3.3. Article 3.

Translocation (11;14)(q13;q32) and preferential involvement of chromosomes 1, 2, 9, 13 and 17 in mantle cell lymphoma. Cancer Genet Cytogenet 111: 92-98, 1998.

ELSEVIER

Translocation (11;14)(q13;q32) and Preferential Involvement of Chromosomes 1, 2, 9, 13, and 17 in Mantle Cell Lymphoma

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ABSTRACT: *We have studied 13 cases of histologically confirmed mantle cell lymphomas (MCL) combining cytological-immunological features with conventional cytogenetics and in situ hybridization (ISH) techniques. Peripheral blood smears and lymph node biopsies expressed the typical mantle zone pattern with a B-cell phenotype. Most of the cases (11 of 13) had lymphomatous cells in the peripheral blood. Chromosome analysis was carried out on lymphoid cells from peripheral blood and/or lymph node biopsies. Phytohemagglutinin (PHA) and phorbol 12-myristate 13 acetate (TPA) were used as mitogens. Biotin-labeled libraries of whole chromosomes implicated in complex karyotypes were used to improve their interpretation. Clonal chromosome abnormalities were found in 10 of 13 patients (77%); 7 of these had a complex abnormality. The most frequent recurrent structural abnormalities were: t(11;14)(q13;q32), involvement of chromosome 1 (der[1], del[1], dup[1]), chromosome 2 (del[2], der[2]), chromosome 9 (der[9], −9), chromosome 13 (add[13], t[13q]), and chromosome 17 (add[17], der[17], t[17q]). The most frequent numerical abnormalities were monosomy 21 and loss of the Y chromosome.*

INTRODUCTION

Mantle cell lymphoma (MCL) is a malignant non-Hodgkin disease of B-cell lineage derived from mature CD5+ virgin B-cells of the follicular mantle zone [1]. This type of lymphoma was described by Gerard-Marchant et al. [2] as centrocytic lymphoma and integrated as an entity in the Kiel classification system [3–5]. Mantle cell lymphoma was recognized by some American groups as an intermediate differentiated lymphocytic lymphoma [6], by the Working Formulation as diffuse small cleaved cell lymphoma [7] and by the BNLI (British National Lymphoma Investigation Group) classification as diffuse lymphocytic lymphoma [8].

Patients usually present themselves with extensive disease and B-cell symptoms, extranodal organ involvement and splenomegaly, which predominantly affect middle-aged to elderly males [9–11]. Clinically, MCL is considered a relatively aggressive lymphoma, with a median overall survival of 2–5 years [1, 12].

In most cases, the tumor is composed exclusively of small- to medium-sized lymphoid cells, usually slightly larger than normal lymphocytes, with more dispersed chromatin, scant cytoplasm, and inconspicuous nucleoli. Generally, the nuclei are irregular or "cleaved"; however, in some cases cells are nearly round, and in others they may be very small and resemble small lymphocytes.

The tumor cells are SIgM+, usually IgD+, $\lambda > \kappa$, B-cell associated antigen positive, CD5+, CD10−/+, CD23−, CD43+ and CD11c− [1].

The chromosomal translocation (11;14)(q13;q32) occurs frequently (40–78%) in patients with MCL [13–18], resulting in a rearrangement of the Ig heavy-chain locus (located in the long arm of chromosome 14), the *BCL-1* gene locus (located in the long arm of chromosome 11) and overexpression of the cyclin D1 (*CCND1/PRAD1*) gene [19–22]. This genetic event has an important role in the pathogenesis of MCL.

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Received June 3, 1998; accepted September 15, 1998.

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0165-4608/99/$–see front matter
PII S0165-4608(98)00228-3

The most common cytogenetic abnormality associated with MCL is t(11;14)(q13;q32), but other variants such as t(11;22) have also been described [23]. Deletion 6q15 and monosomy 13 are the most frequent secondary abnormalities associated with t(11;14) [14].

The aim of the present study was to analyze the cytogenetic findings in a group of patients with MCL, emphasizing the most frequent secondary chromosomal aberrations associated with t(11;14). We have studied 13 MCL patients, combining conventional cytogenetics with in situ hybridization (ISH) techniques. A high incidence of different clonal abnormalities, preferentially complex, with involvement of chromosomes 1, 2, 9, 11, 13, 14, 17, 21, and Y were recorded.

MATERIAL AND METHODS

Patients

Thirteen cases diagnosed as MCL between 1993 and 1997 were studied cytogenetically (Tables 1 and 2). All were diagnosed as diffuse MCL except cases 7, 11, and 13, which were diagnosed as a blastoid variant of MCL. In the present series, we included only patients in whom the diagnosis was histologically confirmed; all of them expressed B-cell markers and were negative for those of T-cells. In all cases, immunophenotyping was performed using the following monoclonal antibodies: CD3, CD5, CD19, CD23, SIg, κ, and λ (Table 3).

The patients included in our study were predominantly males (10 males/3 females). The median age was 63 years, with a range of 43–88 years. The mean hemoglobin was 11.3 g/L, the mean platelet count was 121×10^9/L and the mean WBC count was 22.5×10^9/L. Eight of 13 patients presented adenopathies, 8 splenomegaly, and 3 hepato- and splenomegaly. Ten patients were treated with CHOP and none achieved complete remission. Three of 13 have died (1, 12, and 87 months after being diagnosed) (Table 4).

Cytogenetic Studies

Cytogenetic studies were performed in all patients at diagnosis prior to any treatment. Chromosome analysis was carried out on lymphoid cells from peripheral blood (cases 1, 4–13) and lymph nodes (cases 2 and 3). Cultures were established with 2×10^6 cells per mL in 5 mL of RPMI 1640 supplemented with 17% fetal calf serum, 2% L-glutamine, and 1% penicillin-streptomycin. Phytohemagglutinin (PHA) was used as a mitogen in nine cases and phorbol 12-myristate 13 acetate (TPA) in two (Table 1). Cultures were incubated for 72 hours at 37°C. Colcemid was added at a final concentration of 0.15 μg/mL for the last 2 hours of culture, and cells were fixed in methanol:acetic acid (3:1) after 30 minutes of hypotonic treatment in 0.075 M KCl at 37°C. G-banding was performed after treating the preparations in a slide warmer at 100°C for 1 hour, and they were then stained with Wright solution [24]. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature, ISCN 1995 [25].

In Situ Hybridization (ISH) Techniques

In three patients with a complex karyotype (cases 1, 3, and 7), chromosomal in situ suppression hybridization was performed with biotin-labeled libraries of whole chromo-

Table 1 Cytogenetic abnormalities in 13 mantle cell lymphomas (MCL)

Case[a]	Karyotype	Mitogen	Whole chromosome probes applied	Modified karyotype
1	46,XY,der(2),der(3),t(11;14)(q13;q32)[14]/46,XY[34]	PHA	2,3,11,14,17,20	46,XY,der(2),+3,t(11;14)(q13;q32), add(17)(p12),−20
2	45,X,−Y,del(2)(q11),der(6),der(10)t(10;?)(q26;?)t(11;14) (q11;q32),−19,−22,+mar[21]/46,XY[9]	PHA	—	—
3	44∼46,XY,del(1)(q32),dup(1)(q25q44),add(4)(p16), add(7)(p22),der(9),−10,t(11;14)(q13;q32),der(12), add(17)(p13),−21[10]/45∼46,+dup(1)(q25;q44), del(14)(q24)[10]	PHA	1,4,7,9,12,14	id.,t(4;9)(p16;q12),inv(12)
4	46,XY[52]	PHA	—	—
5	46,XY,del(7)(q32)[8]/46,XY[15]	PHA	—	—
6	46,XY,der(1),der(2),t(11;14)(q13;q32)[5]/46,XY,del(7) (q22),add(11)(p15)[1]/46,XY[23]	PHA	—	—
7	43,X,−Y,der(1),−9,t(11;14)(q13;q32),der(17),t(?;17) (?;p13),+der(18)t(?;18)(?;q23)[11]/46,XY[4]	PHA	1,9,11,13,14,17,18	id.,t(13;17)(q12;p13),dup(13),der(17)
8	46,XY[27]	PHA	—	—
9	46,XY,t(11;14)(q13;q32)[5]/46,XY[43]	PHA	—	—
10	45,XY,add(1)(p36),dup(1)(q25q44),dic(9;14) (p24;p13),add(13)(q?34),der(20)t(9;20)(q12;p13), −21,+mar[3]/46,XY[9]	TPA	—	—
11	46,XX[13]/46,XX,−1,−2,add(3)(q29),del(6)(q12),t(11;14) (q13;q32),add(13)(q?34),+mar1,+mar2[4]	NM	—	—
12	46,XX[22]	NM	—	—
13	46,XX,del(16)(q22)[8]/46,XX[30]	TPA	—	—

Abbreviations: PHA, phytohemagglutinin; TPA, phorbol 12-myristate 13 acetate; NM, no mitogen.

[a]Peripheral blood: cases 1, 4–13; lymph node: cases 2 and 3.

Table 2 Numerical and structural cytogenetic abnormalities in 13 mantle cell lymphomas (MCL)

Monosomies (%)	Trisomies (%)	Structural abnormalities	Breakpoints
−Y (15)	+3 (8)		
−21 (15)	+5 (8)	t(11;14) (54%)	14q32 (61%)
−1 (8)		der(1) (23%)	11q13 (54%)
−2 (8)		dup(1) (15%)	1q25-q44 (15%)
−9 (8)		der(2) (15%)	13q34 (15%)
−10 (8)		add(13) (15%)	1q32 (8%)
−19 (8)		add(17) (15%)	2q11 (8%)
−20 (8)		del(1) (8%)	3q29 (8%)
−22 (8)		del(2) (8%)	4p16 (8%)
		der(3) (8%)	6q121 (8%)
		t(4;9) (8%)	7p22 (8%)
		del(6) (8%)	7q32 (8%)
		der(6) (8%)	9p24 (8%)
		add(7) (8%)	9q12 (8%)
		del(7) (8%)	10q26 (8%)
		der(9) (8%)	11q11 (8%)
		dic(9;14) (8%)	13q12 (8%)
		der(10) (8%)	14q13 (8%)
		t(10;?) (8%)	14q24 (8%)
		inv(12) (8%)	16q22 (8%)
		dup(13) (8%)	17p12 (8%)
		t(13;17) (8%)	17p13 (8%)
		del(14) (8%)	18q23 (8%)
		del(16) (8%)	20p13 (8%)
		der(17) (8%)	
		der(18)t(?;18) (8%)	
		der(20) (8%)	

somes (Cambio, UK). The chromosome probes used were libraries of chromosomes 1–4, 7, 9, 11–14, 17, 18, and 20.

In situ hybridization was performed on cultured peripheral blood or lymph node cells. Slides were pretreated with RNase/2 × SSC (10 ng/mL) for 1 hour at 37°C, Pepsin/HCl (0.1–1 mg/mL) for 5 minutes, and formaldehyde-free acid (1%) in PBS/$MgCl_2$ for 10 minutes at room temperature. After being dehydrated in ethanol series and air dried, slides were denatured in 70% formamide solution at 70°C for 2 minutes. Biotin-labeled whole chromosome probes were denatured for 5–10 minutes at 72°C. Five microliters of the probe solution were added to each slide, which was covered by a coverslip. The preparations were hybridized at 37°C overnight in a humid chamber. Posthy-

Table 3 Immunological markers in 13 mantle cell lymphomas (MCL)

Case	Sample	Kappa	Lambda	CD19/20	IgS	CD5	CD10	CD23
1	PB	+	−	+	+++	+	ND	−
2	LN	ND	ND	+	ND	+	−	−
	PB			+		+		
3	LN	ND	ND	+	ND	+[a]	−	−
4	LN	ND	ND	+	ND	+	−	ND
5	PB	−	++	+	+++	+	ND	−
6	LN	ND	ND	+	+++	+	−	−
	PB	ND	ND	+	+++	+	−	−
7	LN	+	−	+	+++	+	−	−
	PB	+	−	+	+++	+	−	−
8	PB	+	−	+	ND	+	−	−
9	PB	ND	ND	+	ND	+	ND	−
10	PB	−	+	+	ND	+	−	−
11	PB	−	+	+	ND	+	−	−
12	PB	+	−	+	+++	+	−	−
13	PB	+	−	+	+++	+	−	−

Abbreviations: PB, peripheral blood sample; LN, lymph node sample; ND, not done.

[a]Cytospin sample.

Table 4 Analytical and clinical features in 13 mantle cell lymphomas (MCL)

Case	Sex/age	Hb (g/dL)	WBC (10^9/L)	Plat (10^9/L)	AD	H/S	T	RT	Survival (months)
1	M/73	13.7	16.2	88	−	−/+	NDA	NDA	NDA
2	M/77	13	6.4	198	++	−/−	NDA	NDA	NDA
3	M/65	10	6	107	−	+/+	CHOP	PR	87
4	M/53	15.4	13.6	285	++	−/+	CHOP	PR	+32
5	M/62	14	12	NDA	NDA	NDA	NDA	NDA	NDA
6	M/67	10	94	42	+	−/+	CHOP	PR	+24
7	M/63	13	41	45	+	+/+	CHOP	NR	12
8	M/66	12	4.1	50	+	−/+	CHOP	NR	+32
9	M/59	8.3	4.8	55	+	+/+	CHOP	PR	+42
10	M/88	8.2	61	69	−	−/−	CHOP	NR	1
11	F/53	9.3	8.1	92	+	−/−	CHOP	PR	+42
12	F/51	9.3	11	260	−	−/−	CHOP	IT	+2
13	F/43	10.7	15	164	+	−/+	CHOP	PR	+18

Abbreviations: AD, adenopathies; H/S, hepato/splenomegaly; T, treatment; RT, response to treatment; NDA, no data available; PR, partial remission; NR, no remission; IT, in treatment, no valorable response.

bridization washings consisted of three changes of 5 minutes each with 50% formamide solution at 45°C and three changes of 5 minutes each with 0.1 × SSC at 60°C. The biotinylated probes were detected using the immunoperoxidase method [26]. Ten metaphases per probe and case were analyzed with a light microscope.

RESULTS

Mitoses were obtained in all patients. Ten of 13 patients (77%) showed clonal karyotypic abnormalities, and 6 presented a complex karyotype (Table 1). Structural aberrations with no numerical abnormalities were observed in 4 patients, 6 others showing both numerical and structural abnormalities. Structural rearrangements were detected in 10 patients, the most frequent of which was involvement of chromosomes 11 and 14 implicated in t(11;14)(q13;q32). Other chromosomes frequently involved in structural rearrangements were 1, 2, 13, and 17 (Table 2). Structural changes as a sole abnormality were found in 3 patients, 1 of whom showed t(11;14)(q13;q32) and another del(7)(q32). The last one presented with del(16)(q22).

The most frequent numerical abnormalities were −Y (patients 2 and 7) and −21 (patients 3 and 10); however, other numerical changes such as +3, +5, −1, −2, −9, −10, −19, −20, and −22 were detected in low frequency (Table 2).

With regard to the relative frequency of the different cytogenetic abnormalities, chromosome 14 was involved in eight patients, chromosome 11 in seven, chromosome 1 in five, chromosomes 2 and 17 in four and chromosomes 3, 9, and 13 in three patients. The chromosomal breakpoints 11q13 and 14q32 were found in seven and eight patients, respectively (Table 2).

DISCUSSION

In the present study, we describe the cytogenetic findings in 13 patients with MCL. The overall incidence of clonal chromosome abnormalities in our series was 77%. Brito-Babapulle et al. [13] studied six patients affected with MCL, the most frequent aberration being t(11;14) (50%). It is interesting to note the incidence of del(13q) in two patients. In the recent series of Argatoff et al. [15], they studied 31 patients affected with MCL; in 13 the cytogenetic analysis revealed a normal karyotype or no metaphases were obtained, 14 presented t(11;14)(q13;q32) and 4 showed an abnormal karyotype without t(11;14)(q13;q32). Taking into account the cytogenetically analyzed patients, 18 (58.1%) showed cytogenetic abnormalities among whom, 14 (77.8%) had t(11;14). Other aberrations were involvement of chromosome 9 (monosomy 9 in 2 cases and structural abnormalities in 9q in 3) and involvement of chromosome 17 (monosomy 17 in 2 cases and disruption of 17p11 because of balanced translocations with chromosomes 3 and 15 in 2 cases). In the recent series of Decaudin et al. [16] a typical t(11;14)(q13;q32) was found in 8 cases (40%), a t(11;18)(p11;p11) associated with del(11)(q13q14) in 1, and a normal karyotype in 11 of a total of 20 cases studied.

In our series, the chromosomes most frequently involved were, in decreasing order, chromosome 14 in 8 patients, chromosome 11 in 7, chromosome 1 in 5, chromosomes 2 and 17 in 4, and chromosomes 7, 9, and 13 in 3 patients. Among 13 patients, 7 (50%) showed t(11;14)(q13;q32), but among those who showed an abnormal karyotype, the incidence of t(11;14) was 70%. Up to 73% of MCL patients have been shown to harbor detectable *BCL-1* rearrangements when analyzed with multiple breakpoint probes [27]. Most of the breaks have been reported as occurring within the MTC (major translocation cluster) region, although literature data suggest strong variations of positivity rates for MTC rearrangements [28–31]. The *BCL-1* locus at 11q13 has been sporadically reported to also be involved in recurrent translocations to 14q32 in B-cell chronic lymphocytic leukemia (CLL), chronic prolymphocytic leukemia (CPL), hairy cell leukemia (HCL), and multiple myeloma (MM). In MCL, the breakpoints on 11q13 are clustered in the MTC region, whereas rearrangements involving this region are rare in other lymphoproliferative

disorders showing the t(11;14)(q13;q32). However, the presence of *BCL-1* rearrangements in other B-cell lymphoproliferative disorders (CLPD) indicates that these entities share a biological background and that the t(11;14) is indeed the crucial step in tumorigenesis of these CLPD, irrespective of cytomorphologic variations [31].

Johansson et al. [14] reviewed the recurrent secondary chromosomal abnormalities in NHL in relation to primary aberrations and morphology. They found that secondary abnormalities in MCL are nonrandomly distributed throughout the genome and reported that the chromosome band most frequently involved in secondary changes associated with t(11;14) was 6q15; monosomy 13 was the most frequent numerical abnormality associated with t(11;14). In our series, the involvement of chromosome 6q was found in two patients, and chromosome 13 in three patients, but none showed monosomy. We observed involvement of chromosomes 1, 2, 9, 13, and 17 as the most frequent secondary aberrations.

Loss of the Y chromosome was observed in two patients. This cytogenetic abnormality has also been found in healthy elderly males [32]. If it is associated with other cytogenetic abnormalities, the coexistence with normal metaphases in the same patient suggests that this aberration is not age-related.

Regarding chromosome 7 in MCL karyotypes, we found changes in two patients: one with add(7)(p22) (case 3), and another with del(7)(q32) (case 5) as a sole abnormality. The finding of 7q deletion as a sole abnormality is particularly interesting. Deletion (7)(q32) is seen in several categories of NHL; the majority are low-grade lymphomas with circulating lymphoid cells showing plasmocytoid features. Hernández et al. [33] reviewed del(7q) in chronic B-cell lymphoid disorders and found that del(7q) is associated with a subset of mature small B-cell lymphoproliferative disorders of which some, but not all, showed lymphoplasmocytic features. In the majority of NHLs there are multiple karyotypic abnormalities other than del(7q). We previously described del(7)(q32) as a new chromosomal anomaly associated with mature B-cell chronic lymphoproliferative disorders [34]. In our series of 19 splenic marginal zone B-cell lymphoma (SMZBCL), 1 patient showed a del(7)(q32) as a sole abnormality [35].

Involvement of 17p was observed in three patients (17p12 in case 1, 17p13 in cases 3 and 7); two of them have died (12 and 87 months of survival, respectively). The *Tp53* gene is located in 17p region, and *Tp53* mutations are related to a poor prognosis [36, 37].

In our study, the blastoid variant of MCL was diagnosed in three patients (cases 7, 11, and 13) two of whom showed a complex diploid karyotype [38]. Ott et al. [31] described an interesting finding about the tendency of blastoid MCL subtypes to harbor chromosome metaphases in the tetraploid range. Hernández et al. [36] and Zoldan et al. [37] observed a poor prognosis related to *TP53* gene mutations and its protein overexpression in this variant.

Interestingly, cytogenetic studies performed on lymph nodes yield more complex karyotypes than those carried out on peripheral blood. It would be of interest to compare cytogenetic findings obtained from lymph nodes and peripheral blood. Cytogenetic methods are especially hampered by mature-looking cells with a low mitotic index, poor quality of chromosomes, or absence of mitoses. For this reason, there are only a few series that include a relatively large number of MCL patients studied cytogenetically. Conventional cytogenetic techniques performed preferentially on infiltrated tissues will probably yield more satisfactory results.

Monteil et al. [39] proposed a molecular diagnosis of t(11;14) in MCL using two-color interphase fluorescence in situ hybridization. Recently, Takashima et al. [40] described an in situ hybridization method to detect t(11;14) in interphase nuclei using a 14q32.33 probe. With these methods, it should be possible to detect a higher incidence of translocations than with conventional cytogenetic methods that are dependent on culture conditions. However, molecular techniques inform of the presence/absence of selected rearrangements. Secondary aberrations detected by conventional cytogenetics could be interesting to correlate with lymphoma progression. For this reason, the combination of conventional cytogenetics with molecular studies is the most useful strategy for studying the genetic changes in patients with lymphoma.

Complex karyotypes deserve some comments. In these cases, the chromosomal in situ suppression hybridization of DNA, the recently described multicolor spectral karyotyping [41] and comparative genomic hybridization [42] methods permit a definitive karyotype to be defined correctly. In MCL patients, especially in those with complex karyotypes, these methods are highly recommended.

In conclusion, t(11;14)(q13;q32) is the most frequent cytogenetic abnormality associated with MCL. The involvement of chromosomes 1, 2, 9, 13, and 17 in secondary changes needs to be confirmed in larger series.

Supported in part by the grant FI-2.103 from Researchers' Formation from the "Generalitat de Catalunya" and by the grant FIS 97/0655 from the "Ministerio de Sanidad y Consumo." This report is enclosed in the "Schering España 1996" award of the "Asociación Española de Hematología y Hemoterapia."

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3.4. Article 4.

Frequent involvement of chromosomes 1, 3, 7 and 8 in splenic marginal zone B-cell lymphoma. Br J Haematol 98: 446-449, 1997.

British Journal of Haematology, 1997, 98, 445–449

SHORT REPORT

Frequent involvement of chromosomes 1, 3, 7 and 8 in splenic marginal zone B-cell lymphoma

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Received 7 April 1997; accepted for publication 29 April 1997

Summary. We have studied 19 cases of splenic marginal zone B-cell lymphoma (SMZBCL) combining cytological features, conventional cytogenetics, and in situ hybridization (ISH) techniques.

A clonal chromosome abnormality was found in 11/19 patients (58%). The more frequent recurrent abnormalities were: del(3), del (7q), and involvement of chromosomes 1, 3, 7 and 8. No patient showed the translocation t(11;14)(q13;q32). An outstanding finding was the low incidence of trisomy 3 (36%) compared to patients with MALT lymphoma. These findings support the interpretation that SMZBCL is a distinct lymphoproliferative disorder.

Keywords: cytogenetics, in situ hybridization, splenic marginal zone B-cell lymphoma.

Splenic marginal zone lymphoma is a recently recognized entity of which the clinical, morphological and immunophenotypic characteristics are well established (Harris *et al*, 1994). Nevertheless, uncertainty about the genetic features still exists.

Splenic marginal zone B-cell lymphoma (SMZBCL) has peculiar characteristics and a well-known histologic picture. It is believed that SMZBCL has some overlapping features with splenic lymphomas with circulating villous lymphocytes (SLVL). SMZBCL may express circulating monocytoid B cells, with or without microvilli, and a variable amount of lymphocytes which closely resemble those of SLVL. Bone marrow involvement in the absence of significant lymphadenopathy is also commonly seen at presentation (Melo *et al*, 1987).

Although SMZBCL and SLVL appear to have clinical, immunophenotypic and histological features distinct from other B-cell malignancies, chromosome analysis has been reported previously in only few series (Oscier *et al*, 1993; Dierlamm *et al*, 1996a, b). The series of Oscier *et al* (1993) suggested a relation with mantle cell lymphomas due to the high incidence of t(11;14), and the series of Dierlamm *et al* (1996b) found a high incidence of trisomy 3, similar to that reported in MALT lymphomas. As these cytogenetic findings showed contradictory results, we consider it of interest to report the cytogenetic results in a series of 19 patients with a strict diagnosis based on histomorphologic and immunological studies of spleen and peripheral blood.

We have studied 19 SMZBCL patients, combining conventional cytogenetics with the *in situ* hybridization (ISH) technique. We describe the cytogenetic findings in a relatively large series of patients with SMZBCL.

PATIENTS AND METHODS

Patients. 19 patients with a diagnosis of SMZBCL were studied (Table 1). Only patients in whom the diagnosis had been histologically confirmed were included; all of them were T-cell markers negative and expressed B-cell markers. The patients were included according to the criteria of Matutes *et al* (1995). The diagnosis of SMZBCL was made in all cases after study of the splenectomy specimens. No t(14;18) or cyclin-D1 overexpression was detectable in any of the tissue from splenectomy specimens. In all patients peripheral blood involvement was observed. In 63% of the patients, circulating villous lymphocytes were observed.

Cytogenetic studies. The cytogenetic studies were performed in all patients at diagnosis prior to any treatment. Chromosome analysis was carried out on lymphoid cells from peripheral blood and lymph nodes (one patient). The

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Table I. Cytogenetic findings in 19 patients with splenic marginal zone B-cell lymphoma (SMZBCL).

Pt	Karotype	ISH (centromeric probes 3 and 12)
1	46,XX [20]	Normal
2	48,XX,der(1),t(8;19)(?;q13),+mar(3),+mar [12]/46,XX [8]	+3
3	46,XX [20]	Normal
4	46,XY,ins(3;?)(p23;?) [20]	Normal
5	46,XX,del(1)(q32) [7]/46,XX [13]	Normal
6	46,XX,del(7)(q32) [20]	ND
7	46,XX [20]	Normal
8	46,XX [20]	ND
9	46,XX* [4]	ND
10	46,XX [20]	ND
11	46,XX [20]	Normal
12†	85–90,XXY,1q–,t(1;2),3p–,der(4),5p–,6q–,9p–,dup(10q),der(14q),der(17q),der(20q) [20]	ND
13	44,XY,t(1;3)(q2;q2),t(7;17)(p1;p1),8q–,–20,–21 [20]	ND
14	46,XX [20]/46,XX,t(1;15)(p11;q11),del(8)(q12),del(18q),del(14q) [22]	ND
15	46,XX [20]	ND
16	48,XX,+del(3)(p23),der(4)t(1;4)(q32;q35),+der(4)t(1;4)(q32;q35),add(14)(q32) [18]/46,XX [14]	+3
17	46,XY,del(7)(q22),add(8)(q24),del(13)(q14) [22]/46,XY [17]	Normal
18	46,XY,del(3)(q25),add(6)(q27),–7,del(13)(q14),del(14)(q11),–20,+mar(3),+mar [12]/46,XY [8]	+3
19	48,XY,del(3)(p23),add(8)(q24),add(13)(p11),add(15)(p11),+18,add(21)(p11),add(22)(p11),+mar(3) [18]/46,XY [12]	+3

mar(3): Marker with material from chromosome 3 (found by the painting method). ISH: *in situ* hybridization. ND: not done.
* Only four metaphases analysed.
† Cytogenetic analysis performed from lymph node.

following mitogens were used: phytohaemagglutinin (PHA) and phorbol-myristate-acetate (TPA). The cultures were incubated for 72 h at 37°C.

G-banding was performed after heating the preparations in a microwave oven for 5 min, they were then stained with Wright's solution. A minimum of 25 metaphases were analysed. Karyotypes were described according the International System for Human Cytogenetic Nomenclature (ISCN).

In situ hybridization. ISH was performed in 11 patients with a biotin-labelled chromosome-12-specific alpha-satellite DNA probe pSP12-1, containing a 340 bp EcoRI fragment and the centromeric probe of chromosome 3 (Oncor). Hybridization was performed by our method described elsewhere (Pérez-Losada *et al.* 1991). A minimum of 200 nuclei were analysed. The percentage of positive cells considered by us to represent a true abnormality was > 5%.

In all the patients we performed the chromosomal *in situ* suppression hybridization of DNA from chromosomes 1, 3, 6, 7, 8, 11, 12, 13, 14 and 18 (Cambio). We have used these probes in order to unmask small translocations or rearrangements. In patients with complex karyotypes we also used probes from the chromosomes involved.

RESULTS

Cytogenetic abnormalities

Mitoses were obtained in all patients. Out of 19 patients, 11 (58%) showed clonal karyotypic abnormalities (Table I) which were detected with both mitogens (PHA and TPA). No patient showed non-clonal cytogenetic abnormalities.

Our cytogenetic results are summarized in Tables I and II. The chromosomal breakpoints found in at least two patients were: 1q32, 3p23, 8q24 and 13q14. Gain of material of chromosome 3 was observed in 4/11 patients (36·4%). In three patients a gain of chromosome 3 was also detected by the ISH method. Eight out of 19 patients had a very complex karyotype.

DISCUSSION

The present study deals with the cytogenetic findings in a group of histologically proven SMZBCL.

The overall incidence of clonal chromosome abnormalities in our series of SMZBCL was 58%. In the series of Oscier *et al* (1993) cytogenetic abnormalities were found in 27/31 patients with SLVL (87%), and Dierlamm *et al* (1996b), in a series of 31 SMZBCL, reported 23 patients (74%) with cytogenetic abnormalities. The different incidence of chromosomal abnormalities could be due to the small size of the series.

The chromosomes most frequently involved were 1, 3, 7, 8, 13, 14 and 20; the finding of deletions 1q32, 3p23 and of 7q (7q22 and 7q32) is particularly interesting. Surprisingly, our results regarding the presence of translocation t(11;14) are not in accordance with those of Oscier *et al* (1993). We were not able to detect this translocation in any of our 19

Table II. Frequency of chromosomes implicated in cytogenetic abnormalities in 11 patients with splenic marginal zone B-cell lymphoma (SMZBCL).

Chromosome involved	Frequency
1	6/11
2	1/11
3	7/11
4	2/11
5	1/11
6	2/11
7	4/11
8	5/11
9	1/11
10	1/11
11	0
12	0
13	3/11
14	3/11
15	2/11
16	0
17	2/11
18	2/11
19	3/11
20	3/11
21	2/11
22	1/11
X	0
Y	0

patients, whereas Oscier et al (1993) found it in 5/31 cases. No t(11;14) or cyclin D1 over-expression was detectable in any of the tissue from splenectomy specimens. This discrepancy is difficult to explain, although the same inclusion criteria were used. Additional findings in the series of Oscier et al (1993) were deletions and translocations involving 7q, and 7q and 2p11.

In the series of Dierlamm et al (1996b) no patient showed either translocation t(11;14) or rearrangements of the bcl-1, bcl-2, bcl-3, bcl-6 and c-myc genes. The most frequent clonal abnormalities of this series included whole or partial trisomy 3 (18 patients), trisomy 18 (nine patients) and structural rearrangements of chromosome 1 (13 patients) with breakpoints in 1q21 or 1p34. We found an incidence of trisomy 3 in 4/11 patients (36·4%). In the recent series of Dierlamm et al (1996a) an incidence of trisomy 3 of 55% (6/11) was found in splenic marginal zone B-cell lymphoma (SMZBCL), of 67% (8/12) in extranodal MZBCL and 62% (8/13) in nodal MZBCL. Oscier et al (1993) found only one patient among 27 with a derivative chromosome 3. In 70 MALT lymphomas Wotherspoon et al (1995) found an incidence of trisomy 3 in 60% of the patients. The different frequency of trisomy 3 among the series reported might be due by the small number of evaluated cases (11/19) with ISH. Recently, Brynes et al (1996), in a retrospective study of 36 cases of marginal zone B-cell lymphomas (MZBCL) studied by fluorescence in situ hybridization, identified trisomy 3 in 11 (85%) extranodal MZBCL with monocytoid B cells, in six (50%) of 12 nodal MZBCL of monocytoid B-cell type, and in only two (18%) SMZBCL. In our experience, one out 11 patients with mantle cell lymphoma had trisomy 3 and no patient with follicular lymphoma (10 patients) had trisomy 3 (Solé et al, 1996). This frequency is also lower than that observed in MALT lymphomas.

In our series, and in others (Oscier et al, 1993; Offit et al, 1995; Dierlamm et al, 1996b), the involvement of chromosome 7 is frequent, most commonly in the form of deletions. In myeloid leukaemias and myelodysplastic syndromes the cytogenetic and molecular mapping of the deleted regions have been localized in band 7q22. Deletion del(7)(q32) is found in some low-grade lymphomas with circulating lymphoid cells showing plasmacytoid features. Frequently they share multiple karyotypic abnormalities other than del(7q). This finding suggests a secondary, progression-related role for a tumour-suppressor gene at 7q32. In our series a patient showed a deletion del(7)(q32) as a sole abnormality, suggesting a pathogenetic role.

In conclusion involvement of chromosomes 1, 3, 7 (the chromosome usually deleted in 7q) and 8 are the most frequent findings in SMZBCL. Splenic marginal zone lymphomas have distinct cytogenetic abnormalities from other lymphoproliferative disorders.

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3.5. Article 5.

Isochromosome +i(3)(q10) in a new case of persistent policlonal B-cell lymphocytosis (PPBL). Eur J Haematol 64: 344-346, 2000.

Eur J Haematol 2000: 64: 344–346
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EUROPEAN
JOURNAL OF HAEMATOLOGY
ISSN 0902-4441

Letter to the Editor

Isochromosome +i(3)(q10) in a new case of persistent polyclonal B-cell lymphocytosis (PPBL)

To the Editor:

Persistent polyclonal B-cell lymphocytosis (PPBL) with binucleated or bilobulated lymphocytes is a disorder first described by Gordon *et al.* (1982) in three patients. As far as we know, nearly 60 cases of PPBL have been reported since then (1–12). This entity usually affects young or middle-aged women who smoke heavily and are generally asymptomatic. Patients present moderate but sustained absolute lymphocytosis in the range $(5–15) \times 10^9$/L, with small numbers of circulating bilobulated forms detected in peripheral blood smears. Most patients show a polyclonal increase of serum IgM, with low to normal levels of IgA and IgG. Phenotypically, binucleated cells express both kappa and lambda light chains. Interestingly, most of the patients reported display the HLA-DR7 antigens on their lymphocytes, suggesting a genetic predisposition of the disease (2, 7, 10, 12). Cytogenetically, the presence of an additional isochromosome i(3)(q10) has been reported (3, 9, 11, 12).

A 44-yr-old white female was referred to our institution for investigation of a persistent lymphocytosis present for more than 10 yr. The patient was a heavy cigarette smoker (more than 40 cigarettes a day) and presented hypercholesterolaemia. On physical examination, neither adenopathies nor hepatosplenomegaly were detected. Laboratory evaluation revealed: haemoglobin of 11 g/dL and platelet count of 219×10^9/L. Her leukocyte count was 10.3×10^9/L with 47% segmented neutrophils, 1% band forms, 48% lymphocytes, 1% monocytes, 2% eosinophils and 1% basophils. Peripheral blood smear evaluation of the lymphoid cells showed 50% mature lymphocytes, 40% large lymphocytes and 10% bilobulated lymphocytes. Blood chemistry was normal. Total serum protein was 6.4 g/dL and serum IgM was increased, reaching 794 mg/dL but without peak, while serum IgG and IgA were normal to slighly decreased (595 and 86 mg/dL, respectively). The patient's serum was studied for the presence of antibodies against hepatitis B virus, hepatitis C virus and human immunodeficiency virus, all of them being negative. Low titers of antibodies against Epstein–Barr virus (EBV) and cytomegalovirus (CMV) were found. HLA-DR typing revealed expression of DR3, DR7, DRw52 and DRw53.

Regarding the morphology of the peripheral blood lymphocytes, nearly half of them had a normal appearance; 40% showed an enlarged size, presence of nucleoli and a blue-stained cytoplasm. A minority of nuclei were deeply indented and eventually split into two fragments. Binucleated or bilobulated nuclei connected or not by a slender internuclear bridge were recorded in approximately 10% of the lymphocytes. The most striking ultrastructural findings were nuclear pockets, found in 6 out of 25 cross-sections of mainly bilobulated lymphocytes and an abundance of multivesicular bodies. Bundles of fibrils could also be observed.

Bone marrow aspirate was moderately hypercellular; the myeloid series were qualitatively and quantitatively normal. Lymphocytic series represented only 13% of the total cellularity, and no bilobulated forms were seen.

Immunology studies were performed by the alkaline phosphatase antialkaline phosphatase (APAAP) method on peripheral blood films. The atypical lymphocytes found in peripheral blood were $CD19^+$, $HLA\text{-}DR^+$, $CD25^+$, IgM^+, $CD11c^{+/-}$, $CD3^-$, $CD5^-$, $CD11b^-$ and $CD23^-$. These lymphocytes were clearly of the B-cell type, as they expressed reaction with the CD19 antigen, and both kappa and lambda light-chains were expressed, indicating a polyclonal expansion of the B-lymphocyte pool (Fig. 1). Chromosome studies were carried out on lymphoid cells from peripheral blood according to standard methods. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature, 1995 (13). The patient demonstrated the presence of different clones (Fig. 2, Table 1).

FISH studies, using α-satellite centromeric probe from chromosome 3 (CEP 3, Vysis, Downers Grove, USA), demonstrated the presence of three

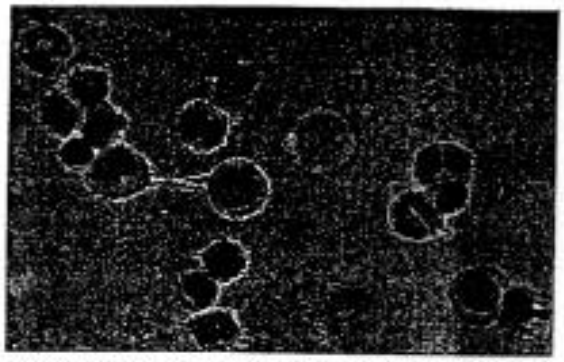

Fig. 1. Bilobulated and stimulated atypical lymphocytes from a PPBL patient showing intense IgM positivity. Cells were immunolabelled with IgM, APAAP stained and giemsa counterstained (× 1000).

Fig. 2. Composite of partial karyotypes showing chromosome 3 abnormalities (+i(3)(q10), trisomy 3, der(3) and der(3)t(3;9)) in a PPBL patient.

spots, consistent with three centromeres of chromosome 3. One thousand interphase nuclei were scored from both PHA and TPA samples, and 11% and 10.9% of interphase nuclei, respectively, showed three hybridization signals. PCR analysis for bcl-2/IgH rearrangements was performed from peripheral blood mononuclear cells' DNA and revealed multiple bands within MBR and MCR regions, indicating the presence of multiple rearrangements.

In PPBL, two types of "atypical" lymphocytes can be detected in peripheral blood: atypical lymphocytes with enlarged size resembling those found in some viral infections such as EBV mononucleosis, and atypical bilobulated lymphocytes with nuclei that can appear deeply indented or bilobed. Both lobes can be connected or not by a fine interlobular nuclear chromatinic bridge. Bilobulation of lymphocytes can also be observed in some malignant lymphoproliferative disorders, especially chronic prolymphocytic leukemias. At an ultrastructural level, the presence of nuclear pockets is the most relevant feature. Nuclear pockets are mainly found in blood cells of leukemic or preleukemic stages, and an association between nuclear blebs and aneuploidy has been demonstrated (14). The main message of this study from a morphological point of view is that this entity can easily be overlooked. Therefore a careful morphological observation of Giemsa-stained peripheral blood films is strongly recommended.

Cytogenetic studies have been documented in 37/60 cases (2, 3, 5–9, 11, 12), and in 22 patients the presence of an additional isochromosome i(3)(q10) has been reported. The present case was studied using two different mitogens (PHA and TPA), and chromosome abnormalities were found with both of them. In the PHA culture we found +i(3)(q10), as it has been described previously. However, in the TPA culture, additional chromosome abnormalities involving chromosome 3, such as trisomy 3 and der(3), were found. FISH analyses using a centromeric probe for chromosome 3 enabled the detection of three copies of the centromeric region of this chromosome, but we could not distinguish between trisomy 3 and +i(3)(q10). We observed the same percentage of trisomic cells in both cultures, indicating that the pathologic clone is stimulated equally with PHA and TPA. As Callet-Bauchu *et al.* (9) reported using the FICTION (Fluorescence Immunophenotyping and Interphase Cytogenetics as a Tool for Investigation of Neoplasms) method (15), we observed that the cytogenetic abnormality

Table 1. Cytogenetic and FISH studies in one patient with PPBL

Mitogen	Karyotype	FISH experiments with 3 centromeric probe (% of trisomic cells)*
PHA	46,XX [194]	
	47,XX, +i(3)(q10) [2]	11%
TPA	46,XX[182]	
	47,XX, +i(3)(q10)[4]	
	47,XX, +3[1]	
	46,XX,der(3)del(3)(q22q25)[2]	
	46,XX,der(3)t(3;?9)(q29;?q13)[1]	10.9%

* Laboratory control values for trisomy ≥5% nuclei with three spot

+i(3)(q10)/+3 (indistinguishable by centromeric FISH) is randomly distributed in the B-cell population, independent of the bilobulated or non-bilobulated aspect of the nucleus. However, Mossafa et al. (12) recently reported +i(3q) predominantly associated with the non-binucleated cells. The majority of patients (21/73) had an indolent course with a follow-up that ranged between 1 and 20 yr. In two cases, a pulmonary blastoma and a non-Hodgkin's lymphoma were diagnosed 11 and 14 yr after the presentation of PPBL (4, 15). Several features may be relevant in the pathogenesis of this entity: expression of HLA-DR7, several clonal anomalies affecting specifically chromosome 3, bcl-2/IgH rearrangements and nuclear pockets in the abnormal B lymphocytes. Female gender and cigarette smoking could also be relevant factors. However, two recent cases where cytogenetic abnormality persisted after stopping tobacco have been reported (12). This observation could suggest no association with cigarette smoking. PPBL may represent a pre-malignant stage with a very slow progression rate, if any. Additional studies are warranted to elucidate this enigma.

Acknowledgements

We thank Helena Almeida and Carol Berger for their help in preparing the manuscript. This work was supported in part by grant FI-2.101 for Researchers' Formation from the Generalitat de Catalunya and by grant FIS 97/0655 from the Ministerio de Sanidad y Consumo.

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