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Original Research Article

Cytogenetic abnormalities in reactive lymphoid hyperplasia: byproducts of the germinal centre reaction or indicators of lymphoma?[†]

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Abstract

Non-random karyotypic abnormalities associated with non-Hodgkin lymphomas (NHLs) have been described in cases of reactive lymphoid hyperplasia (RLH). However, the frequency and types of cytogenetic aberrations detected and their clinical relevance are unknown. To address these questions, we undertook a retrospective analysis of a large series of RLH diagnosed at our institute over 8 years. Cytogenetic abnormalities were identified in 20 of 116 (17%) cases with informative karyotypes, comprising 14 (70%) structural and 11 (55%) numerical changes. Clonal ($n = 14$, 70%) and non-clonal ($n = 6$, 30%) abnormalities were observed. Aberrations of chromosome 14 were the most frequent ($n = 8$, 42%, 7 represented IgH translocations), followed by chromosome 3 ($n = 4$, 3 represented BCL6 translocations), and chromosome 12 ($n = 4$). Abnormal karyotypes were most often associated with florid follicular hyperplasia. Isolated lymphoid organ (lymph node, tonsil or spleen) enlargement (12/20, 60%) was more common, no specific etiology was identified in 10/20 (50%) cases and only 1 of 18 patients with clinical follow-up (range 2–107 months, median 60 months) developed lymphoma. In our experience, cytogenetic abnormalities involving loci associated with B-cell NHL are not infrequently detected in RLH. Their occurrence portends low risk for lymphomagenesis, however longer follow-up is prudent to further evaluate the natural history of such cases. Copyright © 2010 John Wiley & Sons, Ltd.

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Introduction

It is well established that reactive clonal proliferations of B- and T-cells occur in response to antigenic stimulation [1,2] and such lymphocytic expansions can be detected in the peripheral blood of older individuals [3,4]. In healthy individuals, however, reactive clonal B-cell expansions, which are usually restricted to the germinal centres (GCs) of lymphoid organs [5], have been demonstrated in lymph nodes (LN), spleen and acquired lymphoid tissue by analyzing surface immunoglobulin light chain expression [6], in peripheral blood by flow cytometry [7] and by polymerase chain reaction (PCR) [8] or Southern blot analyses for immunoglobulin heavy chain (*IGH*) gene rearrangement in tissue samples [9]. The ability to detect clonal lymphoid proliferations only in a few instances of reactive lymphoid hyperplasia (RLH) could reflect reduced sensitivity of the diagnostic methods utilized or be indicative of the oligoclonal nature of immune responses in the majority of cases.

Numerous recurrent and clonal cytogenetic abnormalities described in B- and T-cell non-Hodgkin lymphoma (NHL) subsets are considered the 'gold standard' for

diagnosing specific entities [10]. However, by using sensitive methods, 'NHL associated translocations', especially *IGH/BCL2* [11,12], *IGH/BCL6* [13], *NPM/ALK* and *AT1C/ALK* [14] can be detected in LN or peripheral blood of healthy individuals. Although, the developmental stage or time of acquisition of *t(14;18)* is still debated, the currently favoured view is that it results from abnormalities of VDJ recombination in bone marrow B-cell precursors [12]. The increased frequency of *t(14;18)* detection in older individuals [15] and HIV or HCV infected patients [16,17], could thus reflect increased genotoxic stress due to chronic antigenic stimulation and/or deregulated immune surveillance allowing persistence of aberrant clones.

A few studies have investigated DNA ploidy alterations in RLH [18], but data regarding karyotypic abnormalities are scarce. Only a few small series and single case reports have described clonal or non-clonal cytogenetic abnormalities in LN from healthy individuals or patients with non-neoplastic diseases [18–24]. We, thus retrospectively analysed a large series of RLH cases to determine the frequency and types of karyotypic abnormalities detected and sought correlations between the occurrence of

chromosomal abnormalities and specific morphologic patterns of RLH, results of PCR analysis for *IGH* and T-cell receptor- γ (*TCR-\gamma*) gene rearrangements and flow cytometry, and clinical outcomes.

Materials and methods

Case selection and clinical characteristics

We searched our departmental cancer cytogenetics database to identify all lymphoid organ specimens submitted for karyotype analysis, irrespective of diagnosis, over a period of 8 years (1997–2004). Morphologic features were evaluated by reviewing H&E stained sections of formalin-fixed, paraffin-embedded tissue. Data regarding patient demographics, immune status and clinical outcomes (until November 2009) were obtained from our laboratory information system. This study was approved by our institutional review board.

Immunohistochemistry and *in situ* hybridization

A comprehensive panel of immunohistochemical stains was performed, which included the following primary antibodies: CD20, CD3, CD21, BCL2, BCL6, CD43 (DAKO, Carpinteria, CA) and kappa and lambda (Cell Marque, Hot Springs, AR), after heat-induced antigen retrieval and visualized with Envision plus (DAKO) and DAB. *In situ* hybridization for EBV-encoded small RNAs (EBER) was performed using the supplied protocol (INFORM EBER, Ventana, Tuscon, AZ).

Flow cytometry

Three or four-colour flow cytometric analysis was performed on lymphoid tissue suspensions using FACS-Calibur (BD Bioscience, San Jose, CA) and data analysed using the CellQuest software (BD Bioscience), according to standard methods. The panel of antibodies included CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD23, CD16 + 56, CD43, CD79a, kappa and lambda (BD Bioscience).

Giemsa-banded karyotype and fluorescence *in situ* hybridization (FISH) analysis

Giemsa (G)-banding was performed on metaphase preparations obtained after short-term (12 h) unstimulated cultures of samples using standard methods. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [25]. FISH analysis was performed on methanol-acetic acid fixed cells (eight cases) and paraffin-embedded tissue (four cases) using *IGH* ($n = 6$) and *BCL6* (3q27) ($n = 3$) dual colour break-apart (VYSIS, Downers Grove, IL, USA) or centromeric 16 (one case) (Abbott Molecular, Abbott Park, IL, USA) probes according to standard protocols; 200–500 cells were analysed and fluorescence signals were captured after counterstaining with DAPI using the Cytovision Imaging

system attached to a Nikon Eclipse 600 microscope (Applied Imaging, Santa Clara, CA).

PCR for *IGH* and *TCR-\gamma* gene rearrangement

DNA was extracted from paraffin-embedded tissue using standard methods, briefly five 10 μ tissue sections were deparaffinized with xylene, washed with ethanol and passed through DNA extraction columns (QIAamp DNA mini kit, Qiagen, Valencia, CA) using the supplied protocol. PCR for *IGH* and *TCR-\gamma* gene rearrangement, followed by heteroduplex analysis and polyacrylamide gel electrophoresis, was performed as previously reported [26,27].

Statistical analysis

Statistical significance of the difference between groups was calculated using Fisher's exact test and a p value < 0.05 was considered significant.

Results

Case selection and clinical characteristics

Cytogenetic analysis was performed on 405 consecutive, unselected, biopsies or resections of enlarged LNs ($n = 375$), tonsils ($n = 22$) and spleens ($n = 8$) submitted to our department over the study period, which included 149 cases (130 LN, 16 tonsils and 3 spleens) of RLH and 256 cases (245 LN, 6 tonsils and 5 spleens) of lymphoma. Successful karyotypes were obtained at a similar frequency for RLH (78.9%) and lymphoma (80.2%). Two of 149 cases previously diagnosed as RLH were excluded after histologic review, as one represented nodular lymphocyte-predominant Hodgkin lymphoma with rare 'popcorn' cells and one LN was from a patient with concurrent T-cell lymphoma in an adjacent node. In the remaining 147 RLH cases (from 139 patients), abnormal karyotypes were observed in 20 (13.6%) and normal karyotypes in 96 (65.3%) cases; karyotype failures accounted for 31 (21.1%) cases. Overall, chromosomal abnormalities were detected in 20/116 (17.2%) specimens (16 LN, 2 tonsils and 2 spleens) with informative karyotypes and a non-neoplastic diagnosis. The demographic and clinical information of patients in these three groups is summarized in Table 1.

The majority of patients (12/20, 60%) had isolated lymphoid organ enlargement, while 8/20 (40%) presented with generalized lymphadenopathy. Five patients with karyotypic abnormalities had additional, prior and/or subsequent, biopsies (time from index case 1–51 months) and karyotypic abnormalities were detected in one of four cases evaluated (case 11, Table 2); two had normal karyotypes and one was a karyotypic failure. No etiology for RLH could be determined in 10/20 (50%) cases; three patients were HIV+, two were liver allograft recipients, one had SLE and four had other immune/autoimmune disorders. Follow-up information was available for 18/20 cases (range 2–107 months, median 60 months, mean

Table 1. Clinical information of 147 RLH cases evaluated

Case/patient characteristics	Karyotype		
	Abnormal	Normal	Failure
Number of cases	20	96	31
Age (median)	2–82 years (43)	0.5–88 years (38)	2–72 years (41)
Sex (M/F)	7/13	46/50	15/16
Lymph nodes (%)	16 (80)	84 (88)	28 (90)
Tonsils (%)	2 (10)	12 (13)	2 (7)
Spleen (%)	2 (10)	0	1 (3)
HIV (%)	3 (15)	10 (10)	5 (16)
S/P Solid organ transplant (%)	2 (10)	6 (6)	4 (13)
SLE (%)	1 (5)	2 (2)	0
Generalized lymphadenopathy (%)	8 (40)	5 (5)	1 (3)

53.3 months). Only one patient, a liver allograft recipient, developed an EBV-associated polymorphic post-transplant lymphoproliferative disorder (PTLD) after 8 months (case 11, Table 2).

Cytogenetic abnormalities detected in RLH

Clonal abnormalities were observed in 14/20 cases with abnormal karyotypes; seven had minor clones (2–4 cells) and seven had a higher frequency of aberrant clones (Table 2), while six cases had non-clonal abnormalities (Table 3). Structural abnormalities included balanced reciprocal translocations ($n = 11$), inversions ($n = 3$), deletions ($n = 2$), addition ($n = 1$), isochromosome ($n = 1$), derivative chromosomes ($n = 4$) and marker chromosomes ($n = 3$) (Tables 2 and 3). The affected chromosomes were: chromosome 14 ($n = 8$, 40%, 7 involving the 14q32/IgH locus), chromosome 12 ($n = 4$), chromosome 3 ($n = 4$, including 3 with 3q27 rearrangements), chromosome 1 ($n = 3$), chromosome X ($n = 3$), chromosomes 8, 10, 11, 13, 16 and 22 ($n = 2$ each) and chromosomes 2, 5, 6, 9 and 18 (18q21) ($n = 1$ each). In three cases, clonal karyotypic abnormalities were present in all cells analysed (cases 12–14, Table 2), suggesting constitutional chromosomal abnormalities, however, this was not further investigated.

Confirmation by FISH analysis

FISH analysis was performed on metaphase spreads in eight cases to confirm karyotypic aberrations. *IGH* break-apart probes confirmed *IGH* rearrangements in two cases (cases 1 and 2, Table 2) with clonal karyotypic abnormalities (Figure 1A and B), but not in the case with a non-clonal 14q32 translocation (case 5, Table 3), as it was below the threshold for detection. *BCL6* rearrangement was confirmed in 1 case (case 3, Table 2) with clonal 3q27 translocation, but not in one case with a non-clonal translocation (case 3, Table 3), again, since the aberration was below the detection threshold of the FISH assay. Trisomy 16 was confirmed by FISH in the single case with this abnormality (case 4, Table 2, Figure 1C).

FISH analysis for *IGH* (cases 1 and 2, Table 2) or *BCL6* (cases 3 and 5, Table 2) was also performed on paraffin-embedded sections (interphase cells), after microdissecting GC using an 18 gauge needle, to determine the

compartment harbouring cells with abnormal karyotypes. Abnormal FISH signals were localized to the GC in 2, 31 and 37% of cells in cases 1, 3 and 5, respectively but the results were non-informative for case 2.

Histologic patterns associated with abnormal karyotypes

Follicular hyperplasia, defined as >90% of lymphoid follicles displaying reactive GC, for this study, was the most frequently encountered pattern ($n = 19$). In 13 of these cases, a variable proportion of follicles showed florid follicular hyperplasia (FFH) (<30% $n = 2$, 30–60% $n = 4$ and >60% $n = 7$) (representative examples are shown in Figure 1A and B). Cases of FFH exhibited markedly expanded GC (CD10+/BCL6+/BCL2–), with tingible body macrophages, attenuated mantle zones and expanded CD21+ follicular dendritic cell (FDC) meshworks. Eleven cases demonstrated some degree of folliculolysis with or without hemorrhage. Eight cases showed focal progressive transformation of germinal centres (PTGC) or PTGC-like changes (enlarged follicles with invaginations or tongue like infiltrates of mantle zone lymphocytes and T-cells into GC), all of these cases also had FFH. Only mild paracortical hyperplasia ($n = 15$), including nodular paracortical hyperplasia ($n = 10$), was observed. Less frequent morphologic features of cases with abnormal karyotypes included, marginal zone/monocytoid lymphocyte hyperplasia ($n = 6$), sinus histiocytosis ($n = 4$), paracortical microgranulomas ($n = 1$) and plasma cell variant of Castleman’s disease ($n = 1$).

The various histologic patterns observed in the three groups (abnormal, normal and failure karyotype) are summarized in Table 4. Of note, although FFH and PTGC and/or folliculolysis were seen in the majority of cases with abnormal karyotypes, six cases only showed morphologic changes encountered in routine cases of reactive follicular hyperplasia (Tables 2 and 3). Only two cases (cases 3 and 4, Table 2, Figure 1C) warranted the designation of ‘atypical lymphoid hyperplasia’. Due to a lack of established criteria, it is unclear whether these cases represent the non-*IGH/BCL2* counterparts of the *IGH/BCL2* translocation-associated situ follicular lymphoma [28], since it is known that non-*IGH/BCL2* follicular lymphomas can lack BCL2 expression [29].

Table 2. RLH with clonal karyotypic abnormalities (n = 14)

Case	Biopsy	Age (year)/sex/ethnicity	Tissue/site	Histologic diagnosis	Karyotype	Metaphases (abnormal/analysed)	FISH ^c (% of cells)	Other clinical conditions	IGH/TCR γ	Outcome/ follow-up
1 ^a	dx bx f/u (1 mo) ^b f/u (11 mo) ^b	14/M/W	LN/Cervical LN/ Site unspecified LN/ Groin	FFH and FL FFH and PTGC FFH and PTGC	46,XY,der(14)t(14;12)(q32;q13) 46,XY 46,XY	3/21 0/20 0/20	IGH (1%) ND IGH NT	None ND Carpal tunnel syndrome	P/P ND ND	A (66 mo)
2 ^a	dx bx	52/F/O	LN/Cervical	FFH, PTGC and FL	46,XX,t(12;14)(q11.2;q32)	5/20	IGH (7%)		P/P	A (68 mo)
3 ^a	dx bx	53/F/H	LN/Cervical	FFH and FL	46,XX,t(3;14)(q27;q32),t(6;22)(p2.4;q11.2), der(18)t(1;18)(q23;q21)	12/20	BCL6 (51%)	HIV+; Sickle cell disease	C/P	A (65 mo)
4	dx bx	33/F/H	LN/Supraclavicular	FFH, PTGC and FL	47,X,add(X)(p22.1),+16	2/20	CEP 16 (1%) IGH NT BCL6 NT	Asthma	C/P	A (62 mo)
5	dx bx f/u (51 mo) ^b	77/F/W	Spleen LN/Axillary	FFH and MZH FFH	46,XX,t(3;22)(q27;q11) 46,XXX	7/20 0/20	ND ND	Hypothyroidism; ITP; Diabetes	P/P	A (60 mo)
6	dx bx f/u (1 mo) ^b	2/F/W	Tonsil LN/Mediastinum	FFH and FL FH	47,XX,+mar1 Failure	3/20 0/0	ND ND	S/P Liver transplant	P/C P/P	A (96 mo)
7 ^a	dx bx	50/F/B	LN/Supraclavicular	FH and SH	45,X,-X,add(8)(p22)	4/22	ND	Pulmonary fibrosis; Eosinophilia; Sarcoidosis	P/P	A (11 mo)
8	dx bx	7/M/B	LN/Lingual	FFH, PTGC, FL and MCP	46,XX,i(1)(q10)[1] and 46,XX,der(12) [2]	2/11	ND	None	P/P	LTF
9 ^a	dx bx	47/M/O	LN/Axillary	FH, Castleman's disease	47,XY,+mar1	3/9	ND	HIV+	P/P	A (53 mo)
10	dx bx	38/F/B	LN/Lingual	FFH, PTGC, FL, PH and MCP	45,X,-X,del(3)(q13.2q25),del(8)(p22)	10/15	ND	Drug abuse	P/P	A (2 mo)
11	dx bx f/u (8 mo) ^b	4/M/W	Tonsil and Adenoids LN/Axillary	FFH, PTGC and FL EBV+ polymorphic PTLD	46,XY,t(1;14)(q21;q32) 46,XY,t(1;14)(q21;q32)	3/20 15/20	IGH NT IGH (20%)	S/P Liver transplant	P/P	A (51 mo)
	f/u (8 mo) ^b		LN/Cervical	EBV+ polymorphic PTLD	47,XY,+3 47,XY,+3	5/20 11/23	CEP 3 (16%) IGH (20%)		P/P	
12	dx bx	56/F/W	LN/Axillary	SH	45,XX,t(13;14)(q10;q10)	10/23	CEP 3 (13%)	Fibradenoma of breast	P/P	A (107 mo)
13	dx bx	79/M/O	LN/Axillary	FFH and PTGC	46,XY,inv(5)(p15;q12)	20/20	ND	Diverticulosis	P/P	A (88 mo)
14	dx bx	18/M/W	Spleen	FH and MZH	46,XY,inv(10)(q11.2;q26)	15/15	ND	None	P/P	A (20 mo)

M, indicates male; F, female; W, white; H, Hispanic; B, black; O, other; LN, lymph node; FFH, florid follicular hyperplasia; FL, folliculolysis; PTGC, progressive transformation of germinal centres; PH, paracortical hyperplasia; MZH, marginal zone hyperplasia; MCP, monocytoid cell proliferation; SH, sinus histiocytosis; dx bx, diagnostic biopsy; f/u, follow-up; mo, month; NT, not translocated; ND, not done; P, polyclonal; C, clonal; A, alive without evidence of disease; LTF, lost to follow-up; ITP, idiopathic thrombocytopenic purpura.

^aGeneralized lymphadenopathy.

^bPatients with follow-up biopsies (time after initial biopsy).

^cThe FISH results listed refer to those obtained from analysis of metaphase spreads only.

Table 3. RLH with non-clonal karyotypic abnormalities (n = 6)

Case	Biopsy	Age (year)/sex/ethnicity	Tissue/site	Histologic diagnosis	Karyotype	Metaphases (abnormal/analysed)	Other clinical conditions	IGH/TCR γ	Outcome/ follow-up
1	dx bx	28/M/B	LN/Inguinal	FFH, FL and MCP	46,XY,inv(14)(q11;q32)	1/20	None	P/P	LTF
2 ^a	dx bx	82/F/W	LN/Inguinal	FFH, PTGC, FL and PH	46,XX,t(4;12)(q21;p13)	1/20	Hypothyroidism; CHF; MI	C/C	A (19 mo)
3 ^a	dx bx	43/F/B	LN/Axillary	FFH, PTGC, PH and MCP	46,XX,t(3;14)(q27;q32)	1/20	HIV+;Kaposi sarcoma, Renal failure	P/P	A (71 mo)
	f/u (2 mo) ^b		LN/Cervical	FH and FL	46,XX	0/20		P/P	
	f/u (4 mo) ^b		LN/Axillary	FFH and MCP	46,XX	0/20		P/P	
4	dx bx	43/F/W	LN/Axillary	FH, FL and SH	Failure	0/0		N/D	
	f/u (16 mo) ^b		LN/Suprahyoid	FH, FL and SH	46,XX,t(9;11)(q22;q22)	1/10	None	P/P	A (60 mo)
5 ^a	dx bx	33/F/B	LN/Axillary	FH and plasmacytosis	40,XX,-1,-4,t(2;14)(q11.2;q32),-8,-10,-11,-13,-16,+mar1	1/20	SLE	P/P	A (26 mo)
6	dx bx	46/F/W	LN/Cervical	FH and SH	48,XX,+mar1-2	1/5	None	P/P	A (34 mo)

M, indicates male; F, female; W, white; B, black; LN, lymph node; FH, follicular hyperplasia; FFH, florid follicular hyperplasia; FL, folliculolysis; PTGC, progressive transformation of germinal centres; PH, paracortical hyperplasia; MCP, monocytoid cell proliferation; SH, sinus histiocytosis; dx bx, diagnostic biopsy; f/u, follow-up; mo, month; P, polyclonal; C, clonal; A, alive without evidence of disease; LTF, lost to follow-up; CHF, congestive heart failure; MI, myocardial infarction; SLE, systemic lupus erythematosus.

^aGeneralized lymphadenopathy.

^bPatients with follow-up biopsies (time after initial biopsy).

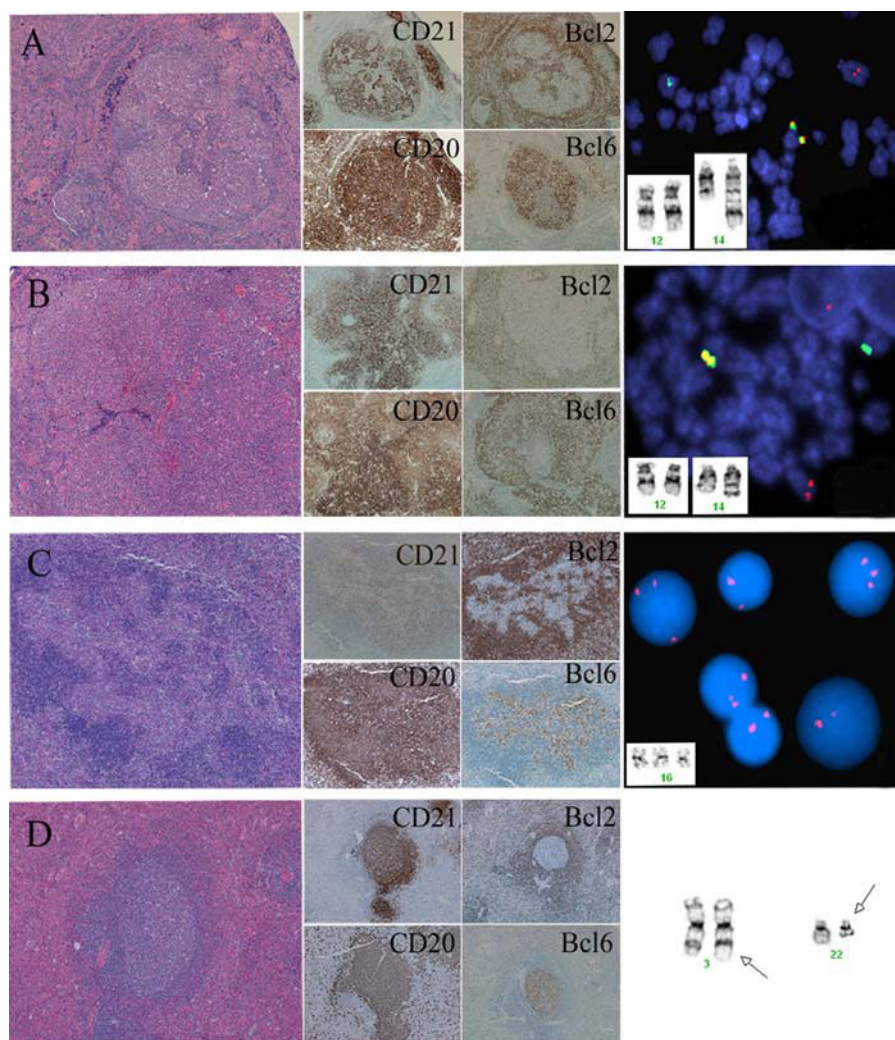


Figure 1. Morphologic features of representative cases of reactive lymphoid hyperplasia with clonal cytogenetic abnormalities, H&E stained sections (left), corresponding immunohistochemical stains (middle) and G-band/FISH findings (right). (A) Case 1, Table 2: cervical LN with FFH and focal folliculolysis. Karyotype analysis demonstrated 46,XY,der(14)t(14;12)(q32;q13)[3/21]. FISH analysis using *IGH* break-apart probes confirmed 14q32 rearrangement of one copy (separate red and green signals) and one normal (fused yellow) signal. Inset shows partial karyotype of aberrant chromosomes 12 and 14. (B) Case 2, Table 2: cervical LN with FFH and focal folliculolysis. Karyotype analysis yielded 46,XX,t(12;14)(q11.2;q32)[5/20]. FISH analysis using *IGH* break-apart probes confirmed rearrangement of one copy of *IGH* (green and red signal separation). A single yellow (fusion) signal representing the normal allele is present on the normal chromosome 14. Inset shows partial karyotype of chromosomes 12 and 14. (C) Case 4, Table 2: supraclavicular LN exhibiting FFH and PTGCs. Clonal karyotypic abnormalities found in this cases were 47,X, add(X)(p22.1), +16[2/20]. Interphase FISH performed with a CEP16 probe shows three copies of chromosome 16 (red signals). Inset shows a partial karyotype comprising three copies of chromosome 16. (D) Case 5, Table 2: splenic white pulp with FFH and mild marginal zone hyperplasia. G-band karyotype showed 46,XX,t(3;22)(q27;q11)[7/20]. Partial karyotype shows abnormal chromosomes 3 and 22 displaying t(3;22)(q27;q11)

Cytogenetic abnormalities were significantly more frequently observed in cases presenting with generalized, compared to, isolated lymphadenopathy (8/14, 57% vs. 12/133, 9%, $p = 0.00005$). Overall, abnormal karyotypes were noted in 13/39 (33.3%) cases of FFH with informative karyotypes. Statistically significant differences were noted with regard to the presence of follicular hyperplasia ($p = 0.023$), FFH ($p = 0.0003$) and PTGC-like changes ($p = 0.003$) in cases with abnormal karyotype compared to those with normal karyotype and karyotypic failures combined; significance was maintained even after removing the three cases with putative constitutional abnormalities from analysis. The triad of FFH, folliculolysis and focal PTGC was also more commonly seen in cases with karyotypic aberrations ($p < 0.01$). Comparisons between

cases with abnormal karyotypes and those with normal karyotypes and karyotypic failures, individually, are listed in Table 4.

Immunohistochemistry and *in situ* hybridization

Immunohistochemical staining did not detect BCL2 or CD43 expression in GC B-cells in all cases. On staining for kappa and lambda light chains, a monotypic plasma cell population was only observed in the case of HHV-8 associated Castleman's disease, which had the characteristic lambda + plasma cells restricted to the follicles. *In situ* hybridization for EBER was performed in all 20 cases. Only scattered EBER+ cells (<5%) were present in 2/6 cases with non-clonal karyotypic abnormalities, including

Table 4. Histopathologic features of RLH cases

Histologic features/karyotype groups	Abnormal (n = 20)	Normal (n = 96)	p-value	Failure (n = 31)	p-value
Follicular hyperplasia (%)	19 (95) ^a	68 (71)	0.0432	7 (23)	0.0003
Florid follicular hyperplasia (%)	13 (65) ^b	26 (27)	0.0018	3 (10)	<0.0001
PTGC (%)	8 (40) ^c	12 (13)	0.0068	0	0.0002
Folliculolysis	11 (55) ^d	33 (34)	0.1270	2 (7)	0.0002
Nodular paracortical hyperplasia (%)	10 (50) ^e	50 (52)	1	16 (52)	1
Granulomas (%)	1 (5) ^f	13 (14)	0.4585	9 (29)	0.0673

The *p* values in the columns correspond to differences between cases with abnormal karyotypes and those with either normal karyotypes or karyotypic failures. The *p* values for comparisons between cases with abnormal karyotypes and normal and failed karyotypes combined were as listed (a-f).

^a*p* = 0.023.

^b*p* = 0.0003.

^c*p* = 0.003.

^d*p* = 0.084.

^e*p* = 1.

^f*p* = 0.28.

a LN from an HIV+ patient, and 4/14 of cases with clonal karyotypic abnormalities, including LN from two HIV+ patients. All except one post-liver transplant FFH (case 11, Table 2), described previously [30], were deemed to be EBV negative lymphoproliferations.

Immune receptor gene rearrangement and flow cytometry

PCR analysis for *IGH* and *TCR-γ* genes was undertaken for all 20 cases with abnormal karyotypes and a subset of cases with normal karyotypes (*IGH*, *n* = 25 and *TCR-γ*, *n* = 21) and karyotypic failures (*IGH*, *n* = 13 and *TCR-γ*, *n* = 12). Cases with normal or failed karyotypes submitted for PCR analysis had histologic patterns similar to the abnormal karyotype group. Of the evaluated cases, three cases with abnormal karyotypes (Tables 2 and 3) and one case each with normal karyotype and karyotype failure showed clonal products on *IGH* gene rearrangement analysis, while two cases with abnormal karyotypes (Tables 2 and 3), one case with a normal karyotype, and none of the karyotype failure cases showed clonal products on *TCR-γ* gene rearrangement analysis. There was no significant difference in the frequency of detecting a clonal *IGH* or *TCR-γ* rearrangement between the three groups. No correlation between the presence of a clonal product and frequency of abnormal metaphases was observed. Flow cytometry was performed on all 20 cases with abnormal karyotypes, 82/96 (85.4%) cases with normal karyotypes, and 28/31 (90.3%) cases with failed karyotypes. No monotypic (clonal) B or aberrant T-cell populations were detected in any case (kappa:lambda—range 0.9–2.4:1 for cases with an abnormal karyotype).

Discussion

We report a spectrum of chromosomal abnormalities in 17% of RLH with successful G-band karyotypes, which were detected on analyzing the largest series of cases described to date in an unbiased manner. Both clonal and

non-clonal changes and structural as well as numerical aberrations were identified. Follicular hyperplasia, often accompanied by FFH, was the most frequent morphologic correlate of cases harbouring cytogenetic abnormalities, but other structural alterations of the GC, for example PTGC-like changes or folliculolysis were not infrequent. Phenotypic aberrations were not observed in any case. The majority of patients had localized lymphadenopathy, however the frequency of karyotypic abnormalities was significantly higher in those presenting with generalized lymphadenopathy. None had evidence of lymphoma at presentation, despite the detection of aberrations that are known recurrent abnormalities in B-NHL.

Karyotypic abnormalities of chromosome 14 were the most frequent (40% of our cases), the majority representing translocations involving the *IGH* locus (band 14q32). *IGH* rearrangements are not unique to any entity and are seen in a wide variety of lymphomas. Similarly, rearrangements of *BCL6* (3q27), seen in 20% of our cases, have also been described in follicular lymphoma, DLBCL, nodular lymphocyte predominant Hodgkin lymphoma, and rarely other types of B-NHL [10]. Other aberrations (1q, 22q11 and 12q), though less frequently observed, have also been documented in different B-NHL [31–33]. A notable exception in our series was a failure to detect *t*(14;18). It is unclear if this reflects the relative insensitivity of G-band analysis compared to PCR analysis, since the published literature is based on the latter detection method [11]. A low proliferation rate of rare non-neoplastic cells harbouring this translocation might not allow detection on analysis of metaphase preparations.

Karyotypic abnormalities have been reported in 30–45% of RLH cases in prior, smaller, studies of healthy individuals [18,20]. The types of chromosomes affected and kinds of abnormalities described in some differ from ours. Grace *et al.* [18] reported 20 cases of RLH and unlike our study, detected frequent chromosome 9 and 22 (20%) aberrations, as well as del 8q and del 3q, also known NHL-associated changes [10]. Chromosome 14 abnormalities were found in only two cases, but no *IGH* translocations were identified. Clinical data or results of clonality

assessment were not provided. A recent report by Au *et al.* [20] described 30 cases of apparent RLH with 10 cases demonstrating clonal and 3 cases non-clonal karyotypic abnormalities. Slightly more frequent translocations involving *IGH* (54%) and *BCL6* (46%) loci were described in this study compared to ours, as were cases with $t(14;18)(q32;q21)$, $t(11;14)(q13;q32)$ and $t(3;14)(q27;q32)$. Interestingly, clonal *IGH* gene rearrangement products by PCR were detected at a much higher frequency (50% of cases with abnormal karyotypes, including one case with a non-clonal karyotypic aberration) than our study. Despite detecting similar cytogenetic abnormalities, only one of our patients developed lymphoma, compared to concurrent or subsequent NHL observed in all 10 cases with clonal karyotypic abnormalities in the series of Au *et al.* [20]. That said, the median duration of follow-up of our cases (5 years) is insufficient to determine if, and how many of, the RLH cases could progress to low grade lymphomas. This question can only be answered by significantly longer follow-up evaluation.

Case reports of RLH with abnormal karyotypes have also been published, but follow-up information was lacking in all. Chenevix-Trench *et al.* [21] described $t(2;19)(p11.2;19q13)$ in a case of atypical lymphoid hyperplasia. Alterations of the transforming growth factor alpha or beta genes that map to 2p11-p13 and 19q13, respectively, or the kappa locus (2p11-p12) were not detected by Southern blot. Zhang *et al.* [24] described a case of persistent, isolated inguinal LN enlargement demonstrating follicular hyperplasia, sinus histiocytosis and clusters of epithelioid cells with 46,XY,del(8)(p11), der(14)t(8;14)(p11;q31). No *IGH* or *TCR-γ* gene rearrangement was detected by Southern blot. Similar to one of our cases, Pinkerton *et al.* [23] reported $t(1;14)(q21;q32)$, along with trisomy X, in an enlarged mandibular LN showing marked follicular and interfollicular hyperplasia. Flow cytometry did not detect a monotypic B-cell population, but a clonal *IGH* rearrangement was identified by Southern blot. Translocations or aberrations of chromosome 1q21, a gene-rich region, are frequently encountered as secondary alterations in a variety of lymphomas [32,33] and genes located in cytoband 1q21-23 have also been implicated in susceptibility to autoimmune diseases [34]. Only a few recurrently targeted genes have been identified at this locus thus far. Identification of chromosome 1q aberrations as isolated abnormalities in RLH suggests the requirement of additional abnormalities for lymphoma development or progression.

In our series, non-clonal cytogenetic abnormalities were seen in 30% of cases. Non-clonal changes are considered artefacts of tissue culture, mostly stimulated or long-term. Hence, although all our cases underwent unstimulated short-term culture, *ex vivo* occurrence of such changes cannot be completely ruled out in all cases. The observed non-clonal changes were varied, but included 14q32 and 3q27 rearrangements and a single case showed inversion 14(q11;q32). Interestingly, the latter aberration has been described previously as a minor clonal abnormality in NHL

(type unspecified), observed only upon mitogenic (PHA and IL2) stimulation [35].

The histologic patterns observed have been previously described in autoimmune diseases, infections (HIV, EBV, HCV, *etc.*) and inflammatory disorders [36,37], which are associated with an increase risk of lymphoma [38]. Immune deregulation due to altered immunity [39] and immune senescence [40] are a few hypothesized mechanisms. Persistent or excessive antigen stimulation or an inability to limit the humoral or GC response due to acquired or inherited defects could contribute to the development of lymphomas. Defects in Fas–Fas ligand interactions are associated with autoimmune syndromes and Fas mutations have been reported in lymphomas [41]. Errors in immunoglobulin remodelling during the GC reaction have been implicated in generating chromosomal translocations [42]. Rearrangements of *IGH*, *BCL6* and other oncogenes, as well as acquisition of somatic mutations in these genes [43,44] are believed to play crucial roles in lymphomagenesis [42]. The fusion transcripts themselves appear insufficient for generating lymphomas, requiring additional genetic or epigenetic alterations. Transgenic mice expressing NHL-associated translocations support this notion. FFH is observed in many models and lymphomas occurring later harbour additional genetic alterations [42,45–47]. Yang *et al.* [13] recently also described *IGH/BCL6* rearrangements in GC B-cells of healthy humans by PCR-based assays. Our findings of frequent 14q32 and 3q27 aberrations, localized to the GC in a few cases analysed, are supportive of the aforementioned etiopathogenic concepts. These changes could represent a ‘first hit’, perhaps providing a mild proliferative advantage, though cells harboring such abnormalities are likely eliminated by a functional immune system. Faulty immune surveillance might allow accrual of genetic alterations and outgrowth of lymphoma, as proposed earlier and observed in one case [30]. Discovery of polymorphisms in immune receptor or inflammatory pathway genes or in DNA repair components could also shed more light on the relationship of RLH with B-NHL [48].

The etiology of RLH could not be determined in half of our cases. An infectious trigger is a possibility for some, since most of the cases involved superficial LN. Only a minority of patients were immunosuppressed (HIV infection and allograft recipients) or had autoimmune diseases. Interestingly, however these etiologies accounted for all except one of our eight cases presenting with generalized lymphadenopathy. Alonso *et al.* [19] described chromosomal abnormalities in 3/7 cases of AIDS-associated lymphadenopathy without histologic evidence of lymphoma, including one case each of ‘non-specific chromosome breakage’, hypodiploid karyotype, and $t(8;14)(q24;q32)$. Two of the patients developed NHL after an unspecified duration. Offit *et al.* [22] reported a $t(3;22)(q27;q11)$, trisomy 7 and inversion/duplication of chromosome 1 (q44-q31) in HIV-associated atypical lymphoid hyperplasia, clinical outcome data, however, were not provided. In our series, *BCL6* translocations were observed in both, immunocompetent and immunosuppressed (HIV infection) individuals who had no clinical evidence of lymphoma. Osborne and Butler described

concurrent or subsequent (1 month–9 years later) lymphomas in one-third of 58 patients >60 years with reactive follicular hyperplasia [49]. Of interest, we also observed a high frequency of abnormal karyotypes in cases of FFH (1/3 with this pattern), however only one patient with this reaction pattern, a liver allograft recipient, subsequently developed an EBV + PTLD.

Flow cytometry and PCR analysis of immune receptor gene rearrangements are useful tools in the diagnostic work up of NHL. Recent investigations using these modalities, however have also cautioned against interpreting clonal populations alone as evidence of neoplasia. Kussick *et al.* [7] described prominent monotypic GC B-cell populations by flow cytometry in RLH. Clonal *IGH* rearrangements were detected by PCR or Southern blotting, but no *BCL2* expression or *t*(14;18) was observed (karyotype data were not provided). No individual developed lymphoma on follow-up of 13–56 months. Intriguingly, monotypic populations were not detected by flow cytometry in any of our cases. A study by the BIOMED-2 Concerted Action Committee described clonal lymphoid populations in 10% of RLH by PCR analysis [50]. Using different PCR protocols, clonal products were detected in 25% of our RLH cases with karyotypic abnormalities (both *IGH* and *TCR-γ*), but without significant difference in frequency compared to cases with normal or failed karyotypes. Importantly, there was no correlation between detection of clonality by karyotype and PCR analysis.

In conclusion, we describe a variety of chromosome abnormalities in RLH, many involving loci associated with B-NHL. The aberrant clones were often small and not detectable by flow cytometry or PCR analysis. Although the frequency of developing NHL was low in our series, longer-term follow up is required to fully evaluate the neoplastic potential of such cases.

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