Myeloma V<sub>L</sub> and V<sub>H</sub> Gene Sequences Reveal a Complementary Imprint of Antigen Selection in Tumor Cells

By Surinder S. Sahota, Regine Leo, Terry J. Hamblin, and Freda K. Stevenson

In multiple myeloma, sequence studies of V<sub>H</sub> genes used to encode clonal Ig in neoplastic plasma cells have shown a common pattern of extensive somatic hypermutation. A further consistent feature of these V<sub>H</sub> sequences is a complete lack of intraclonal variation. These findings indicate that the malignant cell arises at a mature, postfollicular stage of B-cell development. However, only a minority of cases have a distribution of somatic mutations in V<sub>H</sub> consistent with a prior role for antigen in selecting the B cell of origin. To complement these studies, and to take further the investigation of a role for antigen in the clonal history of myeloma, we have investigated tumor-derived V<sub>L</sub> sequences from bone marrows of 15 patients. All sequences (9V<sub>C</sub> and 6V<sub>A</sub>) were potentially functional and 5 of 15 had evidence for N-region additions. All had undergone extensive somatic hypermutation, and showed no intraclonal variation. In 4 of 15 cases, the distribution of mutations revealed a significant (P < .05) clustering of replacement mutations in the CDR sequences, indicating a role for V<sub>L</sub> in selection by antigen. Comparison with the V<sub>H</sub> sequences used by the same tumor cells showed that, if significant clustering was present, it was in either V<sub>H</sub><sub>4</sub> or V<sub>H</sub><sub>5</sub>, but not both. Altogether, 10 of 15 V-regions showed evidence for antigen selection, suggesting that the B cell of origin has behaved as a normal germinal center B cell. Deductions concerning a role for antigen selection may require both V<sub>H</sub> and V<sub>L</sub> sequences for validation.

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MATERIALS AND METHODS

Patients and cell preparation. Heparinized BM aspirates from unselected patients with multiple myeloma at different stages of the tumor cell. If the cell of origin has been through somatic hypermutation, and antigen selection, before neoplastic transformation, this experience should be reflected in the V-gene sequences. For V<sub>H</sub> regions of antibody molecules, it is known that recognition of antigen can involve several sites, with CDR3 having a major influence. However, replacement mutations in CDR1 and CDR2 have a significant role in affinity maturation. For B-cell tumors, where the putative antigen is generally unknown, it is difficult to estimate involvement of CDR3 in recognition. In contrast, the possible clustering of replacement mutations in CDR1 and CDR2 which could be involved in affinity maturation can be analyzed. Rules to assess the significance of apparent clustering of replacement mutations compared with silent mutations have been developed. When these rules were applied to the large panel of V<sub>H</sub> sequences from myeloma cells, only a minority of cases (10 of 52) showed statistically significant clustering in CDRs. However, the antigen-binding site is known to involve both VH and VL, and we have investigated V<sub>L</sub> sequences from a group of 15 patients both to extend our knowledge of VL and VH gene usage in myeloma, and to assess the role of V<sub>L</sub> in the selection of the cell of origin by antigen.

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Somatic mutation. 
performed from each sample. Haven, CT). At least two independent PCR amplifications were 
GenBank and V-BASE sequence directories 21 using MacVector 4.0 somatic mutations in each sequence (Table 5) has been car-

* strand cDNA synthesis kit (Pharmacia). A sample of the cDNA (1/ mutations were identified in the V L sequences, with 9 of ... by polymerase chain reaction (PCR) having additional identifiable mutations in J L , even though using a mixture of 5 

transcription was carried out with an oligo dT primer, using a ®rst- sequences are shown in Figs 2 and 3. In all cases, somatic 
m mutations are shown in Tables 3 and 4. Deduced amino acid 
m to 10 k 

RNAzol B (Cinna Biotecx Labs Inc, Houston, TX). Reverse mutations are shown in Tables 3 and 4. Deduced amino acid 
m k 

same light chain type (9 

had an identifiable monoclonal Ig in serum (13 IgG, 2 IgA) of the available for V 

Clinical and laboratory features are shown in Table 1. All patients are known to be insigni®cant in V 

V 

k 

indicated in Tables 3 and 4 for V 

V 

sequences were seen in all cases, at variable frequency, as 

repeated identical V L-J L 

the tumor cells were identi®ed as repeated identical V L-J L 

aspirates, 20 were different from each other. Repeated se-

MNC) were isolated by centrifugation on Ficoll- signi®cantly from the closest germline genes in the ... 3 and 4), with a mean percent mutation of 5.3 forment was assessed by direct immuno¯uorescent staining for surface V 

V 

k 

have been submitted to the EMBL/GenBank database (ac-

* Durie and Salmon staging. 

Patient Stage* Status Class Level (g/L) Cells in BM

1 

IIIA 

Relapsed progressive 

IgGk 

24.0 

11.0 

2 

IIA 

Stable 

IgGk 

18.0 

21.0 

3 

II 

Progressive 

IgGk 

43.0 

5.0 

4 

II 

Stable 

IgGk 

28.0 

30.0 

5 

III 

Progressive 

IgGk 

15.0 

18.0 

6 

IIIA 

Presentation 

IgGk 

59.0 

14.0 

7 

IIIA 

Progressive 

IgGk 

71.0 

30.0 

8 

IB 

Presentation 

IgGk 

40.7 

60.0 

9 

III 

Progressive 

IgGk 

48.0 

30.0 

10 

IIIA 

Progressive 

IgGl 

20.0 

19.0 

11 

I 

Stable 

IgGl 

16.0 

4.0 

12 

III 

Progressive 

IgGl 

60.0 

5.0 

13 

III 

Progressive 

IgGl 

21.0 

10.0 

14 

I 

Progressive 

IgGl 

3.0 

1.8 

15 

IIIA 

Presentation 

IgGk 

79.0 

50.0 

RESULTS 

V 

k 

gene usage by tumor cells. The V 

k 

genes used by the tumor cells were identi®ed as repeated identical V L-J L 

sequences obtained after cloning of PCR products.4 Re-

remaining sequences, presumably from normal B cells in the aspirates,20 were different from each other.Repeated 

sequences were seen in all cases, at variable frequency, as indicated in Tables 3 and 4 for V 

V 

k 

and V 

l 

respectively. The pro®le of V 

k 

genes used by the 9 

k-positive tumors indicates that 5 of 9 use V 

k1 

and 4 of 9 V 

kII 

frequencies in line with normal B cells.22,23 Among the V 

k1 

group, 4 of 5 use the O8/18 gene, which is commonly rearranged in B-cell tumors,24 and 3 of 4 of the V 

kIII 

group use the A27 gene, found to be used frequently in chronic lymphocytic leukemia.25 The V 

k 

genes (Table 4) used three different families. There appeared to be no preferential use of particular 

J 

k 

genes for either light chain type. 

V-J joining region. Clonality of tumor-derived sequences was con®rmed by analysis of the V-J junction (Fig 1), which showed intraclonal identity. In 9 of 15 sequences, there were base additions at the junction which were not encoded by the V or J genes. In some cases, these appeared to be derived from flanking regions of the genes, and could therefore be accounted for by imprecision at the joint. In 5 of 15, there were additional nucleotides which may represent N-region additions, contributed by TdT activity. In a majority (11 of 15) of cases, nucleotides had been lost by trimming from either V or J genes. 

Somatic mutation. Nucleotide sequences of all V 

k 

genes have been submitted to the EMBL/GenBank database (ac-

cession numbers Z70253-255; Z70258-261; Z70263-264; Z75558;X98894-898). To assess the degree of somatic hypermutation, comparison with the closest counterparts in the database of germline sequences has been made. This does not take into account any polymorphisms in V 

k 

, but these are known to be insignificant in V 

k.26 Less information is available for VA genes, but again suggests only limited polymorphic variation.27 The V 

k 

sequences obtained deviated signi®cantly from the closest germline genes in the database (Tables 3 and 4), with a mean percent mutation of 5.3 for V 

k 

and 6.2 for V 

l 

There was evidence for block mutations, involving two or more adjacent nucleotides, in both light-

chain types. For V 

k 

the high incidence of block mutations (6 of 9 sequences [67%]) compares with the reported ®gure of ~50%.28 The numerical distribution of the mutations in FWRs and CDRs, and the ratio of replacement to silent mutations are shown in Tables 3 and 4. Deduced amino acid sequences are shown in Figs 2 and 3. In all cases, somatic mutations were identi®ed in the V 

k 

sequences, with 9 of 15 having additional identi®able mutations in J 

k 

even though events at the 3’ end of J 

k 

are obscured by the primer sites. Several sequences derived from the same V 

k 

family member were available for the V 

k 

genes O8/18 and A27. Comparison of these showed no evidence for common sites or “hot-

spots” of mutational activity. Analysis of the distribution of somatic mutations in each sequence (Table 5) has been car-

<table>
<thead>
<tr>
<th>Primer</th>
<th>Location</th>
<th>Orientation</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
</table>
| V
k1&4 | FR1 | Sense | GACATCSWGATGACCCACGTCTCC |
| V
k2&6 | FR1 | Sense | GAWRTTGTGACTGACTAGGTCTCC |
| V
k3 | FR1 | Sense | GAAATTGTGTTGACGCAGTCTCC |
| V
k5 | FR1 | Sense | GAWRTTGTGACTGACTAGGTCTCC |
| J
k3-4 | FR4 | Anti-sense | ACCTTTAATCCAGTGTTGCTCC |
| J
k6 | FR4 | Anti-sense | ACCTTTAATCCAGTGTTGCTCC |
| V
k1 | FR1 | Sense | CAGGTTCTGCTACCCGACTGTC |
| V
k2 | FR1 | Sense | CAGGTTCTGCTACCCGACTGTC |
| V
k3 | FR1 | Sense | CAGGTTCTGCTACCCGACTGTC |
| V
k7&8 | FR1 | Sense | CAGGTTCTGCTACCCGACTGTC |
| V
k9 | FR1 | Sense | CAGGTTCTGCTACCCGACTGTC |
| J
kC | FR4 | Anti-sense | ACCAGGACCGTSASTKGGTSCC |
ried out by the method of Chang and Casali.\textsuperscript{13} In this method, each V\textsubscript{L} or V\textsubscript{H} gene sequence is assessed codon by codon for significance of deviation from germline sequence. A modification of the binomial distribution model is then used to calculate whether the probability ($P$ in Tables 5 and 6) of an excess (in CDRs) or scarcity (in FWRs) of replacement mutations resulted by chance alone.\textsuperscript{17} For the FWRs, there were generally fewer replacement (R) mutations than expected due to chance, with significant ($P < .05$) conservation of sequence in 10 of 15 sequences, a feature commonly seen for V\textsubscript{H}.\textsuperscript{14} For the CDRs, there were more R mutations than expected due to chance alone, with significant ($P < .05$) clustering indicative of antigen selection in 4 of 15 (3V\textsubscript{K} and 1V\textsubscript{\lambda}).

Comparison with V\textsubscript{H} genes. For 6 cases (patients 1, 3, 6, 8, 14, and 15), tumor-derived V\textsubscript{H} gene sequences were known.\textsuperscript{4,27} V\textsubscript{H} sequences from the remaining 9 patients were obtained as described\textsuperscript{4} and deduced amino acid sequences are shown in Fig 4. Nucleotide sequences have been submitted to EMBL/GenBank database (accession numbers: Z70256-257; Z75556-5557; X98899-99003). Although the closest germline gene has been obtained from the database, rather than from the patients’ DNA, it appears that in general polymorphism in V\textsubscript{H} is not sufficient to require this approach.\textsuperscript{21} In fact, where we\textsuperscript{4} and others\textsuperscript{4} have analyzed the patients’ germline V\textsubscript{L} genes, the sequence has been found in the majority of cases to correspond exactly to that obtained from the database. However, in 1 of 9 cases of myeloma, a 2-bp difference from the published sequence of a VII-5 germline gene was found also in the patient’s germline sequence, indicating that this was probably caused by a polymorphism.\textsuperscript{5} The distribution of somatic mutations in V\textsubscript{H} of 6 of 15 of these cases indicated a significant clustering in CDRs (Table 6). Comparison of patterns in V\textsubscript{H} with those in V\textsubscript{L} (Table 6) showed that clustering in CDRs of V\textsubscript{H} was not paralleled by clustering in CDRs of V\textsubscript{L}. In addition, the clustering in V\textsubscript{H} observed in 4 of 15 cases was not paralleled by clustering in V\textsubscript{L}. Therefore, from the 10 cases where clustering was evident, it was localized in either V\textsubscript{H} or V\textsubscript{L}, but not both. However, in 5 of 15 cases, there was no significant clustering in either V\textsubscript{H} or V\textsubscript{L}.

DISCUSSION

Analysis of V-genes used by neoplastic B cells is extending our understanding of the origin and progression of B-cell tumors. Now that a complete map of the V\textsubscript{H} gene germline repertoire is available,\textsuperscript{21,30} it is possible to compare a V\textsubscript{H} sequence from a tumor cell to the germline gene of origin with confidence. Although some nucleotide changes may reflect polymorphic variation, particularly for certain V\textsubscript{H}3 genes,\textsuperscript{31} it can be assumed that the majority of deviations from germline sequence in V\textsubscript{H} genes of a B cell represent somatic mutations.\textsuperscript{21} In some cases this has been proved by comparing the tumor-derived sequence with the germline counterpart in the patient.\textsuperscript{13,33} Accumulation of such mutations indicate that the cell of origin has been exposed to the hypermutation mechanism in the germinal center.\textsuperscript{11,18,32} Heterogeneity of mutations within the tumor clone indicates that the tumor cell is still under the influence of the mutation mechanism, subsequent to neoplastic transformation.\textsuperscript{9,10} Finally, concentration of replacement mutations in CDRs can suggest a role for antigen in selection of the B cell.\textsuperscript{17,33}

In the case of multiple myeloma, V\textsubscript{H} gene analyses from several laboratories have shown that the malignant cell has

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Ig Light Chain</th>
<th>V\textsubscript{L} Family</th>
<th>GL Donor</th>
<th>% Homology</th>
<th>R/S Mutations</th>
<th>Tumor-Derived Sequences/ Clones Sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FWR</td>
<td>CDR</td>
</tr>
<tr>
<td>10</td>
<td>\lambda</td>
<td>V\textsubscript{I}</td>
<td>DPL2</td>
<td>95.6</td>
<td>3/3</td>
<td>4/2</td>
</tr>
<tr>
<td>11</td>
<td>\lambda</td>
<td>V\textsubscript{I}</td>
<td>DPL3</td>
<td>91.0</td>
<td>3/6</td>
<td>9/6</td>
</tr>
<tr>
<td>12</td>
<td>\lambda</td>
<td>V\textsubscript{II}</td>
<td>IGLV3S2</td>
<td>96.5</td>
<td>0/2</td>
<td>6/1</td>
</tr>
<tr>
<td>13</td>
<td>\lambda</td>
<td>V\textsubscript{I}</td>
<td>DPL11</td>
<td>95.9</td>
<td>2/4</td>
<td>5/0</td>
</tr>
<tr>
<td>14</td>
<td>\lambda</td>
<td>V\textsubscript{II}</td>
<td>HSLV2046</td>
<td>94.4</td>
<td>4/3</td>
<td>6/2</td>
</tr>
<tr>
<td>15</td>
<td>\lambda</td>
<td>V\textsubscript{III}</td>
<td>DPL23</td>
<td>92.9</td>
<td>3/6</td>
<td>7/2</td>
</tr>
</tbody>
</table>
Fig 1. Nucleotide sequences of the V-J junctional regions. Junctional regions are identical in the repeated sequences from each individual case (Tables 3 and 4). Base additions from flanking regions and losses by trimming are indicated. Remaining bases are presumed to have arisen via N-region addition.

**Fig 2.** Deduced amino acid sequences of the V\(_k\) regions of the tumor-derived clones from patients with myeloma. Comparisons are made with the closest germline V\(_k\) genes. Uppercase, replacement mutations; lowercase, silent mutations. Replacement mutations in J\(_k\) are underlined.

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**Table:** Nucleotide sequences of the V-J junctional regions.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>LIGHT CHAIN</th>
<th>V</th>
<th>N</th>
<th>J</th>
<th>JL</th>
<th>TRIMMING V</th>
<th>J</th>
<th>TOTAL</th>
<th>ADDN BASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>ATCACCTTCGG</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>CATTTTCG</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
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<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
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<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>κ</td>
<td>TAT GAT AAT CTC CCT CC</td>
<td></td>
<td></td>
<td></td>
<td>TACACTTTTCG</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>λ</td>
<td>GAC AGC GAT TGG CCT CC</td>
<td>GA</td>
<td></td>
<td></td>
<td>GTGTTCGG</td>
<td>7</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>λ</td>
<td>GAC AGC GAT TGG CCT CC</td>
<td>GA</td>
<td></td>
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<td>GTGTTCGG</td>
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<td>3</td>
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<td>12</td>
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<td>0</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>λ</td>
<td>GAC AGC GAT TGG CCT CC</td>
<td>GA</td>
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<td>GTGTTCGG</td>
<td>7</td>
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<td>14</td>
<td>λ</td>
<td>GAC AGC GAT TGG CCT CC</td>
<td>GA</td>
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<td></td>
<td>GTGTTCGG</td>
<td>7</td>
<td>0</td>
<td>3</td>
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<tr>
<td>15</td>
<td>λ</td>
<td>GAC AGC GAT TGG CCT CC</td>
<td>GA</td>
<td></td>
<td></td>
<td>GTGTTCGG</td>
<td>7</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

* nucleotide encoded by either V\(_L\) or J\(_L\) gene segment

N denotes mutation event
undergone extensive somatic hypermutation. There is further agreement that there is no intraclonal heterogeneity among the tumor cell population, and there is evidence that the $V_{H}$ sequence is stable from diagnosis through plateau phase. These findings strongly suggest that the malignant cell has exited from the germinal center, and is no longer susceptible to the mutation mechanism.

The germline repertoire of $V_{L}$ genes has also been mapped, but there have been fewer studies of usage in B-cell tumors. In myeloma, using DNA as a source, $V_{k}$ sequences were obtained from 29 cases, with 4 of 7 potentially functional. A second study investigated $V_{k}$-gene usage in 3 cases of myeloma. Together, these studies showed that 3 of 7 functional genes were derived from the O8/18 gene, and we have confirmed this incidence (4 of 9 cases). Although the $V_{k}$ family is often used by normal B cells, the level of usage of the O8/18 gene appears high in myeloma. However, frequency

### Table 5. R and S Mutations in Myeloma $V_{L}$ Genes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Ig Class</th>
<th>Germline Gene</th>
<th>$R:S$ (CDR)</th>
<th>$R:S$ (FWR)</th>
<th>$P$/CDR</th>
<th>$P$/FWR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgGc</td>
<td>O8/18</td>
<td>4.00 (4:1)</td>
<td>4</td>
<td>.22</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.63 (5:4)</td>
<td>10</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IgGc</td>
<td>O8/18</td>
<td>1.70 (5:2)</td>
<td>4</td>
<td>.74</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.00 (5:3)</td>
<td>9</td>
<td>.00</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IgGc</td>
<td>O8/18</td>
<td>1.50 (3:2)</td>
<td>3</td>
<td>.35</td>
<td>.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.80 (4:9)</td>
<td>3</td>
<td>.04</td>
<td>.02</td>
</tr>
<tr>
<td>4</td>
<td>IgGc</td>
<td>O8/18</td>
<td>0.50 (2:4)</td>
<td>3</td>
<td>.20</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.60 (5:3)</td>
<td>8</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>IgGc</td>
<td>A30</td>
<td>5.00 (5:1)</td>
<td>3</td>
<td>.90</td>
<td>.09</td>
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<td></td>
<td></td>
<td></td>
<td>1.00 (4:4)</td>
<td>7</td>
<td>.19</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>IgGc</td>
<td>A27</td>
<td>1.50 (6:2)</td>
<td>4</td>
<td>.12</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.00 (6:2)</td>
<td>10</td>
<td>.16</td>
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<tr>
<td>7</td>
<td>IgGc</td>
<td>A27</td>
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* Probability calculations according to Chang and Casali.

### Table 6. Comparison of Antigen-Driven R Mutations Locating to Myeloma $V_{H}$ or $V_{L}$ Genes

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* Probability calculations according to Chang and Casali.
of this gene in other B-cell tumors has also been reported to be high, and it is not yet clear if there is a difference among the tumor categories.22

The current results have focused on functional \( V_\kappa \) genes, obtained from RNA. Identification of repeated sequences in the cloned PCR product supports the derivation from tumor cells, which can be a problem otherwise. We have analyzed the pattern of both \( V_\kappa \) and \( V_\lambda \) sequences. These confirm the high level of somatic hypermutation, with the level of 5.8% mutation for \( V_\kappa \) being comparable with that of 8.2% for \( V_\lambda \). There is also a lack of intraclonal heterogeneity in \( V_\kappa \) of all selection should be strengthened by including analysis of \( V_\lambda \). patients, again confirming findings in \( V_\kappa \), and supporting the concept that the final event in malignant transformation has occurred at a postfollicular stage.3,4,14 In contrast, the \( V_\kappa \) genes in the benign counterpart of myeloma (monoclonal gammopathy of undetermined significance or MGUS), showed intraclonal heterogeneity in 3 of 7 cases.29 This could indicate that the clonal plasma cell in MGUS is less mature, and may have undergone some, but not all the events leading to malignant behavior.29

If the final neoplastic event is late in maturation of the B cell, it might be expected that the myeloma precursor cell will have been subjected to the same processes of development as a normal B cell. Even if there is an IgM+ clonal precursor, which has undergone some neoplastic event, the few cases available for analysis have indicated that it has a homogeneous \( V_\kappa \) gene sequence identical to the isotype-switched plasma cell.)11,13,38 This would argue that neoplastic transformation in myeloma begins in a mature B cell immediately before isotype-switch. Because a B cell would have reached this point following antigen selection, the imprint of this procedure should remain as a clustering of mutations in CDRs of \( V\)-gene sequences.11,17,32

In fact, analysis of the stable sequences in myeloma should be particularly useful, because the selected sequence will not be obliterated by continuing posttransformation mutations. However, analysis of \( V_\kappa \) sequences in myeloma has given mixed results, with only 21% of the tumor-derived sequences from a large series showing significant clustering in CDRs.5,14 This leaves open the question of the clonal history of the tumor cells in the remaining 79% of the cases. Because \( V_\kappa \) sequence is also known to be involved in recognition of antigen,18 deductions from \( V\)-gene sequences that relate to a role for antigen in selection should be strengthened by including analysis of \( V_\kappa \).
out a role for antigen selection, because optimal binding may occur via CDR3. Clearly we need more information on how normal human B cells generate antibody, but this study would suggest that deductions concerning a role for antigen in the clonal history of neoplastic B cells should take into account mutational events in both V_{\text{H}} and V_{\text{L}}.

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Myeloma V_L and V_H Gene Sequences Reveal a Complementary Imprint of Antigen Selection in Tumor Cells

Surinder S. Sahota, Regine Leo, Terry J. Hamblin and Freda K. Stevenson