Somatic Hypermutation Introduces Insertions and Deletions into Immunoglobulin V Genes

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Summary

During a germinal center reaction, random mutations are introduced into immunoglobulin V genes to increase the affinity of antibody molecules and to further diversify the B cell repertoire. Antigen-directed selection of B cell clones that generate high affinity surface Ig results in the affinity maturation of the antibody response. The mutations of Ig genes are typically base-pair substitutions, although DNA insertions and deletions have been reported to occur at a low frequency. In this study, we describe five insertion and four deletion events in otherwise somatically mutated V<sub>H</sub> gene cDNA molecules. Two of these insertions and all four deletions were obtained through the sequencing of 395 cDNA clones (~110,000 nucleotides) from CD<sub>38</sub><sup>+</sup>IgD<sup>+</sup> germinal center, and CD<sub>38</sub><sup>+</sup>IgD<sup>+</sup> memory B cell populations from a single human tonsil. No germline genes that could have encoded these six cDNA clones were found after an extensive characterization of the genomic V<sub>H</sub>4 repertoire of the tonsil donor. These six insertions or deletions and three additional insertion events isolated from other sources occurred as triplets or multiples thereof, leaving the transcripts in frame. Additionally, 8 of 9 of these events occurred in the CDR1 or CDR2, following a pattern consistent with selection, and making it unlikely that these events were artifacts of the experimental system. The lack of similar instances in unmaturated IgD<sup>−</sup>CD<sub>38</sub><sup>−</sup> follicular mantle cDNA clones statistically associates these events to the somatic hypermutation process (P = 0.014). Close scrutiny of the 9 insertion/deletion events reported here, and of 25 additional insertions or deletions collected from the literature, suggest that secondary structural elements in the DNA sequences capable of producing loop intermediates may be a prerequisite in most instances. Furthermore, these events most frequently involve sequence motifs resembling known intrinsic hotspots of somatic hypermutation. These insertion/deletion events are consistent with models of somatic hypermutation involving an unstable polymerase enzyme complex lacking proofreading capabilities, and suggest a downregulation or alteration of DNA repair at the V locus during the hypermutation process.

During the course of a T cell–dependent antibody response, B cells hone the specificity of their antibody molecules through a process of random somatic hypermutation of their V genes, followed by antigen driven selection. This is collectively referred to as affinity maturation. This process occurs within the germinal centers (GCs) of secondary follicles from peripheral lymphoid organs when antigen stimulated B cells receive proper signals from T and accessory cells. In the human system, GC B cells are characterized by the surface expression of CD38 and, in most cases, the loss of IgD (1–3). We have previously shown that the initiation of somatic hypermutation occurs within the CD77<sup>−</sup> subset of these IgD<sup>−</sup>CD38<sup>+</sup> B cells (4). Mutated V genes can be isolated from all subsequent stages of B cell differentiation and in cells from all IgD<sup>−</sup> and certain IgD<sup>+</sup> B cell subsets (4, 5). The molecular process of somatic hypermutation remains elusive, primarily due to the lack of a good in vitro model until very recently (6). Much of what

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A abbreviations used in this paper: FM, follicular mantle; FW, framework; GC, germinal center.
is known concerns: (a) localizing the somatic hypermutation process to particular B cell subsets and anatomical settings (4, 7–10); (b) delineating the limits and rates of mutational activity (11); (d) determining the minimal substrate through transgenic technology (12, 13); and (d) analyzing the mutations themselves in the context of the surrounding sequence to reveal tendencies such as strand polarity and “hotspots” of somatic hypermutation (for reviews see references 12 and 13).

Although somatic hypermutation is typically described as the generation of bp substitutions, insertions and deletions have been sporadically described. As with somatic point mutations, the analysis of these events can provide valuable information concerning somatic hypermutation itself. Analysis of human V_{\mu}4 family genes generated from the amplification of cDNA from somatically mutated GC (IgD–CD38+) and memory (IgD–CD38–) B cell subpopulations led us to identify a number of cDNA clones from the mutated cell populations that contained insertions and deletions. We provide evidence that these events are linked to the somatic hypermutation process. Additionally, these events occur in a predictable fashion relative to the surrounding sequence, suggesting a model for their occurrence with implications for the molecular process of somatic hypermutation.

Materials and Methods

Isolation, Labeling, and Sorting of Tonsil B Cells. Human tonsils were obtained during routine tonsillectomy. B cell isolation and sorting for CD38 and IgD expression were performed as previously described (4, 14). In brief, human tonsillar B cells were separated into IgD–CD38+ follicular mantle (FM) B cells, IgD–CD38+ GC B cells, and IgD–CD38– memory B cells to 95–98% purity as predicted by FACS analysis, as previously described (13). The mutation state of the V_{\mu} gene cDNA clones from the various subpopulations was in agreement with our previous study (4). Clones were considered somatically mutated if they contained two or more bp substitutions, well beyond the expected error rates for 96 nucleotides in AMV-RT, and PFU polymerases used in these analyses (this mutation rate is based on our previous analyses; reference 4).

Sequencing the IgV_{\mu} Transcripts. Total RNA was extracted from 1–5 × 10^8 B cells using guanidinium thiocyanate-phenol-chloroform in a single step using the Ultraspec RNA isolation system (BIOTECX Laboratories, Houston, TX), and was reverse transcribed using oligo-d(T) or specific V gene constant region gene-specific oligonucleotides (V_{\mu}m family–specific probes) for IgG transcripts and SuperScript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD). First strand cDNA was used directly for second strand synthesis and amplification via the avian myeloblastosis virus reverse transcriptase (AMV-RT) and a primer specific for all V_{\mu}4 gene family heptamer–nonamer spacers as previously described (16). PCR products were agarose gel purified, then cloned into E. coli as described above for the cDNA clones. Clones identified in the cDNA analysis that contained insertions or deletions were used to design PCR primers to amplify both the exact sequence of clones with insertions/deletions as found and the predicted sequences based on the germline counterparts. Oligonucleotides used in this analysis (Format, is as follows: clone: exact/predicted): g645: 5'-GGACGGGTGGTACTTGGTTCC-3'/5'-GGACGGGTGGTACTTGGTTCC-3'; g1445: 5'-CTGGTCAAGGAGGGGTGGTGTG-3'/5'-CTGGTCAAGGAGGGGTGGTGTG-3'; g1875: 5'-CAGCTCCATAGTAAAGCCCG-3'/5'-CAGCTCCATAGTAAAGCCCG-3'; g885: 5'-GGAGGATTGATGGTGGGCC-3'/5'-GGAGGATTGATGGTGGGCC-3'; g925: 5'-GGAGGATTGATGGTGGGCC-3'/5'-GGAGGATTGATGGTGGGCC-3'; and g805: 5'-CCGAGTACCAATACCTCACACT-3'/5'-CCGAGTACCAATACCTCACACT-3'/5'-CCGAGTACCAATACCTCACACT-3'/5'-CCGAGTACCAATACCTCACACT-3'.

Sequence Availability. All cDNA sequences with insertions or deletions, and any genomic sequences unique to the literature as described in the results section are available from EMBL (Genbank/ DDBJ) under accession numbers AF013615 through AF013626. A say for Screening V_{\mu} Gene Lengths. To facilitate the analysis of large numbers of V_{\mu} gene transcripts for the presence of insertions or deletions, first strand cDNA produced as described above was PCR amplified using Expand high fidelity polymerase (Boehringer Mannheim) to reduce errors resulting from Taq polymerase alone. The products of this PCR amplification were cloned as described above and screened using 32P-labeled, gene-specific oligonucleotides (V_{\mu}m 4-39: 5'-ATTTGGGATCTTATCTTATG-3'; L-6 as above). Positive colonies were picked and used to inoculate overnight cultures. A 1 μl aliquot from each 24-h culture was used to directly inoculate 25 μl PCR amplification mixtures in 96-well-format PCR strips. The internal PCR reactions used 32P-labeled, gene-specific oligonucleotides to amplify a 230-base fragment including the V_{\mu}m 4-39 CDR1 (L-4, as above, and V_{\mu}m 4-39: 5'-
A) Insertion events from a single tonsil:

GCTCCCACTATAATAGATACT-3' or for analysis of VH-6 genes a 166-nucleotide fragment including the CDR1 and CDR2 of VH-6 VH-6FW1: 5'-TGCCATCTCCGGGACAGTGTTAT-3', VH-6FW3: 5'-TGTGCTCTGGTGATGGTTAT-3', which the inserts were sequenced in either direction. Gated bands were used to produce plasmid preparations from expected size and those clones in lanes adjacent to aberrantly明代 these events in IgM transcripts, although we have observed such events in IgM transcripts.

B) Deletion events from a single tonsil:

In the PAGE analysis, each VH4-39 FM clone included only the CDR1 and CDR2 (Fig. 1, A and B). The six clones with insertions isolated from various sources. Sequence data available from GenBank/DDBJ under accession numbers AF013615 through AF013626.

R results

Insertions and Deletions into Immunoglobulin VH Genes.

In a large scale analysis of VH genes from both the IgM and IgG compartments of B cell subpopulations separated from a single human tonsil, six clones that contained DNA insertions or deletions were isolated. These insertions and deletions were apparently selected in that they involved nucleotide triplets or multiples of nucleotide triplets, leaving the cDNAs (transcripts) in frame, and they were localized to the IgG compartments of B cell subpopulations, resulting in a frequency of <2% of clones analyzed (~1 event/18,000 nucleotides). All six events were in IgG transcripts. Two events were obtained from IgD-CD38- GC and four events from IgD-CD38- memory B cell populations. None of the IgM VH cDNAs analyzed from this tonsil had insertions or deletions, although we have observed such events in IgM transcripts in the past and in subsequent analyses, as described below.
The Insertions and Deletions Are Not Germline Encoded. The analysis described above focused on the V_{H4} gene family, which consists of 10-14 members per genome, varying slightly between individuals (16, 18). As shown in Fig. 2, the major difference between V_{H4} genes involves the length of CDR1. Because genomic diversity between V_{H4} family members resembles the events described in this paper we had to rule out possible alternative explanations for these events, such as (a) different alleles of the detected genes; (b) rarely expressed or otherwise unknown V_{H4} gene family members or (c) hybrids between known and detected V_{H} genes and/or other artifacts of the experimental system. To address these issues, both the expressed and genomic repertoires from this tonsil were characterized. As indicated in Table 1, 2 out of 118 V_{H4}-39, 2 out of 49 V_{H4}-31, 1 out of 87 V_{H4}-34, and 1 out of 45 V_{H4}-59 cDNA clones contained insertion/deletion events. cDNA clones were judged as negative results (data not shown). The unique nature of these events relative to both the expressed and genomic repertoire and our inability to amplify genomic counterparts for these events by PCR suggest that they are not germline encoded, two sets of PCR primers were designed to specifically recognize: (a) the exact sequence of the events; (b) the predicted, unmutated, germline sequence corresponding to the cDNAs containing insertion and deletion events. These primers were used to amplify genomic DNA from this individual, yielding negative results (data not shown). The unique nature of these events relative to both the expressed and genomic repertoire and our inability to amplify genomic counterparts for these events by PCR suggest that they are not germline encoded.

To characterize the genomic repertoire of the initial tonsil, 80 germline V_{H4} gene clones were isolated and sequenced (Table 1), which encompassed all 14 V_{H4} family members or alternate alleles represented in the 446 cDNA clones analyzed from all of the tonsillar B cell subsets. In the course of this study, we isolated the germline counterpart of a novel V_{H4} gene segment for which transcripts had been found. In addition, germline genes corresponding to two apparently functional V_{H4} genes not found as cDNA clones in this analysis were isolated, as well as one nonfunctional V_{H4} gene and a divergent polymorphism of a known V_{H4} pseudogene. The proposed germline counterparts of each of the V_{H4} genes containing insertion/deletion events were isolated from 4 to 11 times (Table 1). 8 independent genomic isolates of V_{H4}-31 and of V_{H4}-39 were cloned. V_{H4}-34 and V_{H4}-59 were isolated 11 and 4 times, respectively. No germline genes were isolated that could have encoded the insertion/deletion events described.

To further be certain that the insertion/deletion events described herein were not germline encoded, two sets of PCR primers were designed to specifically recognize: (a) the exact sequence of the events; (b) the predicted, unmutated, germline sequence corresponding to the cDNAs containing insertion and deletion events. These primers were used to amplify genomic DNA from this individual, yielding negative results (data not shown). The unique nature of these events relative to both the expressed and genomic repertoire and our inability to amplify genomic counterparts for these events by PCR suggest that they are not germline encoded.

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The Proposed Insertion/Deletion Events Are Not the Result of Recombination. As in most V gene repertoire analyses, we detected hybrid V_{H} sequences that could be the result of either PCR splicing by overlap extension artifacts, or reciprocal homologous recombination between unarranged V genes (19). However, none of these likely artifactual events were altered in size such that they resembled the insertion or deletion of DNA described above. A number of artifacts of this type had been isolated in the cDNA analysis as well; such artifacts are common to V gene analyses (20). The cDNA isolates with deletion and insertion events were stringently compared to all germline and cDNA isolates and were found to be unique relative to both the expressed and germline V_{H4} gene repertoires of this individual, supporting a somatic origin for their occurrence.

The Insertions and Deletions Are Not Associated with Somatic Hypermutation. To determine whether or not these inser-

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**Table 1. cDNA and Germline Clones Isolated**

| V_{H4} gene alleles isolated | cDNA clones with ins/del isolated | Total cDNA clones isolated | Germline clones isolated |
|-----------------------------|---------------------------------|--------------------------|-------------------------|
| V_{H4}-39                    | 2                               | 113                      | 7                       |
| V_{H4}-31                    | 2                               | 49                       | 8                       |
| V_{H4}-34                    | 1                               | 45                       | 4                       |
| V_{H4}-34 related            | 0                               | 0                        | 4                       |
| V_{H4}-55 pseudogene§        | 0                               | 0                        | 12                      |
| V_{H4}-55-related pseudogene§| 0                               | 0                        | 3                       |
| V_{H4}-04                    | 0                               | 17                       | 7                       |
| V_{H4}-04-related pseudogene§| 0                               | 0                        | 2                       |
| V_{H4}-61                    | 0                               | 25                       | 7                       |
| New V_{H4} gene‡             | 0                               | 33                       | 3                       |
| V_{H4}-04B                   | 0                               | 72                       | 1                       |
| V_{H4}-28                    | 0                               | 0                        | 1                       |

* Nomenclature based on Matsuda and Honjo (37).
† Nine unusual isolates were also cloned consisting of hybrids of two of the indicated genes, presumably do to PCR artifact. No one of these artifacts was altered in size or resembled any of the insertion or deletion events observed.
‡ Pseudogenes contain stop codons or frameshift mutations and are not expressed.
§ Newy identified V_{H4} gene is most closely related to V_{H4}-04-04.
tion/deletion events were associated with somatic hypermutation, we analyzed their occurrence in unmutated FM transcripts. This was done using either direct sequencing or PCR amplification of portions of the V_H genes spanning the CDRs, followed by size comparisons on polyacrylamide gels (Fig. 3). Any clones that ran aberrantly, and the clones in adjacent lanes, were sequenced (75 out of the 485 clones). None of these 75 clones were related based on CDR3 homology. To ensure that the remaining 410 FM clones were polyclonal, the CDR3s were PCR amplified and loaded on the sequencing gels simultaneously to the V_H gene amplification products for size comparisons (Fig. 3A). The size distribution of these CDR3s was similar to that of ~500 V_H gene sequences analyzed in this study (Fig. 3B), providing evidence that our FM sample is polyclonal.

The six events detected from a single tonsil were isolated from 395 mutated cDNA clones (25,482 CDR nucleotides), corresponding to a frequency of 2.35 events/10^4 CDR nucleotides. This is significantly different (p = 0.014 by a one-sided x^2 test) from the analysis of unmutated FM-derived clones (25,515 CDR nucleotides) that yielded no insertions or deletions (Table 2).

In the course of the analysis described above, we isolated one IgM clone containing a 6-nucleotide insertion into framework (FW)3 (see below). We believe that this clone is part of the mutated GC or memory repertoire because it contained 4 bp substitutions in addition to the insertion. In this study, the B cell populations analyzed were 95–98% pure, and the FM B cell subpopulation could therefore include between 2 and 5% contaminating clones, that is, IgM-expressing cells not from the naïve population that can therefore be somatically mutated. However, none of

![Figure 3. Polyacrylamide gel assay to identify insertions or deletions into V_H genes. (A) Phosphorimage of a polyacrylamide gel: each lane contains the hot-PCR products (32P-labeled) of the V_H gene and the CDR3 of an individual clone. (B) A comparison of the distribution of CDR3 sizes of the 485 CDR3s assayed to the distribution of 500 CDR3s observed in sequences from this report indicates that the clones assayed by electrophoresis were a polyclonal population. CDR3 sizes were measured from the most 3' Tyr residue (common to all V genes analyzed) to the most 5' C_m or C_g residue. CDR3 lengths for those assayed by electrophoresis were extrapolated based on sequencing of 75 out of the 485 clones assayed. The x-axis is the number of amino acids greater than the shortest CDR3 found.](image)

Table 2. Analysis of Unmutated FM cDNA Clones for Insertion or Deletion Events

| Clone type | Clones assayed | CDR nucleotides | Events observed | Frequency^§ | Expected (events/10^4 CDR nucleotides)^i |
|------------|----------------|-----------------|----------------|------------|-----------------------------------|
| Mutated V_H clones (GC and memory B cells) | 395 | 25,482 | 6 | 2.35 events/10^4 mn | |
| Unmutated clones: | | | | | |
| V_H4–FM, CDR1* | 265 | 5,565 | 0 | 0 | 1.31 |
| V_H6 IgM FM V_H genes* | 220 | 16,500 | 0 | 0 | 3.88 |
| V_H4 family FM sequences | 51 | 3,450 | 0 | 0 | 0.81 |
| Total unmutated values | 25,515 | | 0 | (P = 0.014)* | 2.35 events/10^4 CDR nucleotides |

*Clones analyzed by hot-PCR/PAGE assay as described in the text.

CDR nucleotides are those within the customary bounds of the CDR1 and CDR2. (See Materials and Methods for a more detailed explanation of this unit).

^iEvents per 10^4 CDR nucleotides.

^§Expected frequency (events/10^4 CDR nucleotides) derived from sequencing data: 6 events in 25,482 CDR nucleotides; 6/(25,482 CDR nucleotides/10^4) = 2.35.

*Statistical analysis: x^2 test for independence.
A Insertion events:

\[ \begin{align*}
W_6: & \quad \text{GTC TCT AGC} \quad \text{AAC AGT GCT} \\
W_{64}: & \quad \text{GTC TCT AGC AGG AAG AGT GCT} \\
W_{439}: & \quad \text{TAC TAC AAC} \quad \text{CCG TCC CTC} \\
W_{144}: & \quad \text{TAC TAC AAC CCC TCC CTC} \\
W_{139}: & \quad \text{TAC TAC AGC} \quad \text{TGG GGC TGG} \\
W_6: & \quad \text{TCC AAG AAC} \quad \text{--- CAG TCC} \\
tm121: & \quad \text{TCC AAG AAC AAG AAC CAG TCC}
\end{align*} \]

B Deletion events:

\[ \begin{align*}
W_{64}: & \quad \text{GGG AGC ACC TAC TAC AAC CCG} \\
g64: & \quad \text{GGG Acc Aag --- TAC ACG CCG} \\
W_{631}: & \quad \text{TCC ATG AGC AGT GGT GTG TAC} \\
g187: & \quad \text{TCC ATG AGC --- GGG GTG TAC} \\
W_{459}: & \quad \text{TAC ATG GGG AGC ACC AAC TAC} \\
g189: & \quad \text{TAC ATG GGG --- cCC AAC TAC} \\
W_{434}: & \quad \text{TAC TAC TGG AGC TGG ATC CCG} \\
g80: & \quad \text{agg Tac --- --- TGG ATC CCG}
\end{align*} \]

C Long duplication/insertion:

\[ \begin{align*}
W_{64}: & \quad \text{GGG AGC ACC TAC TAC AAC CCG} \\
g64: & \quad \text{GGG Acc Aag --- TAC ACG CCG} \\
W_{631}: & \quad \text{TCC ATG AGC AGT GGT GTG TAC} \\
g187: & \quad \text{TCC ATG AGC --- GGG GTG TAC} \\
W_{459}: & \quad \text{TAC ATG GGG AGC ACC AAC TAC} \\
g189: & \quad \text{TAC ATG GGG --- cCC AAC TAC} \\
W_{434}: & \quad \text{TAC TAC TGG AGC TGG ATC CCG} \\
g80: & \quad \text{agg Tac --- --- TGG ATC CCG}
\end{align*} \]

Figure 4. The insertions and deletions are related to the surrounding DNA sequence. (A) The insertions involve repetitions of the immediately adjacent sequence. (B) The deletions are deletions of tandem repeats. (C) The 18-base insertion in clone pg86 is a duplication of the adjacent sequence. N nucleotides that mutated before the duplication/insertion are indicated. Sequence data available from EMBL/GenBank/DDBJ under accession numbers AF013615 through AF013626.

Other Insertions and Deletions into \( \gamma \) Genes. We have observed similar instances of insertions and deletions into the coding regions of apparently functional immunoglobulin \( \delta \) genes, including: (a) a \( \gamma_6 \) IgM isolate containing a triplet duplication/insertion into the CDR1 in addition to several bp substitutions (Figs. 1C and 4A), which was derived from a human hybridoma secreting high affinity mAb against Bordetella pertussis (21, 22); (b) a 6-nucleotide insertion into the FW3 region of a mutated IgM \( \gamma_6 \) gene, representing the only insertion or deletion observed outside of the CDRs (Figs. 1C and 4A, clone tm121); and (c) an 18-nucleotide duplication/insertion into a human plasma cell cDNA transcript at the boundary between the FW1 and CDR1 (Figs. 1C and 4C), doubling the length of this hypervariable loop. The viability of clone pg86 was tested by expressing it in the baculovirus system in association with a \( \kappa \) light chain encoding construct (FS-6k; Fig. 5). The efficient expression, secretion, and pairing with light chain in the baculovirus system suggest that the product of clone pg86 is a functional heavy chain despite the large duplication/insertion.

The Insertions and Deletions Are Related to the Surrounding Sequence. As shown in Fig. 4, the insertions reported are duplications of the immediately adjacent sequence, and the deletions involve elements of repetitive tracts. In addition, a higher incidence of these events involve sequence motifs that resemble intrinsic hotspots of somatic hypermutation (12, 23–27): (a) four of eight events involved the serine codon AGC that has been reported as the “hottest” of hotspots (24–27) (Fig. 4, sequences HBp2, g187, g188, and g86); (b) two events involved TAC motifs (Fig. 4, g192 and g64); and (c) two events involved the motif AAC (Fig. 1, g144, and tm121). In general all of the clones found to contain insertions and deletions were highly mutated (Fig. 1). Several of these clones had bp substitutions clustered with the insertions or deletions (Figs. 1 and 4). The plasma cell transcript depicted in Fig. 4C contained an 18-nucleotide insertion that duplicated the 5' adjacent sequence. The central nine nucleotides of the duplicated sequence form a partial palindrome (..GGtGaCtCC..). This clone was mutated (G to A at position 80 and an A to T at position 85) before the duplication/insertion event, as these mutations were perpetuated in the inserted sequence.

Discussion.

Somatic modification of \( \gamma \) genes encoding immunoglobulin and T cell receptors recapitulates most mechanisms observed in the evolutionary diversification of DNA: (a) \( \gamma \) gene recombination, including imprecise junctions, P nucleotides, and untemplated N nucleotide addition; (b) gene conversion; and (c) bp substitutions in Ig somatic hypermutation. The insertion and deletion of nucleotides is another means for the evolutionary diversification of DNA, and has been proposed as an explanation for unusual \( \gamma \) gene sequences in the past (Table 3). In this study, we show that insertions and deletions are associated with the somatic hypermutation process.
Complexities of the Analysis of Insertions and Deletions into VH Genes. The formal characterization of these events has been a daunting task because of their low frequency, and the complexity of the germline VH repertoire. According to our study, these events occur in <2% of somatically mutated clones. As shown in Fig. 2, the primary variability between VH family members is 3–6-bp size variances in the CDR1s, which is comparable to the short insertions and deletions that we attribute to somatic hypermutation (in selected B cell populations). The similarity between evolutionary diversity and somatic diversification was expected, as the molecules are likely subject to the same functional and structural constraints. This has made it difficult to determine whether these events were generated somatically, versus germ-line encoded, or if they were artifacts of the experimental system: they could result from homologous recombination between alternate alleles or imperfect recombination between identical alleles, or they could have occurred during B cell replication independent of somatic hypermutation. In fact, VH genes may exhibit particularly unstable sequence characteristics evolved to help support both germ-line diversity and the generation of somatic mutations, as suggested by the identification of intrinsic hotspots of somatic hypermutation within the CDRs of VH genes (25, 26).

Perhaps the area of greatest contention in this complex system remains the possibility that these low frequency events are artifacts of the experimental manipulations performed, the AMV-RT, Taq, or PFU polymerases, and/or the cloning in E. coli. The Insertion/Deletion Events Are the Result of the Somatic Hypermutation Process. Our system addresses several key issues that associate the occurrence of insertions and deletions to the somatic hypermutation process. (a) Six of the nine insertions/deletions were identified within the VH family members, providing an experimental system that could be characterized extensively as described below. (b) All of the insertion/deletion events reported involved triplets or multiples of triplets, leaving the transcripts in frame and therefore functional, and eight of nine events reported were localized to the CDRs. As with somatic point mutations, no insertions or deletions were observed in the 80 to 120 nucleotides of constant region (C_{\mu} or C_{\gamma}) DNA sequenced with each cDNA clone. These hallmarks of somatic hypermutation and selection argue strongly that these events are not artifacts. (c) The B cell clones analyzed were processed and separated into highly pure, mutated B cell populations including GC (IgD-CD38+) and memory (IgD-CD38+) B cells, and an unmutated FM B cell population (IgD-CD38-), making it possible to focus our analysis on the mutated populations and use the unmutated population as a negative control, which in turn allows the statistical association of the observed insertion and deletions to the somatic hypermutation process (P = 0.014). In addition, the isolation of four of the insertion/deletion events from memory B cells provides evidence that these events did not result from artifacts related to contamination from endonucleolytically cleaved DNA from the apoptotic GC cells. (d) Seven of nine events reported in this study involved \gamma heavy chains that contain nearly twice the mutations of \mu heavy chains (4), further correlating the events described here to somatic hypermutation. (e) As discussed below, the insertion/deletion events described tended to involve sequence motifs resembling previously described hotspots of somatic hypermutation, providing evidence that these events occur by the same process. (f) Finally, we extensively analyzed the VH4 gene family of the tonsil donor at both the expressed and genomic levels, facilitating the assignment of the insertions/deletions as somatic rather than germline encoded. 6 of the clones with insertions and deletions were unique among 395 VH4 cDNA clones sequenced from a single tonsil, including many independent isolates of each of the VH4 genes expressed (Table 1). In addition, we were unable to isolate genomic templates for any of the insertion or deletion events either by PCR or through the extensive characterization of the genomic VH4 repertoire of the tonsil donor (Table 1). Templating of these events from any other VH4 gene family can also be ruled out as members of the seven human VH4 gene families differ significantly in the CDR sequences where the events described had occurred.

Structural and Functional Considerations of Insertions and Deletions into VH Genes. The events involving the insertion or deletion of a single amino acid from the CDR1 or CDR2 would not be expected to profoundly alter the backbone structure of these molecules, as the CDRs are the most malleable portions of antibodies. The clone g80 has two of the five amino acids that are customarily considered its CDR1 deleted, leaving only three amino acids to form this hypervariable loop (Fig. 1B). Thus, this is one of the shortest CDR1s reported to date. The clone tm121 has two amino acids inserted into the CDR3 region. The portion of the FW3 where this insertion occurred is believed to be solvent exposed and corresponds to the region where the B cell superantigen staphylococcal protein A binds to most VH3-encoded Ig molecules (28); therefore, it is likely that the insertion into this VH6 clone can be tolerated as a bulge on the molecule's surface. The most complex structural change observed in our study involved clone pg96, with a single amino acid insertion at the FW1/CDR1 junction that would presumably double the length of this hypervariable loop and require dramatic structural accommodation. However, we were able to express this heavy chain and found it paired with light chain, indicating that it is likely functional (Fig. 5). The clone HBp2, containing a triplet insert into its CDR1, is particularly interesting because it has a known specificity. This VH6 gene was isolated from a human B cell hybridoma with anti- Bordetella pertussis specificity (21, 22). Clone HBp2 has also been expressed in the baculovirus system and is fully functional. We are currently performing mutational analysis of this heavy chain molecule to determine if the additional inserted amino acid plays a role in the affinity and/or specificity of this antibody.

A Analysis of Insertions and Deletions Reported in the Literature. Various groups have reported a number of insertion and deletion events (Table 3). Virtually all of the insertions
| Name                        | Source Description | Ins/Del (position) | Relation to surrounding sequence | References |
|-----------------------------|--------------------|-------------------|-----------------------------------|-------------|
| **Selected populations or coding regions:** |                     |                   |                                   |             |
| L4-1e                       | Human VH4–34 (4.21) | ACC insert (within CDR2) | 4-34: AGC ACC AAC (RT) 38 |             |
| 3B62                        | Murine VH 186.2    | GTT deletion (CDR2) | VH186.2: AGT GGT GGT ACT (RT) 39 |             |
| **Unselected populations or untranslated regions:** |                     |                   |                                   |             |
| 3B62                        | Murine VH 186.2/D/JH2 to JH4 | ACT deletion (3' untranslated) | GL: GTG ACT ACT TTG (RT) 40 |             |
| 3B44                        | Murine VH 186.2    | 4 single-base deletions (leader intron) | VH186.2: GCC, GGT (RT) 39, 40 |             |
| M167                        | Murine VH 107/DFL16.1/JH1 | 2 single-base insertions (leader intron) | GL: ATAG AAGATTAGTAG (RT) 41 |             |
|                             |                    | (3' untranslated)  | M167: ATAGTAAGATTAGTAG           |             |
|                             |                    |                   | M167: TTTGAGGTAGTAGA             |             |
|                             |                    | 5 single-base deletions | M167: GCTTTC TGTA...CCGAGAAAAGA (IR) |             |
|                             |                    | (all in 3' untranslated region) | M167: GCTTTC TGTA...CCGAGAAAAGA (IR) |             |
|                             |                    |                   | M167: CTATTG (RT)                |             |
|                             |                    |                   | M167: TCAT GG                    |             |
|                             |                    |                   | M167: GT ACTACTTTGACTACTG (RT)   |             |
| Name             | Source                  | Ins/Del (position)                                      | Relation to surrounding sequence                                      | Reference |
|------------------|-------------------------|--------------------------------------------------------|------------------------------------------------------------------------|-----------|
| TA deletion (3') | None found (possible hotspot) | GL: TCTGTTGCTGTTGAT (RT)                               | M167: TCTGTT GTAT                                                     |           |
| GTGT deletion (leader intron) | GL: GTGTGTGTGTTT (RT) | M603: TTTTCCTGCTGTGTTT                                  | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    |           |
| M603             | Murine VnS10.7/DFL16.1/JH1 | TC Deletion (leader intron) GL: GTGTGTGTGTTT (RT)       | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    | 42, 41    |
| AAAT deletion (3' Ei/MAR) | GL: AAAT | M603: GCATTCTAAAAATAGTGAGGA (IR)                       | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    |           |
| MC101            | Murine VnQ52/D/Jn 3     | GG deletion (3’ untranslated) GL: AAACGCGAATC (RT)      | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    | 42, 43    |
| M511             | Murine Vn167/Jk5       | GAA deletion (3’ untranslated) GL: TTTGAAGATAAA (RT)    | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    | 42, 44    |
| H37-65           | Murine Vk V521E/Jk1-Jk2 | 11 base deletion (Jk1/Jk2 intron) GL: AGGGACACCAGTGTTGTTACAC (IL) | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    | 45        |
| 296.4C11, 253.12D3 | Murine JkC intron     | 7 base deletion and a 154 base deletion GL: CTTGAAAGAT..(N 30)...CAGATCAAG (RT) | M603: GCATTCTAAAAATAGTGAGGA (IR)                                    | 46        |
| 2G7              | Murine transgene       | single base deletion, and a 49 nucleotide deletion     | (R repeats form ends of deleted “loop”) (IL)                         | 47        |
| 85k              | Human myeloma Vn genes | single-base (T) insertions into the CDR1/FW2 junction rendering genes out-of-frame | N o relation, however, event followed the proposed hotspot motif TAC | 48        |
| HF-1 clone A6:   | Human lymphoma (Jn untranslated) | AG insertion into Vn3' untranslated region               | Consensus: GGGCCAG GGC (RT)                                           | 49        |
| several          | Human lymphoma (Jn untranslated) | 30 base deletion                                      | clone A6: GGGCCAGAGGCG                                              |           |

RT, repetitive tract; IR, inverted repeat (loop with local DNA); IL, internal loop. *Secondary structure reported by Golding et al. (29). ‡This study is difficult to interpret in the context of the current report as the genomic Jn locus was not available. The 10 clones were only 80% homologous to the closest Jn locus reported in the literature with most alterations being similar between all of the isolates. Therefore, only 2 of the 26 proposed insertions/deletions can be attributed to somatic mutation with certainty, as they were unique to the consensus of the individual clones.
Somatic Hypermutation.
The evidence for the involvement of repetitive elements in the daughter strand, or a deletion event reported in our study resembled one of these sequence elements that can be predicted to cause secondary structural changes in the DNA seems to be a hallmark of somatically mutated VH genes. Insertions and deletions reported from somatically mutated V genes involved the untranslated regions or occurred in silent passenger transgenes. 19 out of 25 insertions or deletions into somatically mutated genes involved predominantly repetitive elements, or in several cases other sequence patterns associated with secondary structures such as internal homologies or inverted repeats (Table 3). With the inclusion of the 9 events described in this work, 28 out of 34 insertions and deletions involved such elements. Thus, the proximity of sequence elements that can be predicted to cause secondary structural changes in the DNA seems to be a hallmark of insertions and deletions into somatically mutated V<sub>H</sub> genes.

A possible correlation to intrinsic hotspots. A higher frequency of somatic hypermutation has been reported for immunoglobulin VH genes in freshly sorted GC B cells (CD38<sup>−</sup>CD19<sup>−</sup>) compared to mantle zone B cells (CD38<sup>+</sup>CD19<sup>+</sup>); reference 34). In a recent study by T ran et al. (35), it was shown that tract instability of homonucleotide runs associated with mismatch repair defects occurs more frequently in long than in short runs. These authors suggested that if loop intermediates occur in long repetitive tracts (>8 bp for a homonucleotide run) they could involve a distal repetitive element out of reach of the polymerase proofreading activity and only be subjected to mismatch repair. However, for short repetitive tracts, as for the events reported in this analysis, loop intermediates can only occur proximal to the polymerase complex and are therefore subjected to both polymerase proofreading and mismatch repair mechanisms.

All 9 events in this analysis, and 19 out of 25 events from the literature (28 out of 34 insertions and deletions reported), appeared to result from secondary structural intermediates. Loop intermediates proximal to the polymerase complex during DNA polymerization should be repaired by the polymerase proofreading mechanisms immediately, or by the postreplicative DNA repair systems. This analysis suggests the following characteristics for the polymerization process during somatic hypermutation. (a) The polymerase interacts with the V locus in a particularly unstable or “loose” fashion, especially when hotspot motifs or elements capable of forming secondary structures are encountered, allowing bp substitutions in most instances, and insertions or deletions via polymerase slippage at a much lower frequency; (b) it has limited proofreading capabilities; and (c) there is a downregulation of postreplicative mismatch repair. An efficient mean to downregulate mismatch repair during somatic hypermutation could be through the lack of differentiation of the template and progeny strands for the mismatch repair system; lack of strand differentiation has been shown to increase the rate of mutations introduced (36).

The analysis of selected populations may have influenced this tendency because seven out of eight of these events occurred in the CDRs where it has been shown that hotspot motifs are preferentially found (25, 26). Furthermore, only a weak correlation to hotspots could be found for the previously reported insertions/deletions involving unselected regions of V loci (Table 3). However, the single event found in this analysis that occurred outside of the CDRs in FW3 (clone tm121, Figs. 1C and 4A), also involved a tandem of possible hotspots (AAAG, AAC). A more extensive and directed analysis is required to fully address this issue.

Implications for the Molecular Mechanism of Somatic Hypermutation. The instability of repetitive tracts during DNA replication is a hallmark of defects in postreplicative mismatch repair (33), and the locus-specific downregulation of DNA mismatch repair in response to UV irradiation has recently been reported for immunoglobulin V<sub>H</sub> genes. Loop intermediates proximal to the polymerase complex rather than multiple systems (proofreading and mismatch repair) allow bp substitutions in most instances, and insertions or deletions via polymerase slippage at a much lower frequency. The polymerase interacts with the V locus in a particularly unstable or “loose” fashion, especially when hotspot motifs or elements capable of forming secondary structures are encountered, allowing bp substitutions in most instances, and insertions or deletions via polymerase slippage at a much lower frequency; (b) it has limited proofreading capabilities; and (c) there is a downregulation of postreplicative mismatch repair. An efficient mean to downregulate mismatch repair during somatic hypermutation could be through the lack of differentiation of the template and progeny strands for the mismatch repair system; lack of strand differentiation has been shown to increase the rate of mutations introduced (36). Such a system would be advantageous for the locus-specific V gene somatic hypermutation in that it could involve alterations of a single enzymatic complex (polymerase complex) rather than multiple systems (proofreading and mismatch repair). Another system, which would have the
same advantage, i.e., the alteration of a single complex, would be the alteration of a DNA repair system such as transcription-coupled repair to be the somatic mutator, as suggested in recent studies (13). Alternatively, the insertions and deletions might result solely from a downregulation of postreplicative mismatch repair at the V locus in the rapidly proliferating centroblasts that are undergoing somatic hypermutation or due to a polymerase enzyme with a high fault rate as to overwhelm any repair.

All currently accepted models of somatic hypermutation, whether related to DNA excision-repair–like systems or transcription-repair, or to DNA polymerization or reverse transcription, involve transcriptional activation involving α-factors in the V locus (enhancers, etc.) followed by the activity of unknown polymerase enzymes of some type. This analysis does not refute or corroborate any of these models directly, but it does provide further characterization of the polymerization system involved, based on the types of mutations observed and on the molecular biology that is known to cause such mutations. This analysis and the model presented here provide further information or criteria to be contemplated as the various possible polymerase systems involved are considered.

Conclusions. Insertions and deletions into immunoglobulin V<sub>H</sub> genes during somatic hypermutation are additional means by which the immunoglobulin repertoire can be diversified. These events display characteristics supporting models of somatic hypermutation involving a particularly unstable or error-prone polymerase to allow the introduction of mutations, and involving the downregulation of DNA repair to allow the perpetuation of these mutations. Additionally, we show that these events tend to involve sequence motifs resembling intrinsic hotspots of somatic hypermutation, suggesting that the polymerase complex is destabilized in a sequence-specific manner to allow preferential mutation at these sequence elements.

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References

1. Clark, E.A., and J.A. Ledbetter. 1994. How B and T cells talk to each other. Nature. 367:425–428.
2. Dorken, B., P. Moller, A. Pezzutto, R. Schwartz-Albiez, and G. Moldenhauer. 1989. B-cell antigens. In Leukocyte Typing IV. A.J. McMichael, editor. Oxford University Press, London. pp. 131–140.
3. Ling, N.R., I.C.M. MacLennan, and D.Y. Mason. 1987. B-cell and plasma cell antigens new and previously defined clusters. In Leukocyte Typing III. A.J. McMichael, editor. Oxford University Press, London. pp. 302–308.
4. Pascual, V., Y.J. Liu, A. Magalski, O. de Bouteiller, J. Banchereau, and J.D. Capra. 1994. Analysis of somatic mutation in five B cell subsets of human tonsil. J. Exp. Med. 180:329–339.
5. Liu, Y.J., O. de Bouteiller, C. Arpin, F. Brierie, L. Galibert, S. Ho, H. Martinez-Valdez, J. Banchereau, and S. Lebecque. 1996. Normal human IgD+ IgM− germinal center B cells can express up to 80 mutations in the variable region of their IgD transcripts. Immunity. 4:603–613.
6. Denepoux, S., D. Razanajaona, D. Blanchard, G. Meffre, J.D. Capra, J. Banchereau, and S. Lebecque. 1997. Induction of somatic mutation in a human B cell line in vitro. Immunity. 6:35–46.
7. Berck, C. 1993. Somatic mutation and memory. Curr. Opin. Immunol. 5:218–222.
8. Kelsoe, G. 1996. Life and death in germinal centers (redux). Immunity. 4:107–111.
9. Liu, Y.J., G.D. Johnson, J. Gordon, and I.C.M. MacLennan. 1992. Germinatal centres in T-cell-dependent antibody responses. Immunol. Today. 13:17–21.
10. MacLennan, I.C.M., Y.J. Liu, and G.D. Johnson. 1992. M aturation and dispersal of B-cell clones during T-cell-dependent antibody responses. Immunol. Rev. 126:143–161.
11. McKeen, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. Proc. Natl. Acad. Sci. USA. 81:3180–3186.
12. Neuberger, M.S., and C. Milstein. 1995. Somatic hypermutation. Curr. Opin. Immunol. 7:248–254.
13. Storb, U. 1996. The molecular basis of somatic hypermutation of immunoglobulin genes. Curr. Opin. Immunol. 8:206–214.
14. Liu, Y.J., O. de Bouteiller, C. Arpin, I. Durand, and J. Banchereau. 1994. Five human mature B cell subsets. Adv. Exp. Med. Biol. 355:289–296.
15. Marks, J.D., M. Tristem, A. Karpas, and G. Winter. 1991. Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable genes and design of family-specific oligonucleotide probes. Eur. J. Immunol. 21:985–991.
16. Sanz, I., P. Kelly, C. Williams, S. Scholl, P. Tucker, and J.D. Capra. 1989. The smaller human V<sub>H</sub> gene families display remarkably little polymorphism. EMBO (Eur. Mol. Biol. Organ.) J. 8:3741–3448.
17. Potter, K.N., Y.C. Li, and J.D. Capra. 1994. The cross-reac-
tive idiotopes recognized by the monoclonal antibodies 9G4 and LC1 are located in framework region 1 of two non-overlapping subsets of human \( V_\gamma 4 \) family encoded antibodies. Sand J. Immunol. 40:43–49.

18. Tomlinson, I.M., G. Walter, J.D. M arks, M.B. Llewelyn, and G. W inter. 1992. The repertoire of human germline \( V_\gamma \) sequences reveals about fifty groups of \( V_\gamma \) segments with different hypervariable loops. J. Mol. Biol. 227:776–798.

19. U mar, A., and P.J. Gearhart. 1995. Reciprocal homologous recombination in or near antibody VDJ genes. Eur. J. Immunol. 25:2392–2400.

20. Ford, J.E., M.G. M cHeyzer-Williams, and M.R. Lieber. 1994. Chimeric molecules created by gene amplification interfere with the analysis of somatic hypermutation of murine immunoglobulin genes. Gene. 142:279–283.

21. Andris, J.S., B.R. Brodeur, and J.D. Capra. 1993. Molecular characterization of human antibodies to bacterial antigens: utilization of the less frequently expressed VH2 and VH6 heavy chain variable region gene families. Mol. Immunol. 30:1601–1616.

22. Brodeur, B.R., J. H amel, D. Martin, and P.R. Rondeau. 1991. Biological activity of a human monoclonal antibody to Bordetella pertussis lipopolysaccharide. Hum. Antib. Hybrid. 2:194–199.

23. Betz, A.G., C. R ada, R. Pannell, C. Milstein, and M.S. N euberger. 1993. Passenger transgenes reveal intrinsic specificity of the antibody hypermutation mechanism: clustering, polarity, and specific hot spots. Proc. Natl. Acad. Sci. USA. 90:2385–2388.

24. R ogozin, I.B., and N.A. K olchanov. 1992. Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighboring base sequences on mutagenesis. Biophys. Biophys. Acta. 1171:11–18.

25. G oyenechea, B., and C. Milstein. 1996. Modifying the sequence of an immunoglobulin V-gene alters the resulting pattern of hypermutation. Proc. Natl. Acad. Sci. USA. 93:13979–13984.

26. W agner, S.D., C. Milstein, and M.S. N euberger. 1995. Codon bias targets mutation. Nature. 376:732–733.

27. Smith, D.S., G. C readon, P.K. Jena, J.P. Portanova, B.L. K otzin, and L.J. W ysocki. 1996. Di- and trinucleotide target preferences of somatic mutagenesis in normal and autoreactive B cells. J. Immunol. 156:2642–2652.

28. Potter, K.N., Y. Li, and J.D. Capra. 1996. Staphylococcal protein A simultaneously interacts with framework region 1, complementarity-determining region 2, and framework region 3 on human \( V_\gamma 3 \)-encoded \( lgs \) J. Immunol. 157:2982–2988.

29. G olding, G.B., P.J. G earhart, and B.W. G lickman. 1987. Patterns of somatic mutations in immunoglobulin variable gene segments. Genetix. 115:169–176.

30. Streisinger, G., Y. O kada, J. E mrich, J. N ewton, A. T sugita, E. T erzaghi, and M. I nouye. 1966. Frameshift mutations and the genetic code. Th is paper is dedicated to Professor T heodosius D ozhansky on the occasion of his 66th birthday. Cold S pring Har bor. Symp. Q uant. Biol. 31:77–84.

31. R ipley, L.S. 1990. Frameshift mutation: determinants of specificity. Annu. Rev. Genet. 24:189–213.

32. M odrich, P., and R. L ahue. 1996. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem. 65:101–133.

33. S trand, M., T.A. Prolla, R.M. L iskay, and T.D. P etes. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature. 365:274–276.

34. F airhurst, R.M., Y. V alles-A youb, M. N eshat, and J. B rau. 1996. A DNA repair abnormality specific for rearranged immunoglobulin variable genes in germinal center B cells. Mol. Immunol. 33:231–244.

35. T ran, H.T., J.D. Keen, M. K ricker, M.A. R esnick, and D.A. G ordin. 1997. Hypermutability of homonucleotide runs in mismatch repair and DNA polymerase proofreading yeast mutants. Mol. Cell Biol. 17:2859–2865.

36. M acPhee, D.G. 1996. Mismatch repair as a source of mutations in non-dividing cells. Genetica. (The H agaeul. 97:183–195.

37. M atsuda, F., and T. H onjo. 1996. Organization of the human immunoglobulin heavy-chain locus. Adv. Immunol. 62:1–29.

38. D unn-W alters, D.K., P.G. I saacson, and J. S pencer. 1997. Sequence analysis of human IgV genes indicates that Ieal lamina propria plasma cells are derived from Peyer's patches. Eur. J. Immunol. 27:463–467.

39. B oth, G.W., L. T ailor, J.W. P olland, and E.J. S teele. 1990. Distribution of mutations around rearranged heavy-chain antibody variable-region genes. Mol. Cell Biol. 10:5187–5196.

40. A llen, D., T. S imon, S. S altitzky, K. R ajewsky, and A. C umano. 1988. Antibody engineering for the analysis of affinity maturation of an anti-hapten. EMBO (Eur. Mol. Biol. Org. j.) 7:1995–2001.

41. K im, S., M. D avis, E. S inn, P. P atten, and L. H ood. 1981. Antibody diversity: somatic hypermutation of rearranged \( V_\gamma \) genes J. Exp. Med. 152:1717–1727.

42. L ebecque, S.G., and P.J. G earhart. 1990. Boundaries of somatic mutation in rearranged immunoglobulin genes: 5’ boundary is near the promoter, and 3’ boundary is approximately 1 kb from V(D)J gene. J. Exp. Med. 172:1717–1727.

43. K ataka, T., T. N ikaido, T. M iyata, K. M oriwaki, and T. H onjo. 1982. The nucleotide sequences of rearranged and germline immunoglobulin \( V_\gamma \) genes of a mouse myeloma MC101 and evolution of \( V_\gamma \) genes in mouse. J. Biol. Chem. 257:277–285.

44. G earhart, P.J., and D.F. B ogenhagen. 1983. Clusters of point mutations are found exclusively around rearranged antibody variable genes Proc. Natl. Acad. Sci. USA. 80:3439–3443.

45. R ickert, R., and S. C harles. 1993. Low frequencies of somatic mutation in two expressed V kappa genes: unequal distribution of mutation in 5’ and 3’ flanking regions Int. Immunol. 5:255–263.

46. W eber, J.S., J. B erry, S. L itwin, and J.L. C laflin. 1991. Somatic hypermutation of the JC intron is markedly reduced in unarranged kappa and H alpha and is unevenly distributed in rearranged alleles J. Immunol. 146:3218–3226.

47. R ogerson, B., J. H ackett, J.R., A. P eters, D. H aasch, and U. S torb. 1991. Mutation pattern of immunoglobulin transgenes is compatible with a model of somatic hypermutation in which targeting of the mutator is linked to the direction of DNA replication. EMBO (Eur. Mol. Biol. Org.) J. 10:4331–4341.

48. K osmas, C., N.A. V iniou, K. S tamatopoulos, N.S. C ourtenay-L uck, T. P apadaki, P. K ollia, G. P arterakis, D. A nagnostou, X. Y ataganas, and D. L oukopoulos. 1996. Analysis of the kappa light chain variable region in multiple myeloma. Br. J. H aematol. 94:306–317.

49. W u, H.Y., and M. K arntinen. 1995. The somatic hypermutation activity of a follicular lymphoma links to large insertions and deletions of immunoglobulin genes. Sand J. Immunol. 42:52–59.