Follicular lymphoma grade 3B includes 3 cytogenetically defined subgroups with primary t(14;18), 3q27, or other translocations: t(14;18) and 3q27 are mutually exclusive

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Chromosomal translocations involving t(14;18)(q32;q21) and the chromosome 3q27 region are common in B-cell non-Hodgkin lymphoma of germinal center cell origin. Grade 3B follicular lymphoma (FL), consisting almost exclusively of centroblasts, is a distinct subgroup of follicular lymphomas that has more in common clinically with the aggressive diffuse large B-cell lymphomas than with their indolent FL grade 1 and 2 counterparts. We studied the cytogenetic and molecular genetic aberrations by classic cytogenetics, polymerase chain reaction, Southern blot hybridization, and fluorescence in situ hybridization, with special emphasis on t(14;18), affecting *bcl-2*, and 3q27 rearrangement, affecting *bcl-6*, in 32 cases of FL grade 3B. Three distinctive subgroups were identified based upon the existence of breakpoint 3q27, a translocation t(14;18), or the absence of both. Group I involved a t(14;18) and no 3q27 aberrations (n = 13); group II was without a t(14;18) and without 3q27 aberrations (n = 9), but had other cytogenetic aberrations; and group III was without a t(14;18) but with aberrations involving 3q27 (n = 10). None of the FL grade 3B cases harbored both a t(14;18) and 3q27 aberration. These results, in particular the finding of a mutual exclusiveness of *bcl-2* and *bcl-6* rearrangement, indicate at least 3 different pathways of oncogenesis in FL grade 3B. FL grade 3B with *bcl-2* rearrangement probably is part of the same entity as the other follicular lymphomas (1, 2, 3A), whereas the cases with 3q27 abnormalities or other unrelated translocations are more closely related to the majority of diffuse large-cell lymphomas of germinal center cell origin. (Blood. 2003;101:1149-1154)

Introduction

Malignant lymphoma, follicular, large cell in the Working Formulation, or follicular lymphoma (FL) grade 3 according to the Revised European-American classification of lymphoid neoplasms (REAL classification), has been grouped together with the other follicular lymphomas (grades 1 and 2) in the REAL and World Health Organization (WHO) classifications.[1,2] Follicular large-cell lymphomas can be distinguished by the presence of at least a focal follicular pattern. In contrast to the other follicular lymphomas, they are mainly composed of, or contain a high proportion of, large noncleaved cells (centroblasts), which are also the typical cytologic feature of most diffuse large B-cell lymphomas (DLBCLs). In the Kiel classification, a category of centroblastic lymphoma, follicular, was recognized.[3] In the WHO classification, the so-called Berard criterion has been adopted. This means that cases with more than 15 centroblasts per high-power field are included (so-called grade 3A) in addition to cases with almost pure populations of large cells (so-called grade 3B).[4] Clinically, follicular large-cell lymphomas have more in common with DLBCL than with their indolent FL grade 1 and 2 counterparts. Treatment of follicular large-cell lymphoma according to the guidelines used for DLBCL (ie, doxorubicin-containing chemotherapy) has an outcome that is comparable in terms of remission and freedom from tumor progression rates to that of DLBCL.[5-7]

FLs and a large proportion of DLBCLs are B-cell lymphomas of germinal center origin. A frequent chromosomal aberration of lymphomas originating in the germinal center is the translocation t(14;18)(q32;q21), involving the immunoglobulin heavy chain (IgH) gene on chromosome 14q32 and the *bcl-2* gene on chromosome 18q21. This translocation has the highest incidence in FL grades 1 and 2 and the lowest incidence in DLBCL.[8] Translocation of *bcl-2* results from the process of the IgH variable region gene recombination, which takes place during B-cell development in the bone marrow and probably also infrequently in the germinal center. The result of this translocation is the juxtaposition of the antiapoptotic *bcl-2* gene, located on chromosome 18q21, and the IgH locus on chromosome 14q32, leading to up-regulation of bcl-2 protein expression in most cases of FL.

Another important chromosomal aberration in B-cell lymphomas of germinal center origin involves translocation of the chromosome 3q27 region. The translocation at 3q27 may also involve the IgH locus at 14q32 but has various other counterparts and results in a disruption of the *bcl-6* gene located at this breakpoint.[9-11] Translocations involving the *bcl-6* gene and the IgH gene occur during the process of isotype switching in the germinal center. *Bcl-6* encodes a protein that is involved in the control of cell proliferation and differentiation and plays an important role in germinal center formation and function.[12] Translocation of *bcl-6* must be distinguished from mutations of *bcl-6* that result from the process of somatic hypermutation in normal germinal center cells.[13] Mutations of *bcl-6* are frequently found in

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DLBCL as well as in FL grade 3, but much less frequently in FL grade 1 or 2.[14]

In a recent study, 8 of 11 cases of FL grade 3A were found to be t(14;18) positive, whereas only 2 of 16 cases of FL grade 3B had this translocation. On the other hand, only 2 of the 11 FL grade 3A cases had chromosomal breaks involving 3q27, in contrast to 7 of the 16 FL grade 3B cases. This finding suggests that FL grade 3A is closely related to FL grades 1 and 2, whereas FL grade 3B is different and may be more closely related to DLBCL.[15]

To further elucidate the oncogenesis of follicular large-cell lymphomas and their position in the spectrum of germinal center–derived B-cell lymphomas, we investigated the cytogenetic and molecular genetic aberrations of FL grade 3B and compared the findings with those in indolent FL grades 1 and 2 as well as in DLBCL.

Patients, materials, and methods

Patients

Biopsy material obtained between 1983 and 1998 for the diagnosis and treatment of patients with lymphoma in Groningen, The Netherlands and Edmonton, Canada forms the basis of this study. Cytogenetic investigation was included in the workup of all patients with lymphoma, provided that enough material was available for cell culture. Tumor samples for this study were selected from a group of 215 germinal center cell–derived lymphomas from which representative cytogenetic results were available. In almost all cases, an involved lymph node was the site from which material for this study was obtained. In 3 cases, material was obtained from an extranodal site: case no. 2 and case no. 30 from extradural tissue and case no. 3 from pleural tissue. From this series, 32 successive cases in 30 patients with the histologic diagnosis of centroblastic lymphoma, follicular or FL grade 3B were selected and studied in further detail. It should be emphasized that this series does not include cases that would be included in FL grade 3A based on a number of centroblasts greater than 15. Approval was obtained from the institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Histology

All cases were reviewed by one of us (S.P.) before inclusion in the study. FL grade 3B was defined according to the WHO classification[2] as a follicular lymphoma, that is, a neoplasm derived from follicle center B cells, with at least a partially follicular pattern and with neoplastic follicles composed of solid sheets of centroblasts without residual centrocytes. All of the cases had at least 25% follicularity. Most of the cases also had diffuse areas with centroblasts with similar morphology as those in the neoplastic follicles.

Cytogenetic methods

Briefly, minced tissue fragments of fresh biopsy material were washed in culture medium (RPMI-1640 supplemented with 15% fetal calf serum, glutamine, and antibiotics), seeded in T50 tissue culture flasks, and cultured in a 5% carbon dioxide incubator at 37°C in a humidified atmosphere. Harvesting and chromosome preparation of the tumor cells were performed after short-term culture using standard cytogenetic techniques. The chromosomes were G-banded using pancreatin. Karyotypes were expressed in accordance with the International System for Human Cytogenetic Nomenclature 1995 (ISCN 1995).[16]

Molecular genetic methods

For polymerase chain reaction (PCR) and Southern blot hybridization (SB), genomic DNA was isolated from frozen tumor samples according to standard procedures.

PCR. DNA amplification of the major and minor breakpoint regions was performed as previously described.[17] The major breakpoint cluster was amplified using the MBR3+ primer (5′-TTTGACCTTTAGAGAGTTGCTTTACG-3′) and a JH consensus primer (5′-ACCTGAGGAGACGGTGACC-3′). The minor cluster region was amplified using the MC8 primer (5′-GACTCCTTTACGTGCTGGTACC-3′) and the same JH consensus primer.

Each amplification was carried out in a standard PCR buffer containing 2.0 mM $MgCl_2$, 10% dimethyl sulfoxide, and 1 U Taq polymerase (Amersham Pharmacia, Biotech, Roosendaal, The Netherlands). One microliter DNA was added to each reaction. Samples were amplified for 35 cycles, with denaturing at 94°C for 30 seconds, reannealing at 55°C for 30 seconds, and elongation at 72°C for 2 minutes. For each PCR run, control samples included a blank (no DNA), a negative (placental DNA), and a known positive.

SB. SB was carried out with 2 *bcl-2* and 3 *bcl-6* probes, using standard SB protocols. DNA was digested with 2, *Eco*RI, *Hin*dIII, and partly 4, *Eco*RI, *Hin*dIII, *Bam*HI, and *Bgl*II restriction enzymes: Two different *bcl-2* probes (major: MBR2.5 and minor: PFl2), the JH6 IgH gene, and 3 different *bcl-6* probes (pGSc4.0, p486a, and p4860) were used for hybridization. The bands were reviewed by comparing them with known positive and negative controls. The criterion for an mbr or mcr rearrangement was either a rearranged fragment in at least 2 enzyme digests or comigration with the IgH rearranged fragment.

Immunohistochemistry

Immunostaining for bcl-2 and bcl-6 was performed on frozen tissue samples according to standard methods, using the following antibodies: mouse anti–human monoclonal antibody to bcl-2 protein (clone 124; Dakopatts, Glostrup, Denmark) and PG-B6p for bcl-6 protein (a gift of Dr B. Falini, Institute of Hematology, University of Perugia, Italy). The intensity of the staining was graded as strong (+), weak (+/−), or negative (−).

Fluorescence in situ hybridization (FISH)

FISH, using standard in situ hybridization protocols, was carried out in almost all cases for the translocations involving 3q27 and t(14;18). For the detection of chromosomal breakpoints on metaphase preparations, the following combinations of probes were used. For t(14;18), we used segregation of cos8000H2/cos8000F6 (pooled) (r) and pac15o23/ES8073 (for 3′–bcl-2 breakpoints) and segregation of cos8000H2/cos8000F6 (pooled) (r) and pac210c12/ES8048 (for 5′–bcl-2 breakpoints); for comigration, we used cosIg6/ES4237 (g)[17]; (r) shows a red signal and (g) a green signal. For cases 24 to 32, we used nuclear suspension prepared from frozen tissue blocks instead of metaphase preparations. For the detection of t(14;18) on these slides, the following combinations were used: the cosmids 8000H2 (ES4566) and 8000F6 (ES4561) (g) with pac15o23/ES8073 (r) for 3′–bcl-2 breakpoints, and the cosmids 8000H2 (ES4566) and 8000F6 (ES4561) (r) with pac210c12/ES8048 (g) for 5′–bcl-2 breakpoints.[18]

For detecting the 3q27 region rearrangements, the CEPH-Yac Y803g3 (g) was used, with a centromere 3 probe, pα3.5 (r).[19] Detection of a breakpoint by this probe is based on splitting of one into 2 signals, generating 3 instead of 2 single-colored signals in cells with 1 rearranged and 1 normal chromosome 3. Therefore, a breakpoint can be detected only in metaphase cells or intact interphase nuclei in which the number of these 3q27 signals can be compared with the number of chromosome 3–specific centromeric signals. Cases 1 to 23 were studied on metaphase suspensions, whereas cases 24 to 32 were studied on nuclear suspensions prepared from frozen tissue blocks.

Results

Cytogenetics

The cytogenetic findings of the 32 cases in 30 patients with follicular large-cell lymphoma are shown in Tables 1–3. In 2 patients, a sequential biopsy could be studied. Case no. 22 is a lymph node biopsy taken after 4 courses of cyclophosphamide, hydroxydaunomycin, vincristine, and

Table 1. FL grade 3B, cytogenetic t(14;18)-positive cases, without aberrations involving 3q27, n = 13

Case no.	Cytogenetic results
3	46,X,del(X)(q21q22),der(1)t(1;17)(p36;q11)add(1)(q41),del(3)(p11p21),del(6)(q23q25),der(13)t(1;13)(q21;q32),t(14;18)(q32;q21),add(17)(q22) [cp16]/46,XX [6]
7	51≈52,XY,+Y,t(1;15)(q42;p11)c,t(2;15)(p11;q11),+del(5)(q23),+7,der(9)del(9)(p?)inv(9)(p?p?),inv(11)(q21q25),+12,t(14;18)(q32;q21),+18,+mar [cp10]
10	50,XY,+add(1)(p13),+7,add(9)(q22),+12,add(13)(q21),t(14;18)(q32;q21),+16,add(18)(q21)[cp13]/46,XY [7]
11	52,XY,add(1)(p21),+del(1)(p36),+der(1;12)(q10;q10),+5,+7,der(8)t(1;8)(p31;q23),−17,+18,+add(19)(p11),+20,−22,−22,+r,+mar [cp9]/ 52,idem, der(14)t(14;18)(q32;q21) [2] / 46,XY [7]
13	47,XY,+X, t(14;18)(q32;q21) [cp7]/46,XY [17]
14	47≈50,XY,+X,+add(1)(p11),i(6)(p10),t(14;18)(q32;q21),−15,+16,dup(17)(q11.2q23),der(18)t(14;18)(q32;q21),+mar1,+mar2 [cp19]/ 46,XY [1]
20	48≈49,XX,+X,+X,t(1;20)(p36;q13),der(1)t(1;12)(p34;q13),add(5)(p13),add(5)(p14),add(6)(q13),+7,der(12)t(12;17)(q13;q21),t(14;18)(q32;q21), −15,add(16)(q11),−17,der(18)t(14;18)(q32;q21),del(20)(q13.1q13.3),+r,+mar [cp19]/46,XX [1]
21	88,XX,del(6)(q1),t(14;18),inc
23	46≈47,XY,del(2)(q22q24),−4,del(10)(q24q26),−13,t(14;18)(q32;q21),−17,−21,der(22)t(13;22)(q12;p12),+der(?)t(?;4)(?;p12),+der(?)t(?;4) (?;p12),der(?)t(?;17)(?;q11.2),+mar [cp11]/46,XY [5]
26	76≈83<3n>,Y,t(X;16)(p11;q12)×2,+Y,dic(7;17)(p11;p11)x2,+7,+10,+11,add(11)(q25),+12,+13,+14,t(14;18)(q32;q21)x2, −15,+16,+18,+add(19)(q13),+20,+21,+21,+22,+3mar [cp24]
27	92,XXXX,i(1)(q10),−4,i(6)(p10),+7,+7,+9,−10,+12,t(14;18)(q32;q21),+14,+16,+19 [cp24]/ 46,XX [1]
28	50,XY,+5,+7,+9,+16,t(14;18)(q32;q21) [16] / 46,XY [9]
31	84≈89,XX,add(X)(q22),add(X)(q22),del(1)(q25),−2,+3,−4,−5,−6,i(6)(p10),+add(7)(p12),+8,t(14;18)(q32;q21)x2,add(14)(q13)x2,−16,der(17)t(1;17)(q21;q25), del(18)(q21)x2,−19,−20,−21,−22,+4mar[cp22]/46,XX[2] / 92,XXXX [1]

prednisone (CHOP) chemotherapy of case no. 15. Case no. 23 is a relapse of case no. 13 after 10 months and after 6 courses of CHOP treatment. Cases are grouped according to those with t(14;18)(q32;q21) (n = 13) (Table 1), those with translocations involving 3q27 (n = 7) (Table 2), and those without either (n = 12) (Table 3). All karyotypes were abnormal. Normal karyotypes as the only cytogenetic result were not found.

Clonal balanced translocations, isochromosomes, and deletions of, for example, chromosome 6 (i(6)(p10) and del(6)(q1)) and chromosomes 1, 13, 17, and 18 were detected in the t(14;18)-positive group as well as in the t(14;18)-negative group. In addition, chromosome breakpoints not involved in balanced translocations, deletions, and isochromosomes were found in almost all cases. No differences in these abnormalities were observed between t(14;18)-positive and t(14;18)-negative tumors.

A large number of different structural aberrations were found, and these were present relatively more often in the t(14;18)-negative group than in the t(14;18)-positive group. Gain of chromosome 7 was observed in 62% (8 of 13) of the t(14;18)-positive cases and in 32% (6 of 19) of the t(14;18)-negative cases, as well as in 40% (4 of 10) of the 3q27-positive and 22% (2 of 9) of the 3q27-negative cases. Gain of chromosome 3 was observed in 26% (5 of 19) of the t(14;18)-negative group, in 33% (3 of 10) of the 3q27-positive cases, and in 22% (2 of 9) of the 3q27-negative cases. In the t(14;18)-positive group, no gain of chromosome 3 was found. Numerical chromosomal aberrations as the sole abnormality were not found in these cases.

Significant differences were also found in 3q27 aberrations between the t(14;18)-positive and -negative groups. In the t(14;18)-positive group (n = 13), no alterations in the 3q27 region were observed, whereas in the t(14;18)-negative group, 7 of 19 cases had alterations in the 3q27 region (Table 2).

Various, but no significantly different, structural and numerical aberrations were found in cases with and without 3q27 alterations. In case no. 30, we found 3q27 aberrations with cytogenetic testing, but this was not confirmed by SB and FISH. The quality of the karyotypes was rather poor; the breakpoint is possibly outside the 3q27 region. However, this case also did not have a t(14;18).

In 2 patients, a sequential second biopsy could be studied. The first patient, with a second biopsy because of progressive disease after 4 courses of CHOP, showed concordant results of t(14;18) in both biopsies (case nos. 13 and 23). The second patient, who relapsed at 10 months after 6 courses of CHOP, had discordant results. In the first biopsy (case no. 15), neither t(14;18) nor 3q27 abnormality was detected; however, in the second biopsy (case no. 22), a 3q27 rearrangement was present.

Molecular genetics

Molecular analysis was performed in 28 cases. In 4 of the 32 cases (nos. 22, 26, 27, and 28; Tables 1-2), no frozen material was retrievable, precluding analysis by molecular techniques. Three of these cases (nos. 26-28) had a t(14;18) and one case (no. 22) had a 3q27 aberration by cytogenetic analysis. With PCR, *bcl-2*

Table 2. FL grade 3B, cytogenetic t(14;18)-negative cases, with aberrations involving 3q27, n = 7

Case no.	Cytogenetic results
2	79<2n>,XX,dup(1)(q11q42),+add(1)(p11)x2,+der(2)t(2;2)(p23;q13)x2,der(3)t(3;3;3)(pter--> p12::q27->q12::p26->q27::p12->pter),−3,+5,+6,+6,+7,+7, +der(7)t(3;7)(7pter>7q36::3q12->3q27::3p12>3pter),+del(8)(q24.1)x2,der(10)t(10;11)(p15;q13),+der(10)t(4;10)(q21;q24)x2,+12,+12,+der(13)t(8;13) (q22;p1),der(14)t(8;14)(q22;p1)x2,+15,+der(17)t(17;21)(p11;q11)x2,i(18)(q10)x2,+der(18)t(14;18)(q24;q21),−20,−20,−21,−21,+22,+mar1t/m12 [cp18] /46,XX [4]
6	48,X,−X,der(1)add(1)(p36)t(1;8)(q41;q12),+3,t(3;3)(q27;q29),add(6)(q1),der(8)t(1;8)(q41;q12),+13,add(14)(q32),del(17)(p11),+20 [cp19]/46,XX [3]
17	46,XY,add(2)(q32),del(3)(q27),der(3)add(3)(p21)add(3)(q27),add(4)(q31),add(5)(q11.2),add(7)(q31),der(8)t(3;8)(p24;q24),-14,del(15)(q23),−16,add(17)(q24), der(18)t(8;18)(q24;q22),add(19)(p13),der(19)t(1;19)(q11;p13),+mar1,+mar2 [cp13]/ 46,XY [7]
22	47,X,i(Y)(q10),t(1;14)(q21;q32),t(3;7)(q26orq27;q21),del(6)(q23),del(13)(q12q14),del(13)(q12q22),+18[cp20]
25	46,XY,t(3;3)(q12;q27),dup(17)(q12q21) [25]
29	49,XX,der(2)add(2)(p25)add(2)(q31),t(3;14)(q21;q32),+del(3)(q27),+5,del(6)(q14q21),+7[19] /46,XX [11]
30	52,XX,+X,+X,t(2;6)(q35;p12),t(2;3)(q21;q27),i(8)(q12),+12,+18,+20,+mar [cp21]/ 46,XX [4]

Table 3. FL grade 3B, cytogenetic t(14;18)-negative cases, without aberrations involving 3q27, n = 12

Case no.	Cytogenetic results
1	49≈50,X,−Y,dup(1)(q23q41),+der(3)t(3;4)(p13;q27),+i(3)(q10),der(4)del(4)(q13q25)t(3;4)(p13;q27),del(6)(q13q21),+7,+9,del(9)(q11q21),−17,+del(18)(p11.2),+mar [cp20]
4	47,XY.dup(1)(q12q42),del(13)(q12q14),+mar [14] /46,XY [6]
5	49,XY,der(1)add(1)(q41)dup(1)(q23q21)t(1;3)(p34;q23),+3,add(7)(q31),+der(7)t(7;11)(p21;q13),del(17)(p11.2),+18[cp21]/46,XY[2]
8	52,XY,add(2)(p22),+t(3;18)(q23;q12),add(6)(q2),inv(9)(p13q21)c,add(11)(q21)x2,+12,+13,add(14)(p11),add(16)(p13),add(18)(q23),−18,+add(19)(q13),add(21)(p11)x2,+21,+r [cp6]46,XY,inv(9)(p13q21)c [10]
9	47,X,−Y,add(1)(p36),+2,+7 [4] /46XY [14]
12	49,XX,dup(1)(q32q21),+3,+3,dup(12)(q15q24.1),+i(18)(q10) [20] / 46,XX [1]
15	48,XY,t(2;22)(p12;q11.1),i(6)(p10),+10,der(10;13)(q10;q10),+16,+18[cp20]
16	46,XX,−1,add(1)(q43),add(5)(q31),del(7)(q22),der(8)t(8;12)(p11;q12),add(9)(q34),−10,add(14)(q32),del(17)(q23),+mar1,+mar2 [cp19]
18	45≈50,X,−Y,del(6)(q13q25),+del(6)(q13q25),add(8)(q24),add(12)(p11),+add(12)(p11),i(17)(q10),add(18)(q21),+20,−22,+mar1,+mar2,+mar3 [cp13]/46,XY [1]
19	45≈50,XY,+X,−6,+7,+11,der(16)del(16)(q2q2)dup(16)(p11.2p13.3),del(16)(p11),+der(16)del(16)(q2q2)i(16)(q10),r(19)(p13q13),+mar [cp15]/46,XY [5]
24	48,XX,+del(1)(p22),del(4)(q24q27),der(12)t(7;12)(q11;p12)x2,+12,t(19;22)(q13;q12) [24] /46,XX [1]
32	46≈49,XX,+X,der(1)dup(1)(q21q32)ins(1)(q21),+3,t(11;20)(q14;p12)[cp25]

rearrangement was detected in 4 of 10 cytogenetically t(14;18)-positive cases and in none of the cytogenetically t(14;18)-negative cases. By SB, *bcl-2* rearrangement was detected in 5 of 10 cytogenetically t(14;18)-positive cases. Three of the 18 cytogenetically t(14;18)-negative cases had a *bcl-2* rearrangement by SB; however, no *bcl-2*/JH DNA translocation was detected in these cases. *Bcl-6* rearrangement was detected by SB in 2 of 6 cases with and in 3 of 22 cases without cytogenetic 3q27 aberrations (Table 4). None of the cases with a 3q27/*bcl-6* rearrangement (cytogenetically or by SB or FISH) had a *bcl-2* rearrangement/t(14;18) by cytogenetics, SB, PCR, or FISH (Table 4).

Table 4. Comparison of cytogenetics, Southern blot/PCR, and FISH for detection of *bcl-2* and *bcl-6* rearrangements in FL grade 3B

	t(14;18)/bcl-2 positive			3q27 aberrations/bcl-6 positive		
Case no.	Cytogenetics	Southern blot/PCR	FISH	Cytogenetics	Southern blot	FISH
3	+	+	ND	−	−	−
7	+	+	+	−	−	−
10	+	−	+	−	−	−
11	+	−	+	−	−	−
13*	+	−	+	−	−	−
14	+	+	+	−	−	−
20	+	+	+	−	−	−
21	+	+	ND	−	−	−
23*	+	−	+	−	−	−
26	+	ND	ND	−	ND	ND
27	+	ND	ND	−	ND	ND
28	+	ND	ND	−	ND	ND
31	+	+	+	−	−	−
1	−	−	−	−	−	−
4	−	−	−	−	+	+
5	−	−	−	−	−	−
9	−	−	−	−	+	−
8	−	−	−	−	−	−
12	−	−	−	−	+	−
15†	−	−	ND	−	−	−
16	−	−	−	−	−	−
18	−	−	−	−	−	−
19	−	−	+	−	−	−
24	−	−	−	−	−	−
32	−	−	−	−	−	−
2	−	−	−	+	+	+
6	−	−	−	+	−	+
17	−	−	−	+	+	+
22†	−	ND	−	+	ND	+
25	−	−	−	+	−	+
29	−	−	−	+	−	+
30	−	−	−	+	−	−

+ indicates positive; ND, not done; and −, negative.

* Cases with a sequential biopsy.

† Cases with a sequential biopsy.

FISH

Almost all cases were investigated by FISH. For t(14;18)/*bcl-2*, the cytogenetic results were confirmed by FISH in almost all cases (Table 4). The 3 cytogenetically t(14;18)-negative cases that showed a *bcl-2* rearrangement by SB, but no *bcl-2*/JH fusion product, did not show a t(14;18) in the FISH analysis. Case no. 19 also was t(14;18)-positive by FISH, but t(14;18) was not identified by classic cytogenetics. An unidentified chromosome (mar) present in the karyotype of this case (Table 3) possibly contains material of chromosome 18 with a rearrangement at 18q21.

In 3 cases, we found discrepancies between 3q27 aberrations by cytogenetic analysis and *bcl-6* rearrangements in SB. All cases were subsequently investigated by FISH, and in the 3 cases that were positive by SB but had no 3q27 rearrangements with cytogenetics, we found a positive FISH result only in case no. 4. In this case, we observed a disruption of the LAZ3 region, with one green signal on one chromosome 3 and splitting of the signal between the other chromosome 3q27 region and a chromosome with a size consistent with the F/G group. Comparing this finding with the cytogenetic results, it is likely that this chromosome with a size of the F/G group is probably the marker chromosome in the karyotype description (Table 3, case 4).

Immunohistochemistry

All cases were bcl-6 protein–positive, and all but 2 cases (nos. 8 and 9) showed positive staining of the tumor cells for bcl-2 on frozen tissue sections. These 2 negative cases indeed did not have a translocation or rearrangement involving *bcl-2*, but all the other cases with or without *bcl-2* rearrangement or translocation did express bcl-2 protein. There was no clear correlation between the presence of *bcl-2* translocation and the intensity of bcl-2 protein staining. In fact, several cases with translocation had a staining intensity that was less in the lymphoma cells than in the remaining normal mantle cells.

Discussion

Cytogenetic analysis was performed in a cohort of 32 cases of FL grade 3B. To confirm the cytogenetic findings and to find potential cryptic translocations, we also applied Southern blot hybridization (SB), polymerase chain reaction (PCR), and fluorescence in situ hybridization (FISH).

The ability to detect t(14;18) and 3q27 rearrangement was much better with cytogenetics than with SB and PCR. In cases with discrepancies between the cytogenetic and molecular genetic findings, FISH corroborated the cytogenetic results. The high percentage of negative cases by SB and PCR is probably due to relatively frequent breakpoints outside the mbr and mcr region in this subset of FL.[20] By FISH, one additional case with t(14;18) and one additional case with 3q27 aberration were detected in the group that cytogenetically did not have t(14;18) or 3q27 aberration.

Based on the cytogenetic results, the cases can be divided into 3 subgroups: group I, with t(14;18) and without 3q27 aberrations; group II, without t(14;18) and without 3q27 aberrations, but with other cytogenetic aberrations; and group III, without t(14;18) but with aberrations involving 3q27. The third group is of interest because all 10 cases with 3q27/*bcl-6* aberrations lacked a t(14;18). The *bcl-6* translocations involved several different partners, including chromosome 2 (1 case), chromosome 3 itself (3 cases), and chromosome 7 (2 cases). Although there were 2 cases with translocations involving chromosome 2 or chromosome 14, these did not involve the immunoglobulin gene regions. This may explain the low sensitivity of the SB procedure in this study. In 2 cases, only SB showed rearrangement for *bcl-6*. This may be the result of a duplication of the *bcl-6* gene.

In contrast to what might be expected in a group of FLs, none of the cases contained both a t(14;18) and 3q27 aberration. Moreover, in one of 2 patients in whom a sequential biopsy was available for study, a 3q27 aberration was demonstrated in the second biopsy specimen, but both specimens in this patient were t(14;18)–negative. Previous studies in FL as well as in DLBCL did not show the association of t(14;18) and 3q27 rearrangements to be mutually exclusive.[21-23] Our own (unpublished) cytogenetic results in 80 patients with the diagnosis of DLBCL and 90 patients with the diagnosis of FL grade 1 or 2 showed one and 4 cases, respectively, with a simultaneous t(14;18) and 3q27 aberration.

The hallmark of FL is the t(14;18)(q32;q21), but this translocation is not thought to be of prognostic significance. The large proportion of grade 3B cases in this study without a t(14;18) and the frequent presence of 3q27 aberrations are features also frequently seen in DLBCL[11] and are consistent with another recent study.[15] In comparison with FL grade 1 or 2, all 3 subgroups of this series of FL grade 3B showed a large number of cytogenetic abnormalities. The abnormalities included deletions of the long arm of chromosome 1, deletion of the long arm of chromosome 6, gain of chromosome 7, 17p deletions, and abnormalities of 17q. All of these are frequently encountered during progression of lymphoma. In particular, gain of chromosome 7 as a secondary aberration can be considered an indication of progression to a more aggressive disease state.[24,25] These features all suggest that the oncogenesis of a large proportion of FL grade 3B cases is closely related to that of de novo DLBCL.

It is of interest that cases of FL grade 3B with *bcl-2* rearrangement, *bcl-6* rearrangement, or other abnormalities cannot be distinguished based on morphology or immunophenotype. This is also in accordance with the findings in DLBCL. The finding of bcl-2 protein expression in cases without *bcl-2* rearrangement indicates that other mechanisms can lead to bcl-2 protein expression. All cases with t(14;18) or *bcl-2* rearrangement were bcl-2 protein–positive, but not all cases showed overexpression, and in some cases staining of the lymphoma cells was clearly weaker than in the normal B cells. Several cases showed starry-sky macrophages in the histologic slides, indicating the presence of apoptosis despite the presence of bcl-2 protein.

In conclusion, we identified 3 cytogenetically distinct subgroups based on the presence of a breakpoint 3q27, a translocation t(14;18), or the presence of other cytogenetic aberrations in the absence of these 2 relatively frequent abnormalities in a large cohort of consecutively sampled cases of FL grade 3B. We also found mutual exclusiveness of *bcl-2* and *bcl-6* gene rearrangements. These findings point to at least 3 different pathways of tumorigenesis. FL grade 3B with *bcl-2* rearrangement probably is part of the same entity as the other FLs (grade 1, 2, or 3A). The cases with 3q27 abnormalities or other unrelated translocations are probably more closely related to the majority of DLBCLs of germinal center cell origin.

Based on these findings, it appears appropriate to compare the survival of these 3 groups separately. In previous reports, no

difference in survival was found between cases with t(14;18) and those without t(14;18).[26] The latter obviously included cases with 3q27 breakpoints as well as cases with other translocations. The preliminary data on clinical outcome of our 30 patients suggest that the survival of patients with a t(14;18)-positive lymphoma may be intermediate between those with a breakpoint 3q27, who had a better survival, and those with other translocations, who had a worse survival. Because of the relatively small number of patients, no firm conclusions can be drawn. Therefore, we will combine our results with those of other investigators with cytogenetic data on FL grade 3B to obtain a sufficient number of patients for meaningful comparisons of clinical outcome in distinct subgroups of FL grade 3B.

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