after 3-120 months; one patient (#7) had progressive disease despite intensive treatment and died 84 months after diagnosis.

Cytogenetics and FISH

Results are detailed in Table 3. Three patients had a normal karyotype at diagnosis. Six out of twelve patients with clonal karyotypes had a complex karyotype (i.e. more than 5 anomalies), including all 4 cases with a high-grade component. Recurrent chromosomal anomalies in the stemline were: structural anomalies of 7q and 3q anomalies (3 cases), trisomy 3, 6q21 anomalies and a 14q+ chromosome, in 2 cases each. As outlined in Table 3, interphase FISH confirmed karyotypic findings and showed 1-4 anomalies in all cases tested but two (cases #4 and 11): by combining karyotypes and FISH data, total/partial trisomy 12q was found in five cases, 13q14 and 17p13 deletion were found in four cases each, +3, 7q31 deletion and a BCL6 split signal in three cases, deletions at 6q21 and 11q22.3 in two cases each, +7 and a 9p21 deletion were found in one case each.

Discussion

The identification of secondary chromosome changes in specific forms of NHL permitted the identification of chromosome regions containing pathogenetically relevant genes/loci21,22 and allowed stratification of patients for risk assessment.14,23,24

The recently recognized entity of M ZBCL encompasses a spectrum of clinicopathologic manifestations, corresponding to three distinct forms,3 having in common several biological features. It was noteworthy that clinical presentation and disease history in our patients (Table 1) were clearly indicative of a significant degree of overlapping between each entity (extra-nodal, nodal and splenic). Though further study is required to establish definitely that these forms all derive from margin-
al-zone lymphocytes,25 the idea of their identical origin was reinforced by the observation that a similar pattern of chromosomal rearrangements is found in these neoplasias, irrespective of the primary site of involvement.9,26,27

The diagnosis of MZBCL in our series was supported by histologic and immunologic findings. All cases tested were CD5-, CD10-, CD79a+ on biopsy specimen. In 10 cases with a monoclonal population of lymphoid cells in the PB, immunophenotyping showed a profile of reactivity (see Table 2) consistent with splenic lymphoma with circulating villous lymphocytes,28 the leukemic counterpart of SMZBCL.2 However, 4 cases were found with CD5+ cells in the PB despite 3 of them being CD5– at immunohistochemical analysis (patient no. 7 could not be tested). This discrepant finding may be accounted for by the different sensitivity of immunohistochemistry on biopsy samples and immunophenotyping on fresh cells obtained from the PB. The notion that approximately 20% of splenic lymphoma with villous lymphocytes may express the CD5 antigen28 supports this argument.

Using the sensitive interphase FISH technique, we were able to refine the profile of recurrent chromosome lesions marking the development of this lymphoma, showing that a similar pattern of chromosomal rearrangement may be found irrespective of the primary site of disease (spleen, lymph node). The association of trisomy 3 and 7q- with MZBCL, found in previous studies,8,9 was confirmed by our analysis (see Table 3). Recently, attention was drawn to the possible role of 9p21/p16 deletions in B-NHL, especially mantle cell lymphoma30 and, less frequently, in follicle center cell lymphomas,31 in chronic lymphocytic leukemia32 and MZBCL.33 It is noteworthy that two of our three patients with BCL6 involvement had a predominant large cell component and the other featured a relatively aggressive disease. In a previous study, a high frequency of BCL6 rearrangement was noted in primary high-grade gastric lymphomas34 and disseminated disease with a prevailing blast-like component was described in 2/3 BCL6-rearranged cases in another study.33

In this analysis, cryptic chromosome anomalies were seen by FISH that were not recognized by CCA due to mitoses occurring in residual normal lymphocytes (two cases with normal karyotype) or due to the inadequacy of banding resolution (four cases with chromosome anomalies). The most frequent chromosome lesions were total/partial trisomy 12 and 13q14 deletion, found in 5 and 4 cases respectively, including two cases carrying both anomalies. The finding of 13q14 deletion in MZBCL is not surprising, since 13q14 deletion is known to occur at an incidence of approximately 50% in B-CLls, small lymphocytic lymphomas and MCL,35 and at an incidence of 10% in other low-grade and high-grade lymphomas, in which it was predictive of a lower complete remission rate.13 Likewise, the identification of extra chromosome-12 material as a recurrent anomaly in MZBCL is in line with previous studies that this anomaly was associated with evolutive forms of low grade NHL, including B-CLL, MCL and follicle center cell lymphoma.14,36,37 It is noteworthy that 3 out of 4 patients with a large cell component in this series had total/partial +12: this finding is in keeping with a recent study using FISH on paraffin sections of gastric MALT lymphomas, where +12 was associated with histologic switch to aggressive disease.38

Three previously unreported chromosome lesions in

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<tr>
<th>Case</th>
<th>WBC (% lymphs)</th>
<th>CD19</th>
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<th>CD22</th>
<th>CD23</th>
<th>CD11c</th>
<th>CD25</th>
<th>FMC7</th>
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<tr>
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<td>5.6 (42%)</td>
<td>27%</td>
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<td>27%</td>
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<td>20%</td>
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<tr>
<td>2 BG</td>
<td>58.8 (75%)</td>
<td>ND</td>
<td>—</td>
<td>78%</td>
<td>77%</td>
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<td>ND</td>
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<td>3 CS</td>
<td>18.1 (61%)</td>
<td>ND</td>
<td>43%</td>
<td>57%</td>
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<td>4 GAM</td>
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<td>81%</td>
<td>94%</td>
<td>81%</td>
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<td>77%</td>
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<td>6 MV</td>
<td>13.2 (89.9%)</td>
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<td>56%</td>
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<td>7 PF</td>
<td>23.6 (71%)</td>
<td>80%</td>
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<td>9 TA</td>
<td>4.5 (48%)</td>
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*Percentage of positive cells in the lymphocyte gate; — indicates <5% positive cells; (sIg: + low intensity of fluorescence; ++ bright expression).
MZBCL were found in this series which may be associated with evolving disease and/or with high-grade histology: deletions involving 17p13 (four cases) and/or 6q21, 11q22 (two cases each). In all cases a complex karyotype with many chromosomal rearrangements was observed and it is difficult to sort out the relative contribution of each of these anomalies to the observed clinicopathologic features. In this regard the following observations may be pertinent: a) 17p13 deletion involving the p53 gene is a marker of histologic transformation and clinically aggressive disease in several histologic subsets of NHL; therefore a similar role for this anomaly in MZBCL would not be unexpected; b) there is a growing body of evidence linking 11q22-23 deletions involving the ATM locus and B-cell lymphomagenesis; a recent study indicated an association of deletion at the ATM locus, karyotype complexity and severe outcome in indolent as well as in aggressive lymphomas; c) 6q21 deletions occur frequently in other low-grade NHL and they have been associated with a large cell component in small lymphocytic lymphoma.

In conclusion, we used conventional cytogenetics and a large panel of locus-specific probes to characterize MZBCL, showing that a) a similar pattern of chromosome lesions may occur irrespective of primary disease presentation, b) besides +3 and 7q–, 13q14 deletion, total/partial +12, BCL6 rearrangement, and deletions at 6q21, 11q22-23, and 17p13.3 are relatively frequent events in this clinicopathologic entity; c) unlike in MCL, 9p21 deletion occurs unfrequently in MZBCL; d) a switch into high grade histology may be associated with complex chromosomal defects, including BCL6 rearrangement, 6q–, 11q–, +12, and 17p–.

Table 3. Cytogenetic and interphase cytogenetic findings in 14 patients with MZBCL.

| Patient | Sample | Karyotype | Anomalies by FISH *
|---------|--------|-----------|--------------
| 1. B.R. | S      | 46,XY [20] | 7q- (45%); +12 (40%) |
| 2. B. G. | PB     | 46, XY [15] | +3 (78%) |
| 3. C. S. | PB     | 46, XY, del(13)(q22q32) [6/12] | +12 (35%); 13q– (70%) |
| 4. G. A. | PB     | 46, XY, t(11;14)(q13;q22) [13/13] | No lesion |
| 5. D. M. | PB     | 46XX, del(7)(q22q32) / 46, idem, t(11;14)(p11;q12) [12/20] | 7q– (86%) |
| 6. M. V. (*) | S | 46, XX, del(1)(q21q32), +3, +12, +mar [18/20] | +3 (65%); +7 (50%); +12 (55%) |
| 7. F. E. | PB     | 45-46, XY, add(1)(q22), add(3)(q27), -10, add(13)(p13), +12, add(16)(q22), +21 +mar [16/21] | BCL6 split (70%); 11q– (60%); 13q– (80%); 17p– (70%) |
| 8. F. F. | PB     | 47, XY, +3 [2/20] | +3 (20%) |
| 9. T. A. | PB     | 46, XY [20] | 13q– (72%) |
| 10. M. (*UN) | LN | 47-51, XX, del(10)(p11;p12)(p22q22), +del(1)(p11), del(4)(q27), del(6)(q13), add(6)(q21), +12, add(13)(q22), +add(16)(q13), +21 +mar [16/21] | 6q– (70%); 11q– (77%); +12 (50%); 13q– (68%); 17p– (60%) |
| 11. B. V. | LN-PB | 46, XY, del(1)(q22q22) [5/29] | No lesion |
| 13. B. L. (*UN) | LN | 49-51, XX, add (3)(q22), add (3)(q22), +8, +11, add (13)(q21), -6, -10, +12, +13, +18, +mar [11/20] | BCL6 split (75%); 6q– (60%); 11q– (50%); +12 (70%); +13 (60%); +18 (60%); +mar (11/20) |
| 14. Gu.A. (*) UN | LN | 45-48, XX, del(2)(p11)(q31), t(3;14)(q27;27), -4, -8, dup(12)(q13q22), del(17)(p11), add(22)(p12) [12/18] | BCL6 split (75%); 17p– (67%); 6q– (60%); 11q– (50%); +12 (70%); +13 (60%); +18 (60%); +mar (11/20) |

P.B: peripheral blood; B.M: bone marrow; S: spleen; N: node/nodal. * The 10-probe panel (see the Design and Methods section) was tested in all patients: the presence of each anomaly in interphase cells is indicated (% abnormal cells in parentheses); no indication means absence of the anomaly. ° Patient with high-grade histology.
References