Original Articles

Burkitt's Lymphoma Is a Malignancy of Mature B Cells Expressing Somatically Mutated V Region Genes

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ABSTRACT

Background: The developmental stage from which stems the malignant B cell population in Burkitt's lymphoma (BL) is unclear. An approach to answering this question is provided by the sequence analysis of rearranged immunoglobulin (Ig) variable region (V) genes from BL for evidence of somatic mutations, together with a phenotypic characterization. As somatic hypermutation of Ig V region genes occurs in germinal center B cells, somatically mutated Ig genes are found in germinal center B cells and their descendents.

Materials and Methods: Rearranged V_κ region genes from 10 κ-expressing sporadic and endemic BL-derived cell lines (9 IgM and 1 IgG positive) and three κ-expressing endemic BL biopsy specimens were amplified by polymerase chain reaction and sequenced. In addition, V_H region gene sequences from these cell lines were determined.

Results: All BL cell lines and the three biopsy specimens carried somatically mutated V region genes. The average mutation frequency of rearranged V_κ genes from eight BL cell lines established from sporadic BL was 1.8%. A higher frequency (6%) was found in five endemic cases (three biopsy specimens and two BL cell lines).

Conclusions: The detection of somatic mutations in the rearranged V region genes suggests that both sporadic and endemic BL represent a B-cell malignancy originating from germinal center B cells or their descendants. Interestingly, the mutation frequency detected in sporadic BL is in a range similar to that characteristic for IgM-expressing B cells in the human peripheral blood and for μ chain-expressing germinal center B cells, whereas the mutation frequency found in endemic BL is significantly higher.

INTRODUCTION

Burkitt's lymphoma (BL) is a high-grade B cell lymphoma that is cytogenetically characterized by translocation of the c-*myc* proto-oncogene on chromosome 8 into either of the three Ig gene loci on chromosome 14 (IgH locus), 2 (Ig κ locus) or 22 (Ig λ locus) (1,2). Furthermore, in the endemic form of the disease, found predominantly in African children, more than 90% of the cases test positive for the Epstein Barr virus (2). This virus is present in only 30–40% of sporadic tumors of Europeans and from nonendemic areas in North Africa (1). Endemic and sporadic BL also differs in the patterns of chromosomal breakpoint locations: in sporadic BL, translocations are predominantely found within the c-*myc* gene and outside the μ switch region, whereas the reverse is true for endemic BL (3).

The origin of the malignant B cell giving rise to BL is controversial. In a recent proposal for a revised lymphoma classification, the normal counterpart of the tumor cells in BL is classified

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as "B cell of unknown differentiation stage" (4). Based on the expression of germinal center (GC) B cell-associated surface antigens (CD10, CD38, and CD77) a GC-derivation of BL has been proposed (5,6). Since Ig heavy chain class switch is known to take place in the GC-microenvironment (7,8), though not exclusively (9), the finding that in several cases the breakpoint of translocation at chromosome 14 is located in the sμ switch region was taken as support for this hypothesis (10). However, others consider BL to be derived from B lymphocytes corresponding to earlier stages of B cell development (11). Several lines of evidence are compatible with a derivation of BL tumor cells from immature or antigen-inexperienced (naive) B cells: (1) the surface markers CD10 and CD38 are not exclusively found on GC B cells but also on immature B cells in the bone marrow (12,13); (2) the existence of translocations in both the light chain loci and the heavy chain J_H locus suggests that the translocation occurred during V gene rearrangement (i.e., in the bone marrow); (3) most BL and BL cell lines express IgM and not IgG or IgA (1), the isotypes characteristic for "classical" GC-derived memory B cells (14); and (4) the fact that BL is primarily an extra-nodal disease has been taken as an indication of a non-GC derivation of this tumor (1).

A sequence analysis of rearranged V region genes for somatic mutation is a reliable approach to reveal the stage of cellular maturation of the tumor B cells in BL: naive IgM^+IgD^+ B cells express unmutated V region genes (15–17). The process of somatic hypermutation is active in, and probably restricted to, antigen-activated B cells proliferating in the microenvironment of the GC in secondary lymphoid organs (18). Therefore, somatically mutated V genes are found in GC B cells and GC-derived memory B cells (16–18).

Expressed V region genes have previously been cloned and sequenced from several BL cell lines (19–26) and from one BL biopsy (27). Furthermore, V gene sequences of five cell lines established from AIDS-associated BL were determined (28–30). However, in most of the studies on classical BL lines an assignment of somatic mutations was hampered by the fact that the corresponding germline genes could not be unequivocally identified. Somatically mutated V region genes were found in AIDS patients-derived BL lines (28–30). However, the relevance of this finding for classical BL is unclear. Thus, whether classical BL express somatically mutated V region genes or not remains an open question. Since nearly all human V_κ genes have been ordered and sequenced (31), a sequence analysis of rearranged V_κ genes represents a reliable approach to study somatic mutation in antibody genes of BL.

In the present work 10 κ-expressing BL cell lines of sporadic and endemic origin and three κ-expressing endemic BL biopsy specimens were analyzed for somatic mutation in their rearranged V region genes. It turned out that all cell lines and tumors harbor somatically mutated V region genes, indicating that they originate from GC or GC-derived B cells.

TABLE 1. Oligonucleotides used for the amplification of human V_H region genes[a]

Name	Sequence (5′ to 3′)
VH1L	TCACCATGGACTGGACCTGGAG
VH2L	ACCATGGACATACTTTGTTCCACGC
VH2L.2	ACCATGGACACACTTTGCTCCACGC
VH3L	ACCATGGAGTTTGGGCTGAGCTG
VH3L.2	ACCATGGAACTTGGGCTCCGCTG
VH4L	AAGAACATGAAACACCTGTGGTTCTTC
VH5L	ATCATGGGGTCAACCGCCATCCT
VH6L	ACAATGTCTGTCTCCTTCCTCATC

[a]Oligonucleotides VH2L and VH2L.2 as well as VH3L and VH3L.2 were used as a 1:1 mixture, respectively. The V_H oligonucleotides hybridize to the leader region of the respective V_H gene family.

MATERIALS AND METHODS

Amplification of Rearranged V_H and V_κ Region Genes by Reverse Transcriptase PCR

RNA was isolated from 10 Burkitt's lymphoma-derived cell lines as described (32). For first strand cDNA synthesis total cellular RNA of 5×10^4 cells was hybridized to oligonucleotides recognizing the first exon of the Cκ (Cκ [15]) and either the Cμ or a Cγ constant region genes (16) and extended using SuperScript MMLV reverse transcriptase II (Gibco-BRL, Gaithersburg, MD, U.S.A.). Amplification of V_H gene rearrangements was performed with a set of VH leader family-specific primers (Table 1) together with a Cμ or a Cγ primer (see Ref. 16) in separate

reactions for each V_H gene family. The PCR mixtures contained 1/20 of the cDNA mixture, 200 μM each dNTP, 10 mM Tris-HCl pH 8.4, 1.5 mM $MgCl_2$ (2.5 mM for the V_H4L primer) 50 mM KCl, 0.125 μM of one of the V_H primers, 0.125 μM of the Cμ or Cγ primers, and 1.25 units of Taq DNA polymerase. Cycling conditions were: 95°C 2 min, 65°C 4 min, 72°C 1 min, followed by 95°C 60 sec, 61°C 30 sec, 72°C 60 sec for 34 cycles; and finally an incubation step at 72°C for 5 min. Taq DNA polymerase was added after the first denaturation step.

Amplification of V_κ gene rearrangements was performed with a set of V_κ gene family-specific primers (hybridizing to framework region I of V_κ families 1–4; $V_\kappa1,2,4$ see Ref. 18; $V_\kappa3$: 5'TTGTG⟨AT⟩TGAC⟨AG⟩CAGTCTCCAG⟨GC⟩CACC3') together with a C_κ primer (see Ref. 15) in separate reactions for each of the $V_\kappa1$ to $V_\kappa4$ gene families. The PCR reaction mixtures and the cycling conditions were as described above except that the annealing temperature was set to 63°C.

Amplification of Rearranged V_κ Region Genes from Genomic DNA

DNA was isolated from frozen biopsy specimens of three cases of κ-expressing Burkitt's lymphoma by standard methods (33). V_κ region genes were amplified from 100 ng of genomic DNA with a set of four V_κ family-specific primers (see above) and a J_κ primer mix (3' mix [18]) in separate reactions for each of the V gene primers. The PCR reaction mixtures and the cycling conditions were as described above, using 2.5 mM $MgCl_2$ and 61°C as annealing temperature.

Amplification of the B3 Germline Gene from Patient MM

The B3 germline gene was amplified from genomic DNA of the tumor specimen of patient MM with the $V_\kappa4$ FRI primer (see above) and a primer hybridizing to the recombination signal sequence of the B3 germline gene (B3Rev: 5'AGGAGGTTTGTGTTCGAGGCTGAAG3'). The reaction mix contained in 50 μl: 50 ng of genomic DNA, 200 mM each dNTP, 10 mM Tris-HCl pH 8.4, 1,5 mM $MgCl_2$, 50 mM KCl, 0.125 μM of primer VK4 and B3Rev, and 1.25 units Taq DNA polymerase. Cycling conditions were: 95°C 5 min, 63°C 4 min, 72°C 60 sec; followed by 95°C 60 sec, 63°C 30 sec, 72°C 60 sec for 34 cycles. The reaction ended with a 5-min incubation at 72°C. Enzyme was added after the first denaturation step.

Sequence Analysis of PCR Products

PCR products were gel-purified and directly sequenced without subcloning. Sequence reactions were performed either with ^{32}P-labeled oligonucleotides or with Digoxigenin-labeled primers, using the GATC direct blotting system (MWG Biotech, Ebersberg, Germany) and the Dig Taq Sequencing Kit (Boehringer-Mannheim, Mannheim, Germany) in the latter case. The V gene sequences have been submitted to the EMBL Data Library under accession numbers X87437 X8746.

RESULTS

V_κ and V_H Gene Rearrangements of 10 Burkitt's Lymphoma-Derived Cell Lines

V_κ and V_H region genes were amplified from cDNA generated from 10 κ-expressing BL lines. Seven of the lines were derived from sporadic cases of BL, two from endemic cases and one from a North African case which is related to the sporadic form of BL (Table 2). The BL line of North African origin, as well as both lines derived from endemic and two of the lines derived from the sporadic cases are EBV-positive (Table 2).

From 9 of the 10 κ-expressing BL, one potentially functional V_κ gene rearrangement was obtained (Table 2, Fig. 1). Two potentially functional V_κ rearrangements were amplified from cell line BL41 (Table 2, Fig. 1). Products were obtained for each of the V_κ gene families 1–4. The $V_\kappa4$ gene B3 was found to be rearranged three times, the $V_\kappa1$ gene O2, the $V_\kappa2$ gene A3, and the $V_\kappa3$ genes L2 and A27 were each rearranged in two cell lines. The $V_\kappa3$ gene rearrangement of BL41 harbors a deletion of three nucleotides in complementarity determining region (CDR) I (Fig. 1). This should not interfere with the expression of a functional light chain, since sequence length variation is frequently found in CDRs of V_H and V_κ germline genes (31,34). The detection of two potentially functional V_κ gene rearrangements appears to violate the principle of L chain isotype exclusion. However, since not the entire V gene sequences were determined, it is possible that only one of the two V_κ gene rearrangements is functional and can be translated into protein. Indeed, BL41 has been de-

TABLE 2. V_H and V_κ region gene sequence analysis of 10 Burkitt's lymphoma cell lines

Burkitt Line	Class	Origin[a]	V_H Family	V_H[b] Gene	Mutations/bp Sequenced[c]	% Mutation	V_κ Family	V_κ[b] Gene	Mutations/bp Sequenced[c]	% Mutation
Jijoye P79[d]	μ	e					3	L2	15/292	5.1
Daudi[d]	μ	e	3	H11	52/337	15.0	1	O2	29/288	10.1
BL36[d]	γ	s	3	DP58	19/337	5.6	3	L2	5/297	1.7
BL29[d]	μ	s	4	VH4.21	2/332	0.6	4	B3	10/294	3.4
			2	S12-9[e]	18/347	5.2				
JI[d]	μ	s	3	LSG10.1	8/343	2.3	4	B3	4/294	1.4
BL41	μ	s	3	1-9III	14/338	4.1	3	A27[f]	13/294	4.4
							4	B3	0/286	0.0
CA46	μ	s	5	BLK	1/333	0.3	3	A27	3/297	1.0
ST486	μ	s	3	1-9III	13/326	4.0	2	A3	4/295	1.4
CW698	μ	s	3	DP51	15/341[g]	4.4	1	O2	6/299	2.0
BL31	μ	s	4	VH4.21	14/327	4.3	2	A3	2/297	0.7

[a]The origin of the lines is indicated (e, endemic; s, sporadic).
[b]References for V_H germline genes: DP51, DP58 (34), H11 (35), VH4.21, BLK (36), S12-9 (37), LSG10.1 (38), 1-9III (39). References for V_κ germline genes are summarized by Schäble & Zachau (31).
[c]The number of basepairs sequenced represents the sum of the V and J segments, excluding D_H genes and N nucleotides.
[d]EBV positive lines.
[e]deletion of eight nucleotides in FRI.
[f]deletion of three nucleotides in CDRI.
[g]insertion of three nucleotides in CDRII.

scribed to produce only one light chain with a V gene segment belonging to the $V_\kappa3$ family (21).

Potentially functional V_H gene rearrangements were obtained from 9 of the 10 BL lines. Rearrangements of V_H gene families 2–5 were obtained, six of which could be assigned to the V_H3 family. No V_H gene rearrangement could be amplified from cell line Jijoye P79 using either V_H leader or framework region (FR) I primers (not shown). An attempt to amplify the V_H gene rearrangement from this line using genomic DNA also failed. This may be due either to the occurrence of somatic mutations preventing successful amplification, to the usage of a hithero unknown V_H gene or to structural aberrations in the V_H gene. Production of a truncated heavy chain has indeed been described for another Jijoye-subline (41). From cell line BL29 two V_H gene rearrangements were obtained in the RNA analysis: a potentially functional $V_H4.21$, and a nonfunctional V_H2 gene rearrangement carrying a deletion of eight nucleotides in FR I. The V_H2 gene used in this rearrangement shows highest homology to the S12-9 gene (Table 2). Finally, in BL line CW698 a deletion of three basepairs was found within CDRII of the V_H3 gene rearrangement.

Somatic Mutations in V Region Genes of BL Lines

The analysis of the ten BL lines for somatic mutation in their rearranged V region genes is summarized in Table 2. Somatic mutations were found in all lines. Interestingly, there was a clear difference in terms of mutational load between V_κ genes derived from endemic versus sporadic BL cases. While the V_κ genes of the two endemic cell lines Daudi and Jijoye P79 showed mutation frequencies of 10.1% and 5.1%, respectively (29 and 15 basepair differences), the average mutation frequency in the eight sporadic cases was 1.8% (range 0–13 point mutations).

Nucleotide differences to the putative germline genes were also detected in each of the 10 V_H gene rearrangements (Table 2). However, since V_H gene polymorphism may account for some of

```
a)                CDR I        CDR II                 CDR III
          11  28  31  36  50  55  70  77  85  89  92  93  95  95A 96  97  102 105 107 108
JijoyeK3  CTG AAT AGT TAT GCT GTC GAC AGT CTG CAA GAT TAC CCT CCG CCT ACT ACA GAA AAG CGA
L2        --- -G- --C --C -G- -C- --G --C G-T --G A-- A-- --- --
JK4                                                                 --- --C --G --A ---

b)                                CDR I                                   CDR II                          CDR III
          10  14  19  26  27  28  30  31  32  34  38  41  45  46  47  48  51  53  56  61  69  70  78  85  91  93  94  95  96  105 106 108
DaudiK1   TCC TGT GTT GGT CAT AAC ACC AAC TTT AGT CAA GGC ACA TTA TTG ATT GTG AAT CGT AGA GCA GAA CTA ACC AAT AAT TTC TCG TTC GAC AAC CGA
O2        --- -C- --C A-- --G -G- -G- -G- -A- -A- --G --G -AG C-C C-- --C -CA -G- A-- --G A-- --T --G --T -G- -
JK4                                                                                                                                -- --G -T- ---

c)                CDR I                 CDR III
          11  29  30  38  39  85  95  95A 96  108
BL36K3    CTG AAC AAC CAC AAT ATT CCT CCG TGG CGA
L2        --- -G- -G- --G --A G-- --- --
JK1                                       - --- ---

d)                CDR I                                   CDR III
          17  18  28  31  36  43  45  49  66  77  89  93  95  96  108
BL29K4    GAG ANG CTT AAC TAY TCT AAA TAT GGA AAC CAC ACT CCT CTC CGA
B3        --- -G- G-- -G- --C C-- --G --C --G -G- --G -G- ---
JK4                                                                 --- ---

e)                        CDR III
          17  46  92  93  94  95  97  108
JIK4      GAG TTG GAT ACT ATT CCC ACT CGA
B3        --- C-- T-- -G- -C- --
JK4                                - --- ---

f)                        CDR I                   CDR II              CDR III
          11  18  20  31  32  33  40  43  49  50  56  74  76  92  93  95  96  108
BL41K3    CTG AGC ACT A     AC TTG CGT TCT CGT GAT AAT ATC AGT AGT ACC CCG TAC CGA
A27       --- --A --C -GC T-- --A -C- G-- TA- -G- -C- -C- --C G-- -G- --
JK2                                                                         - --- ---

g)            CDR III
          19  95  96  108
BL41K4    GCC CCA CTC CGA
B3        --- --
JK3               -- ---

h)            CDRI  CDR III
          12  31  93  95A 96  103 108
CA46K3    TCT AAC AGT CCG TGG ACC CGA
A27       --- -G- --C --
JK1                       - --- -A- ---

i)                CDR I                 CDR III
          15  21  27  27D 34  36  95  96  97  108
ST486K2   CCT NTC GAG CAA GAC TAT CCT CGG ACT CGA
A3        --- A-- C-- --T --T --C --- -
JK2                                           --- ---

j)            CDRI                  CDR III
          9   31  37  59  70  83  95  96  108
CW698K1   TCC AAT CAA ACA GAC TCT CTG ATC CGA
O2        --- -GC --G C-- --T -T- -
JK5                                   - --- ---

k)                CDR III
          15  40  91  95  96  108
BL31K2    CCT TCA ACT CCG CTC CGA
A3/A19    --- C-- G-- --
Jk4                   - --- ---
```

FIG. 1. V_κ gene sequences of 10 BL lines

V_κ region gene sequences are compared with the most homologous germline genes. Dashes indicate sequence identity. Only the first and last codons of the sequences and those codons of the V_κ and J_κ genes that differ from the respective germline genes are shown. For references for V_κ germline genes, see Schäble and Zachau (31), J_κ sequences are from Hieter et al. (40). The aminoacid code and the codon numbers are given above the sequence. CDR I–III are indicated. a) JijoyeK3, b) DaudiK1, c) BL36K3, d) BL29K4, e) JIK4, f) BL41K3, g) BL41K4, h) CA46K3, i) ST486K2, j) CW698K1, k) BL31K2.

the nucleotide differences between the putative germline genes and the rearranged V_H genes, an unequivocal identification of somatic mutation is not in all cases possible. In particular, sequence polymorphism is common in the V_H3 gene family (38). Members of this family were involved in 6 of the 10 V_H gene rearrangements (Table 2).

Three lines for which the V_κ genes had already been sequenced several years ago were included in the analysis. From the sporadic lines JI and BL41, sequences identical to those previously published (21,22) were determined in the present study. On the other hand, the $V_\kappa1$ sequence which we obtained from the endemic Daudi line differed by three point mutations from the sequence published by Klobeck et al. (20). Interestingly, another group recently reported a V_H3 sequence from the Daudi line that differed from that determined in the present work by 14 point mutations (EMBL accession number HS07987).

V_κ Gene Sequence Analysis of Three Cases of Endemic Burkitt's Lymphoma

V_κ gene rearrangements were amplified from three cases of κ-expressing endemic BL using genomic DNA isolated from biopsy material. From each of the cases one PCR product was obtained. Cases JN and KD use $V_\kappa1$ genes with highest homology to the L1 and L11 germline genes, respectively (5.0% mutation, or 13 bp differences, in both cases; Table 3, Fig. 2). A $V_\kappa4$ gene rearrangement harboring 14 bp differences (5.5% mutation) relative to the $V_\kappa4$ germline gene B3 was amplified from case MM (Table 3, Fig. 2). To exclude that some of the nucleotide differences in the V region genes are due to V gene polymorphism in patients of African origin, the $V_\kappa4$ germline gene was amplified and directly sequenced from the biopsy material of patient MM (see Materials and Methods). A sequence identical to the published B3 sequence was obtained (not shown). This sequence should repre-

TABLE 3. V_κ region gene analysis of three cases of Burkitt's lymphoma

Burkitt Case	V_κ Family	V_κ Gene	% Mutation
JN	1	L1	5.0
KD	1	L11	5.0
MM	4	B3	5.5

For references of V_κ germline genes, see Schäble & Zachau (31).

sent a mixture of the $V_\kappa 4$ sequences from both alleles, which—since a cleary readable sequence was obtained—appear to be identical in patient MM. However, since the fraction of tumor cells in the specimen of patient MM is not known, it cannot be formally excluded that the amplified sequence represents the B3 sequence of the second allele in the tumor cells, if this has not been deleted during rearrangement. Nevertheless, the fact that a sequence identical to the one repeatedly sequenced from Caucasians was obtained supports the assumption that V_κ gene polymorphism does not represent a serious problem in the analysis of V_κ genes for somatic mutation (42).

Replacement Versus Silent Mutation (R/S) Analysis

Taking into account all point mutations in both the BL lines and biopsies analyzed in the present work, the resulting R/S values are 3.8 (23/6) and 9.0 (9/1) for CDRI and CDRII, respectively, and 1.0 (15/15) and 1.0 (13/13) for FRII and FRIII, respectively. For $V_\kappa 3$ genes the calculated R/S values assuming random mutagenesis are 4.3 and 2.5 for CDRI and CDRII, respectively, and 3.8 and 3.4 for FRII and FRIII, respectively. The counterselection of replacement mutations in the FR indicates that these tumors were selected for the preservation of a functional antibody structure. However, it should be noted that some BL lines produce no functional antibody protein (19,43,44).

```
a)          CDRI                CDRII                    CDR III
      12  22  29  36  45  49  53  61  66  70  85  89  91  93  95  96  99
JNK1  TCT AGT ATA TTT AAA TTT ATT AGA AGA AAT ACA TTA AAT ACT CCC CTC GGC
L11   --- -C- --T -A- --G -A- -G- --G G-- G-- --T C-- G-- -A- --
JK4                                                                --- ---

b)            CDR I       CDRII        CDR III
      11  22  28  31  34  48  53  61  89  90  92  93  95  96  100
KDK1  CTG ATG GAC GAT GCG ATT ACT CGA CTA CAA AAC AAT CCG ATC CAA
L1    --- -CT -G- A-- --C --C -G- A-G -A- --G --T -G- --
JK5                                                        - --- ---

c)          CDR I                                   CDR III
      17  27E 27F 28  31  32  36  39  45  61  65  89  90  93  94  95  96  99
MMK4  GAG ACC GCC AAA ACC AAC TTC AGA AAC CGG GGC CAC CAG AGC CCT CCA TTC GGC
B3    --- -G- T-- --C -A- T-- -A- -A- --G --A A-- --G --A --T A-- --
JK3                                                                - --- ---
```

FIG. 2. V_κ gene sequences of three cases of endemic BL

The sequence format is as in Fig. 1. a) JNK1, b) KDK1, c) MMK4.

DISCUSSION

Unusual Patterns of Ig Gene Rearrangement and Expression in BL

In the past, several cases of BL producing heavy chain proteins of abnormal size have been described (19,41,43,44). Such cell lines produce shortened heavy chains due to usage of alternate splice sites within the V_H gene. Furthermore, deletions and insertions were found in the heavy and light chain V genes (19,41,43,44). We also found in the present collection of BL, although the tumor cells appeared normal, unusual patterns of Ig gene rearrangements and expression. Thus, BL29H2 harbored a deletion of 8 nucleotides in FRI, CW698H3 carried a three basepair deletion in CDRII, and an insertion of three nucleotides was found in BL41K3. Although it cannot be ruled out that those V genes represent rare polymorphic forms of the corresponding germline genes, we consider it more likely that the deletions and insertions occurred somatically within the tumor B cells or their precursors. Taking into account that only the expressed V region genes were analyzed in the present study (with the exception of BL29H2 and BL41K4, see below) the occurrence of abnormal rearrangements in BL may be even higher than suggested by the mRNA-based analysis.

Other surprising findings in the present analysis was the detection of both a functional and a nonfunctional heavy chain transcript in BL29, and of two potentially functional κ light chain transcripts in BL41. Usually, a peripheral B cell carries only one functional heavy and light chain gene rearrangement, and stable mRNA is produced only from the functional gene rearrangements (45). A possible explanation for the expression of both V_H gene rearrangements in BL29 may lie in the fact that a translocation of

TABLE 4. Summary of mutations in BL and various B cell subsets

Cell Population	n^a	Range	% Mutation	Reference
Endemic Burkitt	5	13–29	6.0	Present Work
Sporadic Burkitt	7	0–13	1.8	Present Work
$\mu^+\delta^+$ PBL	16	0–3	0.2	15;[b]
μ-Only PBL	28	0–13	1.9	15;[b]
Class-Switched PBL	12	3–23	4.0	15;[b]
μ-Expressing GCC	8	1–10	2.0	16
Class-Switched GCC	9	1–13	3.3	16

[a] *n*, number of sequences.
[b] U. Klein, R. Küppers, and K. Rajewsky, submitted for publication.

the c-*myc* oncogene into the IgH locus has occurred in this cell line (46). This translocation, which put the c-*myc* gene into one of the Ig switch regions downstream of $C\mu$ of the nonfunctional V_H gene rearrangement, may have caused a higher rate of transcription of this heavy chain gene.

Since BL41 has been shown to express only the $V_\kappa 3$ light chain protein on its cell surface (21) it seems likely that the mRNA of the $V_\kappa 4$ rearrangement is not translated into protein. This might be due, for example, to mutations in parts of the gene that were not covered by our sequence analysis.

Different Patterns of Somatic Mutation in Endemic and Sporadic BL

The developmental status of an individual B cell can be determined through the expression of the various Ig heavy chain classes and stage-specific cell surface markers, and most notably the level of somatic mutation in its rearranged V region genes (11,15–17,47,48). Thus, newly arising B cells in the bone marrow are released into the periphery as IgM^+IgD^+ B cells carrying unmutated Ig gene rearrangements. These cells are considered to be precursors of antigen-activated B cells which proliferate and hypermutate their V genes in the GC microenvironment to generate affinity-increased antibodies (18). Eventually, selected B cells leave the GC as either somatically mutated class switched memory B cells or plasma cells (47).

Recently, the existence of another major human B cell subset harboring somatically mutated V region genes has become evident (Ref. 15 and U. Klein, R. Küppers, and K. Rajewsky, submitted for publication). About 10% of peripheral blood B cells are surface IgM-positive/IgD-negative and show a mutation frequency in their V region genes (1.9%; Table 4) that is half of that characteristic for class-switched peripheral blood B cells (4.0%; Table 4). These IgM-only B cells phenotypically resemble classical IgM^-IgD^- memory B cells in several respects: they are $CD23^-$, resting, and have elevated κ light chain mRNA levels compared with IgM^+IgD^+ B cells (U. Klein, R. Küppers, and K. Rajewsky, submitted for publication). This suggests that these cells represent IgM-expressing memory B cells which have been selected in the microenvironment of the GC during T cell-dependent antibody responses. Alternatively however, IgM-only B cells might be generated in a GC-independent pathway. Somatic hypermutation in the absence of external antigens is used in the sheep to diversify the primary antibody repertoire (49). This process takes place within the ileal Peyer's patches (50). In the human, there is as yet no evidence for such a B cell-developmental pathway.

To study Burkitt's lymphoma for somatic mutation of IgV genes, we focused on the expressed κ light chain genes of cell lines and biopsy specimens from sporadic and endemic BL cases. Virtually all human V_κ genes are now known (31) and seem to exhibit little sequence polymorphism, thus representing ideal substrates for a reliable analysis of somatic mutation in peripheral B cell subsets (15,51). Somatic

point mutations were identified in each of the 13 samples. Interestingly, comparing the level of somatic mutation in sporadic ($n = 8$) versus endemic ($n = 5$) cases, a considerably higher number of point mutations per V gene was evident in endemic BL which is confined to African patients. That the higher mutation frequency in endemic versus sporadic cases is not due to V_κ gene polymorphism in Africans is strongly indicated by the fact that a $V_\kappa 4$ gene sequence identical to that found in Caucasians was amplified from the genomic DNA of patient MM.

The Level of Somatic Mutation in Sporadic BL Suggests a Relation to IgM-Only Cells of the GC and the Peripheral Blood

The number of point mutations in the κ light chains of the sporadic BL lines ranged from 0 to 13 with a mean somatic mutation frequency of 1.8%. Both the range of mutations per V gene and the mutation frequency are strikingly similar to those found in two human IgM-expressing peripheral B cell subsets, namely μ chain-positive GC B cells (1–10; 2.0%) and IgM-only peripheral blood B cells (0–13; 1.9%) (Table 4). On the contrary, the corresponding class-switched GC and peripheral blood B cells show considerably higher mutation frequencies (3.3% and 4.0%, respectively; Table 4) (15–17). This observation, together with the fact that most sporadic BLs are IgM-positive/IgD-negative (1; 7/8 sporadic BL lines studied in the present work show this phenotype), clearly indicates a relation of sporadic BL to somatically mutated IgM-expressing B cells. The surface expression of the GC-associated markers CD10, CD38, and CD77 on most BL cases (5,6) suggests that the tumor B cells of BL are derived from GC B cells. On the other hand, a possible relation to IgM-only B cells in the periphery (Ref. 15 and U. Klein, R. Küppers, and K. Rajewsky, submitted for publication) is suggested by the extra-nodal appearance of BL. As discussed in the previous section, such IgM-only cells probably represent GC-derived memory B cells. Thus, the phenotypic resemblance of the tumor cells with GC B cells, together with the extra-nodal location of BL, indicates a derivation of the tumor cells in sporadic BL from a B lymphocyte which is at the transition from the GC to the IgM memory B cell compartment. The extra-lymphatic location and the apparent absence of ongoing IgV gene mutation in sporadic BL (27) clearly distinguish those tumors from the well-characterized GC B cell-malignancy follicular lymphoma (52,53).

Endemic BL Carry V Region Genes with a Higher Load of Somatic Mutation Than Sporadic BL

The five endemic BL cases harbored between 13 and 29 point mutations in their rearranged V_κ genes, yielding a mean somatic mutation frequency of 6.0%. These observations are supported by results recently obtained by Chapman et al. (54), who also found V genes expressed in endemic BL lines to be highly diversified by somatic mutation. The significant higher load of mutations in the endemic versus the sporadic cases suggests that these BL subtypes differ in the developmental stage of the B cell that gave rise to the malignant clone. This is further supported by the finding that sporadic and endemic BL show distinct patterns of c-*myc* translocations (3). Interestingly, the B cell-immortalizing Epstein-Barr virus is found in essentially all cases of endemic BL. Perhaps the presence of this virus in a GC B cell alters the way how these cells are selected and/or proliferate within the GC, resulting in the acquisition of a high load of somatic point mutations. Still, endemic BL phenotypically resembles sporadic BL, also in that most cases are surface IgM-positive/IgD-negative (1). This is surprising since both within the GC and the memory B cell fractions, B lymphocytes carrying highly diversified V genes predominantly express other isotypes (16,17; see also Table 4). The observation that the Daudi cell line seems to mutate in vitro points to an alternative explanation for the higher somatic mutation frequency—in the absence of class switch—in endemic BL cases: the transformed B cell clone continues to diversify somatically its rearranged V genes during extra-nodal tumor growth, and being removed from the GC-microenvironment, the malignant clone would not receive the necessary signals for class switching. Interestingly, also Chapman et al. found evidence for intraclonal heterogeneity in one of the endemic BL lines analysed (54). This heterogeneity is most likely due to ongoing somatic mutation in the tumor cells in vivo (54).

In conclusion, the present results show that classical BL represent lymphomas originating from mature B cells expressing V region genes diversified by somatic mutation. More specifically, their phenotypic resemblance to GC B cells, in conjunction with the extra-nodal location,

suggests that BL is a B cell malignancy originating from GC or GC-derived B cells. The considerable difference between the somatic mutation frequencies of sporadic and endemic BL indicates that these two subtypes of classical BL differ with respect to the developmental status of the cell that upon malignant transformation gave rise to the BL-tumor.

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft through Di184 and SFB 242. We thank H. G. Zachau for helpful discussions and H. Gustav Klobeck for critical reading of the manuscript.

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Contributed by K. Rajewsky on May 9, 1995.