Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2463–2468, March 1998
Immunology

Frequent occurrence of deletions and duplications during somatic hypermutation: Implications for oncogene translocations and heavy chain disease

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Communicated by Klaus Rajewsky, University of Cologne, Cologne, Germany, December 22, 1997 (received for review December 11, 1997)

ABSTRACT **Human naive and germinal center (GC) B cells were sorted by flow cytometry and rearranged V_H region genes were amplified and sequenced from single cells. Whereas no deletions or insertions were found in naive B cells, ≈4% of in-frame and >40% of out-of-frame rearrangements of GC B cells harbored deletions and/or insertions of variable length. The pattern of deletions/insertions and their restriction to mutated V genes strongly suggests that they result from somatic hypermutation. Deletions and insertions account for ≈6% of somatic mutations introduced into rearranged V_H region genes of GC B cells. These deletions/insertions seem to be the main cause for the generation of heavy chain disease proteins. Furthermore, it appears that several types of oncogene translocations (like c-myc translocations in Burkitt's lymphoma) occur as a byproduct of somatic hypermutation within the GC—and not during V(D)J recombination in the bone marrow as previously thought.**

During B cell differentiation in the bone marrow, somatic recombination of antibody gene segments is carried out by B cell progenitors to create functional heavy and light chain variable (V) region genes (1). Antibody diversity is generated by assembling one of the different V, (D), and J genes for each V region gene and by imprecise joining of these gene segments (2). Only B cells expressing functional heavy and light chain genes are allowed to enter the peripheral B cell pool as naive IgM^+ IgD^+ B cells (1). If peripheral B cells are activated by cognate antigen in the course of a T cell-dependent immune response, antibody V genes are further diversified by the introduction of somatic mutations in the microenvironment of the germinal center (GC) (3). The somatic mutations, which are introduced at a high rate (≈10^{-3} to 10^{-4}/bp/generation) to a region of ≈2 kb from the leader region to the J–C intron into rearranged heavy and light chain genes (4), mainly have been described as single nucleotide exchanges, whereas deletions/insertions only rarely have been observed (4, 5).

However, V genes harboring deletions and/or insertions were detected repeatedly in our studies of rearranged V region genes in various human B cell lymphomas (6–12): Deletions and/or insertions were found in 2 of 10 V_H gene rearrangements and 1 of 14 V_κ genes amplified from Burkitt's lymphomas (9), 1 of 4 V_H genes from monocytoid B cell lymphomas (7), 1 of 14 V_H and 1 of 11 V_L genes from diffuse large cell lymphomas (8), 3 of 13 V_H region genes from classical Hodgkin's disease (6, 10, 11), and 3 of 7 V_H region genes from lymphocyte-predominant Hodgkin's disease (6, 12). A common feature of all of these lymphomas is that they carry somatically mutated V region genes and are thus derived from GC or post-GC B cells.

The occurrence of deletions/insertions within rearranged V genes has not been analyzed systematically in normal human B cells, so it is unclear whether their frequent occurrence in the lymphomas is a peculiar feature of malignant B cells or whether they are also present in normal B cells at a comparable frequency. If deletions/insertions were to be present in V genes carried by normal B cells, they might originate either from V(D)J recombination in B cell precursors or somatic hypermutation in GC B cells. To clarify these matters, we used the experimental approach of single cell PCR to analyze rearranged V_H gene segments from human naive and GC B cells. The single cell approach provides, through direct sequencing of the PCR products, reliable information on the rearrangements carried by a given cell, largely avoiding artifacts introduced through errors of the *Taq* DNA polymerase or the formation of hybrid sequences.

The analysis revealed that deletions and insertions are not a peculiarity of malignant B cells but also can be found in rearranged V genes of normal GC B cells at a comparable frequency. Because the occurrence of deletions/insertions in the course of the GC reaction is associated with DNA strand breaks, we were prompted to reevaluate reports of oncogene translocations into the V region of Ig loci in human B cell lymphomas. This reevaluation strongly indicates that several types of oncogene translocations happen as a by-product of somatic hypermutation.

MATERIALS AND METHODS

Isolation of Tonsillar GC B Cells. V gene rearrangements from tonsillar GC cells were isolated in the course of a study of somatic hypermutation in apoptotic cells and cells at different stages of the cell cycle. Tonsils were obtained from routine tonsillectomies performed by the Department of Ear, Nose, and Throat of the Cologne University Hospital. Tonsillar mononuclear cells (MC) were prepared as described (13). For the isolation of apoptotic GC B cells, tonsillar MC first were incubated with anti-CD38-PE (Becton Dickinson) for 10 min. After washing in PBS/BSA, the cells were resuspended in a Ca^{2+}-containing binding buffer, and fluorescein isothiocyanate-conjugated Annexin V (CLONTECH) as well as propidium iodide were added to the cell suspension. Annexin V binds to apoptotic cells (14). After isolation of tonsillar MC, ≈1–2% of human tonsillar MC stain positive for both CD38 and Annexin V and are propidium iodide-negative (not shown). On a FACS 440 (Becton Dickinson), single $CD38^+$ Annexin V^+ propidium iodide-negative cells were sorted into

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Abbreviations: GC, germinal center; MC, mononuclear cells; RSS, recombination signal sequence.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AJ225682–AJ225778).

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PCR tubes containing 20 μl of 1× Expand High Fidelity PCR buffer (Boehringer Mannheim).

Single tonsillar GC centroblasts ($CD77^+$) were enriched by magnetic cell separation (Miltenyi Biotec, Auburn, CA). Tonsillar MC were incubated consecutively with: rat anti-human CD77 (IgM-isotype; Immunotech, Luminy, France); mouse anti-rat IgM (IgG1-isotype; Serotec); and anti-mouse IgG1 microbeads (Miltenyi Biotech). $CD77^+$ cells were enriched to >95%. Isolated $CD77^+$ GC centroblasts were lysed in a 0.1% sodium citrate solution containing 0.05 mg/ml propidium iodide. Single centroblasts in either the G_1 or G_2/M phase of the cell cycle were sorted into PCR tubes (15). The analysis of centroblasts in G_2/M was performed to analyze rearranged V genes in individual cells after DNA replication for somatic mutations separately.

Single-Cell PCR. Ig heavy chain gene rearrangements of the three most frequently used V_H families (V_H1, 3, and 4) were amplified by a seminested PCR approach. Single cells in 20 μl of High Fidelity PCR buffer (Boehringer Mannheim) were incubated with 0.25 mg/ml proteinase K for 1 h at 50°C followed by inactivation of the enzyme at 95°C for 10 min. The first round of amplification was performed by using a V_H/V_κ primer mix (16). PCR was carried out in the same tube containing 1× Expand High Fidelity PCR buffer: 2.5 mM $MgCl_2$/100 μM each dATP, dGTP, dCTP, dTTP/2.8 nM each V_H, V_κ, 3′J_H, and 3′J_κ primer mix in a final volume of 50 μl. Expand High Fidelity Polymerase (1.75 U; Boehringer Mannheim) was added during the first primer annealing step. The PCR program consisted of one cycle at 95°C for 1 min, 64°C for 5 min, and 72°C for 1 min, followed by 34 cycles of 95°C for 50 s, 59°C for 30 s, and 72°C for 60 s. At the end of the program, a 5-min incubation step at 72°C was added. The second round of amplification was carried out by using V_H1, V_H3, and V_H4 family-specific primers together with a 5′J_H primer mix (5′-JH1,4,5, 5′-GACGGTGACCAGGGT<GT>CCCTGGCC-3′; 5′-JH2, 5′-GACAGTGACCAGGGTGCCACGGCC-3′; 5′-JH3, 5′-GACGGTGACCATTGTCCCTTG GCC-3′; 5′-JH6, 5′-GACGGTGACCGTGGTCCCTT<TG>GCC-3′) in three separate reactions. First round reaction mixture (1.5 μl) was reamplified in a volume of 50 μl containing 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM each dATP, dGTP, dCTP, and dTTP; 0.125 μM of one of the V_H primers together with the 5′J_H primer mix and 1.25 units of *Taq* DNA polymerase. The cycle program consisted of 45 cycles of 95°C for 50 s, 61°C for 30 s, and 72°C for 60 s followed by a 5-min incubation at 72°C. To analyze duplicated V_H region genes after DNA replication from single GC B cells separately, aliquots of the cellular DNA from centroblasts in G_2/M phase and—as a control—in G_1 phase of cell cycle were amplified in three different reactions (to be published elsewhere).

Sequence Analysis. PCR products were gel-purified and directly sequenced with the same primers used in the second round of amplification. Sequencing was performed by using the Ready Reaction DyeDeoxyTerminator cycle sequencing kit (Perkin—Elmer) and automatic sequencing on an ABI377 sequencer (Applied Biosystem/Perkin—Elmer). The V gene sequence analysis was done by using DNASIS software (Pharmacia) and the GenBank data library (release 97) as well as DNAPLOT. DNAPLOT is part of the ImMunoGeneTics database (IMGT) (17). From Annexin V^+ GC B cells, 27 unique V_H gene rearrangements were obtained, and a total of 76 unique rearrangements was obtained from GC centroblasts in G_1 or G_2/M. From centroblasts in G_2/M phase, 35 rearrangements were amplified twice in two separate reactions. Among the rearrangements harboring deletions/insertions, four were amplified twice, and in each of these cases the pairs were identical. This result strongly argues against the possibility that the deletions/insertions were artifacts of the PCR.

RESULTS

PCR Analysis of Rearranged V_H Region Genes. Tonsillar GC B cells were analyzed by PCR for rearranged V_H1, V_H3, and V_H4 region genes (i.e., the three largest human V_H families) by using family-specific V_H primers together with J_H primers. Among a total of 103 sequenced V_H rearrangements (11 V_H1, 67 V_H3, and 25 V_H4), 69 rearrangements were potentially functional and 28 were nonfunctional (27 out-of-frame rearrangements, one rearrangement with three stop codons in complementarity determining region III). Twenty of the out-of-frame rearrangements were obtained from cells from which we also amplified an in-frame rearrangement. Four sequences were excluded from the analysis because they showed deletions at the end of the V_H gene segment and it could not be determined whether loss of nucleotides at the 3′ end of those V_H segments occurred during $V_HD_HJ_H$ recombination or at a later time point of B cell differentiation. Two other sequences were also omitted from the analysis; one in-frame rearrangement with an unusually long complementarity determining region III (nearly 200 bp) and one in-frame rearrangement with a stop codon in complementarity determining region III.

Somatic Point Mutations in Nonfunctional and Functional V_H Region Genes. Sequences of 28 nonfunctional and 69 functional V_H gene rearrangements from GC B cells were analyzed for somatic mutations. Among the 69 in-frame rearrangements, 67 V_H gene segments showed a total of 721 somatic point mutations, which resulted in an average mutation frequency of 6.0% (on average, 175 bp of the V_H region gene, ranging from framework region II to the 3′ end of the V_H gene segment, were sequenced in the analysis). Among the 28 out-of-frame rearrangements, 26 had acquired a total of 319 somatic point mutations, resulting in an average mutation frequency of 7.2%. The average mutation frequency within both functional and nonfunctional rearrangements determined in the present study was higher than values previously observed in V gene transcripts of GC B cells (≈2% for μ transcripts and ≈4% for γ transcripts; refs. 13 and 18). One can speculate that this discrepancy might be due to differences in the technical approaches (RNA-based population studies vs. DNA single cell studies).

The point mutations observed in the out-of-frame rearrangements showed the expected pattern for mutations introduced through the mechanism of somatic hypermutation, like preferences for distinct nucleotide exchanges, as previously described (4). The ratio of replacement vs. silent mutations in the framework regions often is used as a criterion for the functionality of a V gene. The replacement vs. silent ratio determined for the 28 nonfunctional rearrangements of 3.0 reflects the calculated value of 3.1 assuming random mutagenesis (disregarding potential influences of hotspots of hypermutation). In contrast, the average replacement vs. silent ratio of 1.8 in the 69 in-frame rearrangements indicates that selection against replacement mutations has taken place.

Deletions/Insertions in Human V_H Region Genes. In addition to point mutations, 12 of the 28 nonfunctional V region genes showed a total of 13 deletions, six duplications, and one insertion (Table 1; Fig. 1). Whereas seven rearrangements have acquired only one deletion/insertion, the other five sequences show two to four deletions/insertions. Of the 69 functional rearrangements, three have acquired four of these events (one insertion and three deletions; Table 1). Two of these rearrangements may still be functional because they showed a one-codon deletion and a three-codon insertion, respectively. The lower frequency of deletions/insertions in in-frame rearrangements is expected because many deletions/insertions are deleterious for a functional V gene.

The length of the deletions/insertions within in- and out-of-frame rearrangements differ from a 1-bp insertion to a

Table 1. Summary of deletions/insertions in V_H gene rearrangements

Cell	V_H gene*	Point mutations, *n*	Aberration	Length, bp	Location, region	Location, aa
Out-of-frame rearrangements						
Ann 33	DP31	15	deletion	38	FRII-CDRII	42-54
Ann 38	DP4	9	deletion	21	FRIII	66-73
Ann 40	DP25	4	deletion	89	CDRI-CDRII	31-59
			deletion	59	FRIII	66-83
Ann 47	V3-35	2	deletion/insertion	195/35	CDRI-FRIII	31-91
G1-8	DP10	9	duplication	80	CDRIII-FRIV	96-8 bp
						JH-intron
G2-29	DP7	20	duplication	131	CDRII-CDRIII	61-100d
G2-55†	V3-43	19	deletion	17	CDRII	52-57
			deletion	16	FRIII	73-78
			duplication	28	FRIII	81-87
			duplication	52	CDRIII	99-100q
G2-67	DP49	16	deletion	20	FRIII	83-89
G2-74†	V3-11	7	insertion	1	FRIII	93
			deletion	206	JH4-JH4/5	109-194 bp
					Intron	JH-intron
G2-80	V3-30	13	deletion	7	FRIII	75-77
			deletion	20	FRIII	84-90
G2-95†	DP75	10	deletion	>268	FRIII-JH4/5	82a-216 bp
					Intron	JH-intron
G2-112†	DP31	15	deletion	26	FRIII	76-82b
			duplication	111	CDRII-FRIII	53-86
			duplication	70	FRIII-CDRIII	80-100
In-frame rearrangements						
Ann 36	DP42	9	insertion	9	CDRII	56
G1-8	V4-4b	7	deletion	1	FRIII	77
			deletion	4	FRIII	80/81
G2-56	DP79	6	deletion	3	FRIII	72/73

FR, framework region; CDR, complementarity-determining region.
*References for germ-line genes: DP4, DP7, DP10, DP25, DP31, DP 42, DP49, DP75, DP79 (19); V3-11, V3-30, V3-35, V3-43, V4-4b (20).
†Replicated V_H region genes were amplified in two separate reactions.

>268-bp deletion (Table 1; Fig. 1). They are distributed throughout the whole sequenced V_H region gene and are not linked in an apparent way to recombination signal sequence (RSS) (like) motifs, the recognition sequences for the enzymes involved in V(D)J recombination. This makes it unlikely that the deletions/insertions are introduced during recombination processes like $V_HD_HJ_H$ recombination or heavy chain replacement (ref. 21; see also below). A comparison of the duplicated regions with each other and the respective germ-line genes revealed that the duplicated parts have both acquired shared and unique point mutations, showing that point mutations were introduced before and after the duplication event took place (for example, see Table 2). The borders of the deleted and duplicated regions were analyzed for sequence homologies like direct or inverted repeats, but no homologies were detected (Table 2). However, one has to keep in mind that the exact sequences of the deleted regions are not known because point mutations could have been introduced into these gene segments before the deletion event happened.

Absence of Deletions/Insertions in Naive IgD^+ B Cells. From different experiments, we acquired a collection of 75 unmutated V_H gene sequences from naive IgD^+ B cells, and among those, 62 represent in-frame and 13 out-of-frame rearrangements (Table 3). In contrast to the frequent finding of deletions/insertions in rearranged V genes amplified from mutated GC B cells, not a single such event was detected in any of those unmutated rearrangements.

DISCUSSION

Deletions and Insertions Frequently Occur During Somatic Hypermutation in the Human. Mutations introduced through the mechanism of somatic hypermutation mainly are described as single nucleotide exchanges whereas deletions/insertions rarely are observed (5, 22). However, we repeatedly observed insertions and/or deletions in V genes carried by various human B cell lymphomas, including Burkitt's lymphoma, diffuse large cell lymphoma, monocytoid B cell lymphoma, and Hodgkin's disease (refs. 6–12 and unpublished observations). Deletions and/or insertions were observed in 6 of 11 out-of-frame and 7 of 72 in-frame V gene rearrangements (unmutated V_κ gene rearrangements were not considered, for reasons outlined in refs. 7 and 23). The present study revealed that deletions/insertions are also present within V gene rearrangements of nonmalignant B cells at comparable frequencies: Deletions and insertions of variable length and location were detected in 43% of out-of-frame and 4% of in-frame rearrangements (Table 3).

Because of the frequent finding of deletions/insertions within rearranged V genes of GC B cells and human B cell lymphomas (this work, refs. 6–12, and unpublished observations), the question arises why such deletions/insertions only occasionally have been observed in other V gene studies (reviewed in ref. 23). There are several potential reasons why deletions/insertions were described only rarely. On the one hand, out-of-frame rearrangements of human GC or post-GC B cells, which harbor the highest load of deletions/insertions, only rarely have been analyzed in previous studies. On the other hand, it is most likely because of technical matters that deletions/insertions rarely were identified also within nonselected V genes like passenger transgenes or untranslated sequences flanking rearranged V genes (22, 24, 25): First, if populations of cells are analyzed, PCR products have to be cloned before sequencing, so it is difficult to distinguish between intrinsic deletions/insertions and those caused by PCR or cloning artifacts (26, 27). Thus, aberrant V genes obtained by such an experimental approach usually are excluded from the analysis. Second, we speculate that PCR

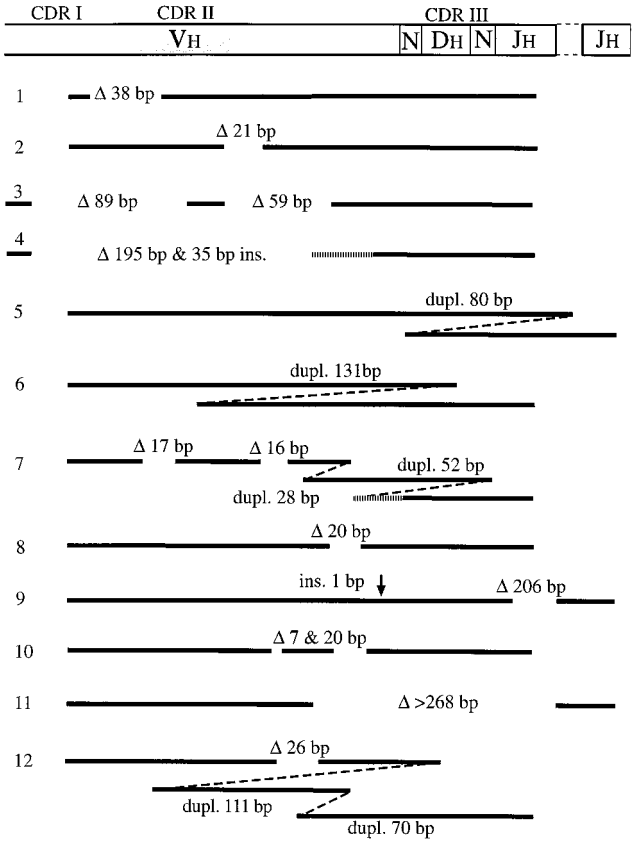

FIG. 1. Deletions and insertions in nonfunctional V_H region genes. The figure schematically shows the deletions (Δ) and insertions (ins., insertion; dupl., duplication) within the 12 nonfunctional V_H gene rearrangements amplified from GC B cells. The lengths of the deletions/insertions are indicated. Sequence 4 shows a long deletion; 35 bp of unknown origin are inserted into the deleted region. In sequence 7, the two parts of the 52-bp duplication are separated by a sequence stretch of 27 bp. Sequence 12 must have acquired the duplications before the deletion event happened because the deleted region is still present in both downstream duplicated regions.

products of unexpected length were either disregarded or lost when PCR products were analyzed on, or purified from, agarose gels. We assume that a restriction of the analysis to PCR products of expected length is the reason why deletions/insertions also were not described in a single cell study of nonfunctional V gene rearrangements (28).

As opposed to the frequent occurrence of deletions/insertions in GC B cells, not a single such event was detected in 13 out-of-frame and 62 in-frame rearrangements from naive IgD^+ B cells (Table 3). This result indicates that such deletions/insertions are introduced only rarely if at all during V(D)J recombination in the bone marrow. In addition, the reliability of the single cell PCR approach is evident from the present results, comparing the data from GC and naive B cells: Deletions/insertions were present in the rearrangements from the former but absent in those from the latter.

The (*i*) absence of deletions/insertions in rearranged V genes from naive B cells, (*ii*) finding that the breakpoints of deletions and insertions detected in GC B cells are not associated with RSS or RSS-like motifs, and (*iii*) finding that most of the duplications have acquired unique as well as shared point mutations strongly indicate that the deletions/insertions happened while the cell was accumulating somatic mutations in the GC microenvironment. This is further supported by the observation that all rearrangements with deletions/insertions described here and occasionally by others also harbor somatic point mutations (reviewed in ref. 23). Deletions/insertions also were detected in rearranged V genes of human intestinal plasma cells, all of which carried a high load of somatic mutations (Table 3; M. Fischer and R.K., unpublished work). Evidence for the introduction of deletions/insertions in the course of the GC reaction is supported further by an analysis of rearranged Ig genes from single GC B cells picked from histological sections (3). Within one GC B cell clone, one of the cells had acquired a deletion of 50 bp in framework region II. This result showed that, in this case, the deletion happened in the course of the clonal proliferation in the GC during which the cell was undergoing stepwise somatic hypermutation.

Given that deletions/insertions appear to be introduced into rearranged V genes in the course of the GC reaction, the question arises by which mechanism this might happen. Based on the recent finding of Rag-1 and Rag-2 activity in GC B cells (29, 30), deletions/insertions could be introduced by V gene editing (31, 32) at this stage of B cell differentiation. However, the absence of RSS-like motifs at the breakpoints of deletions and duplications argues against an involvement of the V(D)J recombination processes. Because GC B cells are proliferating vigorously, one also may speculate that deletions/insertions are introduced by mistakes during DNA replication. However, we consider this unlikely because such a mechanism should affect all genes equally, which would obviously not be tolerable for dividing cells. A more direct argument against this possibility comes from an analysis of rearranged V_κ genes in human B cell lymphomas. In λ- and some κ-expressing B cells, Igκ loci harboring nonfunctional $V_\kappa J_\kappa$ rearrangements usually are inactivated by deletion of the Cκ gene and the κ enhancers (23). We previously obtained evidence that, in B cells having deleted the Cκ gene, remaining $V_\kappa J_\kappa$ joints on the respective chromosomes do not acquire somatic mutations, although rearranged V_H and V_L region genes in the same cell were mutated somatically (7). In a total of 14 unmutated $V_\kappa J_\kappa$ joints amplified from B cell lymphomas harboring somatically mutated V_H and/or V_λ genes (including three cases with deletions/insertions within V_H or V_λ genes), not a single V_κ sequence with a deletion or an insertion was found (Table 3). This strongly argues against a causal relationship of the vigorous proliferation of GC B cells and the introduction of deletions/insertions into rearranged Ig genes and further supports the notion that deletions/insertions and point mutations are introduced into rearranged V region genes by the same mechanism. Moreover, the introduction of deletions/insertions into rearranged V region genes by somatic hypermutation has implications for the so far unknown mechanism of hypermutation: Because the generation of deletions/insertions requires DNA strand breaks, this mechanism appears to be intrinsically associated with such strand breaks.

Taken together, it seems likely that deletions/insertions are introduced into rearranged Ig genes during the GC reaction through the mechanism of somatic hypermutation. On this assumption, comparing the 20 deletions/insertions detected in the present study within the 28 out-of-frame rearrangements to the 319 point mutations seen in these same rearrangements, deletions/insertions account for 6% of all mutations introduced through this mechanism.

Generation of Oncogene Translocations and Shortened Heavy Chains in Heavy Chain Disease as a By-Product of Somatic Hypermutation. The frequency of rearrangements with deletions/insertions in GC B cells as well as in human intestinal plasma cells is comparable to their frequency in the collection of B cell lymphomas (Table 3). Thus, deletions/insertions are not overrepresented in malignant B cells. However, the frequent occurrence and pattern of deletions/insertions in mutated V region genes shed new light on the generation of chromosomal translocations involving the Ig loci in B cell lymphomas, like IgH/c-myc translocations in endemic Burkitt's lymphoma (33–37). It generally is believed that translocations into the J loci, as they are seen in endemic

Table 2. Sequences at the borders of deletions and duplicatons

Cell	Length, bp		Sequences of the junctions*	
		deletions†		
Ann 33	38	5′-CAAGCACCAG	*ggaagggcct/-/gttggaatag*	TGCCTCTATA-3′
Ann 38	21	5′-TCCAGGACAG	*agtcaccattaccagggacag*	GTTTATGAGC-3′
Ann 40	89	5′-CATCTTCACT	*agctatgcta/-/acacaaaata*	TTCACAGAAG-3′
	59	5′-TGCAGGGCAG	*agtcaccatt/-/agcagcctga*	GATCTGAAGA-3′
Ann 47	195	5′-CCTTCAGTAA	*cagtgacatg/-/tgtgtattac*	TGTGCGAGAG-3′
G2 55	17	5′-CTCACGTTTA	*agttgggatggtggtag*	GGTGGACTAT-3′
	16	5′-CCCAGAGACA	*acagcaaaaactccct*	GTATCTGTGA-3′
G2 67	20	5′-AACAGCCTGA	*gagctgaggacacggctgtg*	TGTAACTGTG-3′
G2 74	206	5′-AAACCCTGGT	*caccgtcacc/-/ttcctcctga*	GCCCACCACC-3′
G2 80	7	5′-GACAGTTCCA	*agaacac*	GCTCTGTCTG-3′
	20	5′-CAGCCTGATA	*gctgaggacacggctgtgta*	TTACTGTGCG-3′
G2 95	268	5′-GGAGGTGAAC	*aggctgagat/-/tctggggtcc*	AAGTGCCAAC-3′
G2 112	26	5′-CAACGCCAAG	*aactccctgt/-/aaatgaacag*	TCTGAGACCT-3′
		duplications‡		
G1 8	80	5′-GCGACANAAA	*GGGGACCAGT/-/GTGAGTCCTC*	
			GGGGACCAGC/-/ 3′ end of the sequence	
G2 29	131	5′-GCTACGCACA	*GAAGTTCCAG/-/TATGTGGTTT*	CC
			GAAGTTCCAG/-/GATGTGGTTT	TTTCTGGAGG-3′
G2 55	28	5′-AGTATCTGTG	*AATGAACAGT/-/CGAGGACACG*	
			AATGAACAGT/-/TGAGGACACG	GTCTTGTATT-3′
	52	5-′GTAGTGATGC	*TAGGATATTG/-/GCCGAGCCTT*§	
			TAGGATATTG/-/GCCGAGCCTT	TCGACCCCTG-3′

*The underlined nucleotides are mutated in comparison to the most homologous germ-line gene.
†Shown are 10 bp of the sequence 5′ and 3′ of the deletion in capital letters, as well as the first and last 10 bp of the deleted region in small italic letters.
‡Shown are 10 bp of the sequence 5′ and 3′ of the duplicated regions, as well as (in italics) the first and last 10 bp of the two duplicated parts. The two overlapping duplications in sequence G2 112 are not shown because the complex nature of these duplication does not allow an unequivocal assignment of the duplicated parts. A sequence figure for rearrangement G2 112 is available on request.
§The duplicated parts are separated by 28 nucleotides of unknown origin.

Burkitt's lymphoma, occur during V(D)J recombination (33, 35, 38). However, a closer inspection of chromosomal breakpoints from such cases does not support this view. (*i*) Chromosomal breakpoints are usually not directly adjacent to RSS but are located in J intron sequences (33, 35–37) or within rearranged VJ genes (36, 39, 40), i.e., throughout the region that is target for somatic hypermutation. [The situation is different in the case of bcl-1 and bcl-2 translocations into Ig loci in mantle zone lymphoma and follicular lymphoma, respectively, in which the translocation breakpoints are always directly at the 5′ end of J_H segments (41, 42). This situation indicates that, in the case of translocations that likely result from a mistake of V(D)J recombination, translocation breakpoints are at the 5′ end of the respective gene segments.] (*ii*) V(D)J joints are observed regularly on the chromosome involved in the translocation, strongly suggesting that c-myc/Ig translocation took place after V(D)J rearrangement (35–37, 43). (*iii*) All cases of Burkitt's lymphoma analyzed harbor somatically mutated V region genes and are thus derived from B cells that took part in a GC reaction (9, 44, 45). (*iv*) In two cases of Burkitt's lymphoma, translocations were described that separated the $V_\kappa J_\kappa$ joints from the Cκ locus (including the intron enhancer/matrix attachment region and the 3′ enhancer; refs. 36 and 37). Both rearrangements were found to harbor somatic mutations (36, 37). This would not be expected if the translocation took place before the onset of somatic hypermutation because the Igκ enhancers and matrix attachment region are known to be indispensable for hypermutation (46, 47). Thus, all these features of c-myc/Ig translocations in endemic Burkitt's lymphoma cannot be explained easily by the assumption that translocation takes place in a B cell progenitor. However, one would indeed expect to find them, if the translocations are a by-product of the hypermutation mechanism, which is intrinsically associated with DNA strand breaks, as evident from the frequent occurrence of deletions/insertions. We therefore propose that, in most if not all cases of endemic Burkitt's lymphoma, the c-myc/Ig translocation happens in a mutating GC B cell and results from the process of hypermutation as such. This would unify the picture of events leading to the generation of endemic and sporadic

Table 3. Frequency of deletions/insertions in human V region genes

Population	Mutated V genes	V genes with deletion/insertion		Reference
		Pot. functional	Nonfunctional	
HD and NHL	yes	7/72 (10%)	6/11 (54%)	6-12
Plasma cells	yes	5/32 (16%)	2/4 (50%)	†
GC B cells	yes	3/70 (4%)	12/28 (43%)	‡
IgD B cells	no	0/62	0/13	‡
Unmutated V_κ from mutated NHL and HD	no		0/14	6-8, 11

HD, Hodgkin's disease, NHL, non-Hodgkin's lymphoma; pot., potentially.
†M. Fischer, and R.K., unpublished work.
‡Present work.

Burkitt's lymphoma because, in the latter, translocation of c-myc usually is targeted to the Ig switch regions (43) and thus is assumed to relate to class switch recombination (48), a process also taking place in GC B cells (49).

Chromosomal translocation as a by-product of somatic hypermutation might not be restricted to the c-myc gene. Recently, translocation of the bcl-8 gene into a heavily mutated V_H gene was reported in a case of diffuse large cell lymphoma (50), suggesting that the translocation event happened in a mutating GC B cell also in this case.

The present data also address the origin of heavy chain disease, a human lymphoproliferative disorder in which abnormally short monoclonal Ig heavy chains can be isolated from the patient's serum without associated light chains (51). In several instances, in which the Ig genes encoding the shortened heavy chain genes have been characterized, the truncation originated from deletions and/or insertions within the rearranged V genes that, in addition, all harbor somatic point mutations (51–54). It is thus likely that the deletions/insertions leading to heavy chain disease are caused by the somatic hypermutation mechanism operating in GC B cells.

The involvement of the hypermutation machinery in the generation of oncogene translocations into Ig loci with the potential of subsequent malignant transformation (e.g., c-myc translocations in Burkitt's lymphoma) and aberrant heavy chains leading to heavy chain disease marks the GC apart from its physiological role in affinity maturation and memory cell generation as a microenvironment that plays a decisive role in the generation of lymphoproliferative disorders.

We thank Klaus Rajewsky for many helpful discussions and Julia Jesdinsky for sequencing work. We are grateful to Julia Kurth, Holger Kanzler, and Andreas Braeuninger for sharing unpublished observations. This work was supported by the Deutsche Forschungsgemeinschaft through SFB502, through an SFB243 grant to K.R., and by European Community Grant EU BIO4-CT96–0037 to W.M.

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