

blood

1997 89: 219-226

Myeloma VL and VH Gene Sequences Reveal a Complementary Imprint of Antigen Selection in Tumor Cells

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

Updated information and services can be found at:

<http://bloodjournal.hematologylibrary.org/cgi/content/full/89/1/219>

Articles on similar topics may be found in the following *Blood* collections:

[Neoplasia](#) (4217 articles)

Information about reproducing this article in parts or in its entirety may be found online at:

http://bloodjournal.hematologylibrary.org/misc/rights.dtl#repub_requests

Information about ordering reprints may be found online at:

<http://bloodjournal.hematologylibrary.org/misc/rights.dtl#reprints>

Information about subscriptions and ASH membership may be found online at:

<http://bloodjournal.hematologylibrary.org/subscriptions/index.dtl>

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published semimonthly by the American Society of Hematology, 1900 M St, NW, Suite 200, Washington DC 20036.

Copyright 2007 by The American Society of Hematology; all rights reserved.



Myeloma V_L and V_H 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_H 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_H 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_H 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_L sequences from bone marrows of 15 patients. All sequences (9V_κ and 6V_λ) 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_L in selection by antigen. Comparison with the V_H sequences used by the same tumor cells showed that, if significant clustering was present, it was in either V_H or V_L, 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_H and V_L sequences for validation.

© 1997 by The American Society of Hematology.

MULTIPLE MYELOMA is a malignant tumor involving plasma cells. Neoplastic cells are found in the bone marrow (BM), and typically secrete a monoclonal Ig of IgG or IgA isotype. Although the major identifiable tumor population consists of plasma cells, there has been a great deal of debate concerning the nature of the malignant cell, with some early indications that this may be a less mature B cell capable of "feeding" the plasma cell compartment.^{1,2} The advent of genetic technology aimed at Ig genes has allowed a more incisive investigation of the characteristics of myeloma clones. In fact, there have now been reports of a total of more than 50 sequences of V_H genes used by tumor cells from patients' BM biopsies, and these have revealed common features.³⁻⁵

One conclusion is that usage of V_H genes from the available repertoire appears to reflect no striking bias, with predominance of the large V_{H3} family in line with serological analysis of myeloma proteins.⁶ However, at the level of individual V_H genes there may be some asymmetry in usage. For example, one gene, V₄₋₃₄, commonly used by normal B cells, and mandatory for encoding IgM autoanti-red blood cell antibodies of I/i specificity in patients with cold agglutinin disease,⁷ has so far not been found to be used by tumor cells in myeloma.^{5,8} In all cases of myeloma, the V_H genes have been found to be somatically hypermutated.³⁻⁵ A further common feature is the lack of intraclonal variation in sequence, a finding that contrasts with the heterogeneity found in B-cell tumors of the germinal center.^{9,10} This leads to the conclusion that the final tumorigenic event in myeloma has occurred at a postfollicular stage, when the cell is no longer influenced by the somatic hypermutation mechanism.¹¹ It argues against the concept that there is a "feeder" B cell, unless that cell has escaped the mutator before isotype switching.

However, IgM⁺ B cells with V_H sequences indicating a clonal relationship with the neoplastic plasma cells have been detected in some cases of myeloma.^{12,13} Although there is some controversy about their frequency,¹⁴ it appears that such cells do exist and presumably continue to proliferate. There is uncertainty as to their contribution to malignancy, and it is possible that these cells have undergone some, but not all, of the events leading to malignant behavior.⁴

A further problem in understanding development of myeloma lies in the role of antigen in selecting the V_H sequences

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_H regions of antibody molecules, it is known that recognition of antigen can involve several sites, with CDR3 having a major influence.¹⁵ However, replacement mutations in CDR1 and CDR2 have a significant role in affinity maturation.^{11,16} 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.¹⁷ When these rules were applied to the large panel of V_H sequences from myeloma cells, only a minority of cases (10 of 52) showed statistically significant clustering in CDRs.⁵ However, the antigen-binding site is known to involve both V_H and V_L,¹⁸ and we have investigated V_L sequences from a group of 15 patients both to extend our knowledge of V_κ and V_λ gene usage in myeloma, and to assess the role of V_L in the selection of the cell of origin by antigen.

MATERIALS AND METHODS

Patients and cell preparation. Heparinized BM aspirates from unselected patients with multiple myeloma at different stages of

From the Molecular Immunology Group, Tenovus Laboratory, Southampton University Hospitals, Southampton, UK; and the Division of Clinical Immunology, Medizinische Hochschule, Hannover, Germany.

Submitted June 20, 1996; accepted August 7, 1996.

Supported by the Leukaemia Research Fund, UK, the European Myeloma Research Network (Biomed BMH1-CT93-1407), and the Dr Hiltrud Pulst Myeloma Foundation, Hannover, Germany.

Address reprint requests to Freda K. Stevenson, DPhil, Molecular Immunology Group, Tenovus Laboratory, Southampton University Hospitals, Tremona Rd, Southampton SO16 6YD, UK.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1997 by The American Society of Hematology.

0006-4971/97/8901-0019\$3.00/0

Table 1. Characteristics of Myeloma Patients

Patient	Stage*	Status	Monoclonal Ig		% Plasma Cells in BM
			Class	Level (g/L)	
1	IIIA	Relapsed progressive	IgG κ	24.0	11.0
2	IIA	Stable	IgG κ	18.0	21.0
3	II	Progressive	IgG κ	43.0	5.0
4	II	Stable	IgG κ	28.0	30.0
5	III	Progressive	IgG κ	15.0	18.0
6	IIA	Presentation	IgG κ	59.0	14.0
7	IIIA	Progressive	IgG κ	71.0	30.0
8	IB	Presentation	IgA κ	40.7	60.0
9	III	Progressive	IgG κ	48.0	30.0
10	IIA	Progressive	IgG λ	20.0	19.0
11	I	Stable	IgG λ	16.0	4.0
12	III	Progressive	IgG λ	60.0	5.0
13	III	Progressive	IgG λ	21.0	10.0
14	I	Progressive	IgA λ	3.0	1.8
15	IIIA	Presentation	IgG λ	79.0	50.0

* Durie and Salmon staging.

disease from the Hematology (UK) or Immunology (Germany) clinics were taken for investigation. All patient material was obtained with consent, and with permission from local Ethical Committees. Clinical and laboratory features are shown in Table 1. All patients had an identifiable monoclonal Ig in serum (13 IgG, 2 IgA) of the same light chain type (9 κ , 6 λ) as the major plasma cell population. Mononuclear cells (MNC) were isolated by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden), and plasma cell involvement was assessed by direct immunofluorescent staining for surface CD38 and cytoplasmic κ or λ light chains using the FACS-SCAN (Becton Dickinson, CA).¹⁹ In some cases, cytocentrifuged MNC preparations were stained with the same reagents, and assessed by fluorescence microscopy.

Analysis of V_L genes. For preparation of cDNA, total RNA (2 to 10 μ g) was isolated from the MNC fraction (1 to 6 \times 10⁶ cells) using RNAzol B (Cinna Biotech Labs Inc, Houston, TX). Reverse transcription was carried out with an oligo dT primer, using a first-strand cDNA synthesis kit (Pharmacia). A sample of the cDNA (1/3 to 1/5) was then amplified by polymerase chain reaction (PCR) using a mixture of 5' oligonucleotide FWR1 primers specific for the expressed V κ or V λ families together with a mixture of downstream 3' primers specific for J κ or J λ genes as appropriate (Table 2). Amplification conditions were as described,^{4,20} except that annealing temperature was 65°C for 1 minute. Amplified products were cloned and sequenced as described⁴; alignment was made to current EMBL/GenBank and V-BASE sequence directories²¹ using MacVector 4.0 sequence analysis software (International Biotechnologies Inc, New Haven, CT). At least two independent PCR amplifications were performed from each sample.

RESULTS

V_L gene usage by tumor cells. The V_L genes used by the tumor cells were identified as repeated identical V_L-J_L sequences obtained after cloning of PCR products.⁴ 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 κ and V λ , respectively. The profile of V κ genes used by the 9 κ -positive tumors indicates that 5 of 9 use V κ 1 and 4 of 9 V κ III frequencies

in line with normal B cells.^{22,23} Among the V κ 1 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 κ III group use the A27 gene, found to be used frequently in chronic lymphocytic leukemia.²⁵ The V λ genes (Table 4) used three different families. There appeared to be no preferential use of particular J_L genes for either light chain type.

V-J joining region. Clonality of tumor-derived sequences was confirmed 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_L genes have been submitted to the EMBL/GenBank database (accession 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_L, but these are known to be insignificant in V κ .²⁶ Less information is available for V λ genes, but again suggests only limited polymorphic variation.²⁷ The V_L sequences obtained deviated significantly from the closest germline genes in the database (Tables 3 and 4), with a mean percent mutation of 5.3 for V κ and 6.2 for V λ . There was evidence for block mutations, involving two or more adjacent nucleotides, in both light-chain types. For V κ , the high incidence of block mutations (6 of 9 sequences [67%]) compares with the reported figure 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 identified in the V_L sequences, with 9 of 15 having additional identifiable mutations in J_L, even though events at the 3' end of J_L are obscured by the primer sites. Several sequences derived from the same V_L family member were available for the V κ 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 2. Oligonucleotide V_L PCR Primers

Primer	Location	Orientation	Sequence (5'-3')
V κ 1&4	FR1	Sense	GACATCSWGATGACCCAGTCTCC
V κ 2&6	FR1	Sense	GAWRRTGTGMTGACTCAGTCTCC
V κ 3	FR1	Sense	GAAATTGTGTTGACGCACTCTCC
V κ 5	FR1	Sense	GAAACGACACTCAGCAGTCTCC
J κ 1-4	FR4	Anti-sense	ACGTTTGATHTCACACTTTGGTCCC
J κ 5	FR4	Anti-sense	ACGTTTAATCTCCAGTCGTGTCCC
V λ 1	FR1	Sense	CAGTCTGTSBGTGACKCAGCCRCCTC
V λ 2	FR1	Sense	CAGTCTGCCCTGACTCAGCTCSSYT
V λ 3	FR1	Sense	TCYMTGWGCTGACTCAGSMM
V λ 7&8	FR1	Sense	CAGRCTGTGGTGACYCAGGAGCCMTC
V λ 9	FR1	Sense	CAGCCTGTGTGACTCAGCCACCTTC
J λ C	FR4	Anti-sense	ACCKAGGACGGTSASCTKGGTSCC

Table 3. Analysis of V_κ Genes From Myeloma Patients

Patient No.	Ig Light Chain	V _L Family	GL Donor	% Homology	R/S Mutations		J _L	Tumor-Derived Sequences/ Clones Sequenced
					FWR	CDR		
1	κ	V _κ I	O8/18	93.2	5/8	4/1	J _κ 5	8/11
2	κ	V _κ I	O8/18	93.8	6/2	5/3	J _κ 2	8/12
3	κ	V _κ I	O8/18	94.6	4/5	3/2	J _κ 4	9/11
4	κ	V _κ I	O8/18	94.7	3/5	2/4	J _κ 4	7/9
5	κ	V _κ I	A30	95.0	5/3	5/0	J _κ 1	8/11
6	κ	V _κ III	A27	93.6	6/2	6/4	J _κ 1	8/8
7	κ	V _κ III	A27	95.0	4/1	6/2	J _κ 4	10/12
8	κ	V _κ III	A27	93.9	4/1	11/0	J _κ 2	12/12
9	κ	V _κ III	L6	98.5	0/0	3/1	J _κ 3	6/11

ried out by the method of Chang and Casali.¹⁷ In this method, each V_L or V_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.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_H.¹⁴ For the CDRs, there were more R mutations than expected in 14 of 15 sequences, with significant (*P* < .05) clustering indicative of antigen selection in 4 of 15 (3Vκ and 1Vλ).

Comparison with V_H genes. For 6 cases (patients 1, 3, 6, 8, 14, and 15), tumor-derived V_H gene sequences were known.^{4,29} V_H sequences from the remaining 9 patients were obtained as described⁴ 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_H is not sufficient to require this approach.²¹ In fact, where we⁴ and others⁵ have analyzed the patients' germline V_H 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.⁵ The distribution of somatic mutations in V_H of 6 of 15 of

these cases indicated a significant clustering in CDRs (Table 6). Comparison of patterns in V_H with those in V_L (Table 6) showed that clustering in CDRs of V_H was not paralleled by clustering in CDRs of V_L. In addition, the clustering in V_L observed in 4 of 15 cases was not paralleled by clustering in V_H. Therefore, from the 10 cases where clustering was evident, it was localized in either V_H or V_L, but not both. However, in 5 of 15 cases, there was no significant clustering in either V_H or V_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_H gene germline repertoire is available,^{21,30} it is possible to compare a V_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_{H3} genes,³¹ it can be assumed that the majority of deviations from germline sequence in V_H genes of a B cell represent somatic mutations.²¹ In some cases this has been proved by comparing the tumor-derived sequence with the germline counterpart in the patient.^{4,5} Accumulation of such mutations indicate that the cell of origin has been exposed to the hypermutation mechanism in the germinal center.^{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.^{9,10} Finally, concentration of replacement mutations in CDRs can suggest a role for antigen in selection of the B cell.^{17,33}

In the case of multiple myeloma, V_H gene analyses from several laboratories have shown that the malignant cell has

Table 4. Analysis of V_λ Genes From Myeloma Patients

Patient No.	Ig Light Chain	V _L Family	GL Donor	% Homology	R/S Mutations		J _L	Tumor-Derived Sequences/ Clones Sequenced
					FWR	CDR		
10	λ	V _λ I	DPL2	95.6	3/3	4/2	J _λ 7	12/12
11	λ	V _λ I	DPL3	91.0	3/6	9/6	J _λ 1	4/10
12	λ	V _λ III	IGLV3S2	96.5	0/2	6/1	J _λ 7	8/8
13	λ	V _λ II	DPL11	95.9	2/4	5/0	J _λ 2	6/9
14	λ	V _λ II	HSLV2046	94.4	4/3	6/2	J _λ 2	9/11
15	λ	V _λ III	DPL23	92.9	3/6	7/2	J _λ 2	8/8

PATIENT	LIGHT CHAIN	V			N	J			J _L	TRIMMING			ADD ^N BASES		
		V	N	J	N	V	J	TOTAL							
1	κ	TAT	GAT	AAT	CTC	CCT	CC	G	ATCACCTTCGG	5	0	0	0	3	
2	κ	TAT	GAT	GAT	CTC	CCC		G	TACACTTTTGG	2	0	0	0	0	
3	κ	TAT	GAT	AGT	CTC	CC			CACTTTCGG	4	1	2	3	0	
4	κ	TAT	GAT	AGT	CTC	CCT	C*		TCACCTTTGG	4	0	1	1	1	
5	κ	TAT	AAA	AGT	TAC	CCT	C	T	GACGTTTGG	1	0	2	2	2	
6	κ	TAT	GGG	AGT	TTA	CCT	C		GGACGTTTCGG	1	0	1	1	1	
7	κ	TAT	GGG	AGC	TC			G	CTCACTTTCGG	4	4	0	4	1	
8	κ	CAT	GTT	ACC	GAA	C		A	G	TGCACCTTTTGG	2	2	0	2	2
9	κ	CGT	AGC	AAC	TGG	CCT	CC	GA	TCACCTTTCGG	3	0	1	1	4	
10	λ	GAC	AGC	CTG	AAT	GGT	CC	G	GTGTTCGG	7	0	3	3	3	
11	λ	GAC	AGC	CTG	AGA	GGT*			TATGTCTTGCC	1	0	0	0	0	
12	λ	AGT	AGT	AAN	GAT	CAG		GGG	GTGTTCGGCGG	7	0	3	3	3	
13	λ	AGC	AGC	AGC	ACT	CTC			CTGGTATCCGC	2	0	0	0	0	
14	λ	GGC	AGC	AAC	AAT				GTGATATTCGG	2	3	0	3	0	
15	λ	GAC	CGC	AAC	ACT				TTGATATTCGG	2	3	0	3	0	

Base additions
from V gene 3'
flanking region

Base additions
from J gene 5'
flanking region

* nucleotide encoded by either V_L or J_L gene segment
N denotes mutation event

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.

	5'primer	---CDR1---	---CDR2---	---CDR3---	3'primer	
08/18 P1	DIQMTQSPSSLSASVGDRTTTC	QASQDISNYLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSSTDFFTTISLQPEDFATYYC	QQYDNLP
	-----P-a-----	-----T-----	---q--g--k-----	V---QP	--p-----G--tH-t--G-----Fa-----	-----P
						ITFGQGTKVEIKR Jk5
08/18 P2	DIQMTQSPSSLSASVGDRTTTC	QASQDISNYLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSSTDFFTTISLQPEDFATYYC	QQYDNLP
	-----H--N-H--	---F-V-pg---	---a-I---	-----A-----	-----F-----	-----D-p
						YTFGQGTKVEIKR Jk2
08/18 P3	DIQMTQSPSSLSASVGDRTTTC	QASQDISNYLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSSTDFFTTISLQPEDFATYYC	QQYDNLP
	V-E-----v-----t-	-----s-----	---qH-----Q---	---H---	---r-----L--N-l-----	-----S-
						P TFGGQTKVDIKR Jk4
08/18 P4	DIQMTQSPSSLSASVGDRTTTC	QASQDISNYLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSSTDFFTTISLQPEDFATYYC	QQYDNLP
	--E-----a-----I---	-----i--F---	---I---k-l---	---et---	-----Ift-----	---q--s---
						L tFGGQTKVEIKR Jk4
A30 P5	DIQMTQSPSSLSASVGDRTTTC	RASQGIKNDLG	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSSTDFFTTISLQPEDFATYYC	LQHNSYP
	--E-----D-G-A--	---R-E---R---	-----g--sT-----g-----	-----YK---		
						L TFGQGTKVEIKR Jk1
A27 P6	EIVLTQSPGTLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSSTDFLTISRLEPEDFATYYC	QQYGS
	D--Mt-----V--	-----RNSV-	---k---G---F---	---N---	---V-----V-----	---q-gsL---
						R TFGQGTKVEIKR Jk1
A27 P7	EIVLTQSPGTLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSSTDFLTISRLEPEDFATYYC	QQYGS
	-----L-N---	---NA---	S-N---	-----S-----	---S1-----	---L-g---
						S LTFGGQTKVDIKR Jk4
A27 P8	EIVLTQSPGTLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSSTDFLTISRLEPEDFATYYC	QQYGS
	-----L---	-----N---	---P-----	RTF---P	-----a--F---	---HVTE
						Q CTFGQGTKVEIKR Jk2
L6 P9	EIVLTQSPATLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	DASNRRAT	GIPARFSGSGSSTDFLTISRLEPEDFATYYC	QQRSNWP
	---t-----GN---	-----Y-----	-----y-----			-----PI
						TFGGQTKVDIKR Jk3

Fig 2. Deduced amino acid sequences of the V_κ regions of the tumor-derived clones from patients with myeloma. Comparisons are made with the closest germline V_κ genes. Uppercase, replacement mutations; lowercase, silent mutations. Replacement mutations in J_κ are underlined.

```

      5'PRIMER          CDR1          CDR2          CDR3          3'PRIMER
      |-----|          |-----|          |-----|          |-----|          |-----|
DPL2  QSVLTQPPSASGTPGQRTVISC  SGSSSNIGSNMIVN  WYQQLPGTAPKLLIY  SNNQRPS  GVPDRFSGSKSGTSASLAISGLQSEDEADYYC  AAWDDSLNG  |-----|
P10   -P-----a-----S-----  -----n--G-----  ---H-----V---  TD-----  -----t-----g-----  T-----  P  VFgGGTQLTVLG JA.7

DPL3  QSVLTQPPSASGTPGQRTVISC  SGSSSNIGSNIVY  WYQQLPGTAPKLLIY  RNNQRPS  GVPDRFSGSKSGTSASLAISGLRSEDEADYYC  AAWDDSLSG
P11   -----G-----sK-n--TY-I-  -F-qI-----i-  WTD-r--  g-----sa-----y-  aa-d--R-  YVLPGGTKLTVLG JA.1

GLI*  SYVLTQPPSVSVAPGKTARITC  GGNIGSKSVH  WYQQKPGQAPVLIY  YSDRPS  GIPERFSGSNGNTATLTISRVEAGDEADYYC  QWWDSSDH
P12   -----ET-----  -----v-----  F-T-----e-----  -----d--N-Q  G  VFgGGTQLTVLG JA.7

DPL11 QSALVTPASVSGSPGQSITISC  TGTSSDVGGYNYVS  WYQQHPGKAPKLMY  EVSNRPS  GVSNRFSGSKSGNTASLTISGLQAEDEADYYC  SSYTSSSTL
P13   -----q-----I-A-D-----  -yE-Q-----  D-N-----f-----  -----c-----  LVSAGGNMLTVLG JA.2

GL2** QSALVTPASVSGSPGQSITISC  TGTSSDVGGYNYVS  WYQQHPGKAPKLMY  EVSKRPS  GVPDRFSGSKSGNTASLTISGLQAEDEADYYC  SSYAGSNN
P14   -----A-G-----A-tsG-I--FD---  ---q-p-----V---  --T-----F--A-----  -----  VIFGGTQLTVLG JA.2

DPL23 SYELVTPASVSVSGTASITC  SGDKLGDKYAC  WYQQKPGQSPVLIY  QDSKRPS  GIPERFSGSNGNTATLTISGTQAMDEADYYC  QAWDSST
P15   -----E-----c-g-----VS  ---q-----w-v-y  --AN-s  W-----s-----a-----  ----RN-  LIFGGTQLTVLG JA.2
    
```

GL1* is IGLV3S2
 GL2** is HSLV204

Fig 3. Deduced amino acid sequences of the V_L regions of the tumor-derived clones from patients with myeloma. Comparisons are made with the closest germline V_L genes. Uppercase, replacement mutations; lowercase, silent mutations. Replacement mutations in JA are underlined.

undergone extensive somatic hypermutation.³⁻⁵ There is further wide agreement that there is no intraclonal heterogeneity among the tumor cell population,³⁻⁵ and there is evidence that the V_H sequence is stable from diagnosis through plateau

phase.³⁴ These findings strongly suggest that the malignant cell has exited from the germinal center, and is no longer susceptible to the mutation mechanism.^{4,5}

The germline repertoire of V_L genes has also been mapped,^{27,35,36} but there have been fewer studies of usage in B-cell tumors. In myeloma, using DNA as a source, 7 V_κ sequences were obtained from 29 cases, with 4 of 7 potentially functional.³⁷ Sequences were somatically mutated, with a hint of antigen selection from R:S ratios.³⁷ A second study investigated V_κ-gene usage in 3 cases of myeloma.²⁴ Together, these studies showed that 3 of 7 functional genes were derived from the O8/18 gene,^{24,37} and we have confirmed this incidence (4 of 9 cases). Although the V_κ1 family is often used by normal B cells,^{22,23} 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

Patient	Ig Class	Germline Gene	R:S (CDR) _{obs}	R (CDR) _{exp}	P (CDR)*
			R:S (FWR) _{obs}	R (FWR) _{exp}	P (FWR)
1	IgG _κ	O8/18	4.00 (4:1)	4	.22
			0.63 (5:8)	10	.02
2	IgG _κ	O8/18	1.70 (5:3)	4	.14
			3.00 (6:2)	9	.09
3	IgG _κ	O8/18	1.50 (3:2)	3	.26
			0.80 (4:5)	8	.03
4	IgG _κ	O8/18	0.50 (2:4)	3	.20
			0.60 (3:5)	8	.01
5	IgG _κ	A30	5.00 (5:0)	3	.09
			1.00 (4:4)	7	.19
6	IgG _κ	A27	1.50 (6:4)	4	.12
			3.00 (6:2)	10	.16
7	IgG _κ	A27	3.00 (6:2)	3	.03
			4.00 (4:1)	7	.06
8	IgA _κ	A27	∞ (11:0)	3	.0001
			4.00 (4:1)	8	.02
9	IgG _κ	L6	∞ (3:0)	1	.04
			0.00 (0:1)	2	.00
10	IgG _λ	DPL2	2.00 (4:2)	3	.20
			1.00 (3:3)	6	.06
11	IgG _λ	DPL3	1.50 (9:6)	6	.08
			0.50 (3:6)	12	.0002
12	IgG _λ	IGLV3S2	6.00 (6:1)	2	.006
			0.00 (0:2)	0	.00
13	IgG _λ	DPL11	5.00 (5:0)	3	.08
			0.50 (2:4)	6	.03
14	IgA _λ	HSLV2046	3.00 (6:2)	4	.09
			1.33 (4:3)	8	.04
15	IgG _λ	DPL23	3.50 (7:2)	4	.14
			0.50 (3:6)	10	.0012

* Probability calculations according to Chang and Casali.¹⁷

Table 6. Comparison of Antigen-Driven R Mutations Locating to Myeloma V_H or V_L Genes

Patient	P (CDR)*	
	V _H	V _L
1	.10	.22
2	.03	.14
3	.06	.25
4	.18	.20
5	.11	.09
6	.0009	.12
7	.13	.03
8	.21	.0001
9	.25	.04
10	.0029	.20
11	.0098	.08
12	.10	.006
13	.049	.08
14	.18	.09
15	.04	.14

* Probability calculations according to Chang and Casali.¹⁷

	CDR1	CDR2	CDR3		
V2-26 P2	QVTLEKESGFLVLPKPTETLTLCTVSGFSLN ---V---Q-----S-----EG-----	NARMGVS WIRQPPGKALEWLA	HIFSNDKSYSTSLKS -----S---ISS--Rs	RLTISKDTSKSQVVLIMTNMDFVDTATYYCA r-----N--G-----KV-pT-t---y-V	RVVQLRVP KYHFDHWGGTLVTVSS JH4b
V3-11 P4	QVQLVESGGGLVLPKPGGSLRLSCAASGFTFS -Aq-----D--k-----V--R--R -H---	DYHMS WIRQAPGKGLEWVS	YISSSGSTIYYADSVKG -l-----g-----F-R---ATF---s---	RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR -----D-nTl-----VD---v-----	GRYSTSPR <u>TFNLWGH</u> GLTLVTVSS JH4a
8M27 P5	QVQLVQSGAEVKKPKGSSVKVSCAKSGFTFS --Ni-----A---M-----C-----	SYTIS WVRQAPGQGLEWVG	RIIPILGTANYAQKFGQ --L-----T-V-----T-----S---	RVTITADKSTSTAYMELSSLRSEDVAVYYCA --t---R---a-----N-y-----v-y--	VYYFDSNR YDYWQQGLTVTVSS JH4b
V3-7 P7	EVQLVESGGGLVQPGGSLRLSCAASGFTFS --l-----AEp-----E-----t-b N--H	SYHMS WVRQAPGKGLEWVA	NIKQDGSEKYYVDSVKG -----gk-----R--M--L---	RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR -----VNL-I---C---v-----r	DGSSYARP YWYFDLWGRGTLVSVSS JH2
V3-9P P9	EVQLVESGGGLVQPGGSLRLSCAASGFTFD G-----v-----G-----SN-----	DYAMH WVRQAPGKGLEWVS	GISWNSGSGYADSVKG -----A- -----ET-----	RFTISRDNKNSLYLQMNSLRAEDTALYYCAKD -----Te---yF-v--QARGYSYGNGLFDYWGQGLTVAVSS	JH4b
DP-66 P10	QVQLQESGPELVKPESETLTLCTVSGGSVS --q-----A---M-----C-----V---	SGSYHMS WIRQPPGKGLEWIG	YIYSGSTNYNPSLKS FF--T-H-----Qs	RVTISVDTSKNTQFSLKLSVTAADTAVYYCAR --A-v---n-Ls-R-T-----GRQPD	YYYGMdVWVGGQGLTIVSVSS JH6b
VHVMW P11	EVQLVQSGAEVKKPKGSELRISCKGSGYFT -----N-----	SYWIS WVRQMPGKGLEWVG	RIDPDSYTYNPSPSFGQ K-G-I-----	HVTISADKSISTAYLQWSSLKASDTAVYYCAR -----T-----	HPTYYDSSGYNFDHWGGTLVTVSS JH4b
DP-38 P12	EVQLVESGGGLVLPKPGGSLRLSCAASGFTFS -----I-- TG--s	NAWMS WVRQAPGKGLEWVG	RIKSKTDGGTIDYAAPVKG -----v- r-----g-I---E---	RFTISRDDSKNTLYLQMNSLKTEDTAVYYCTT -----n-f-----v-----ALTRYFFDSSGYPHFDHWGGTLVTVSS	JH4a
b28e P13	QVQLVESGGGVVQPGGSLRLSCAASGFTFS L-----S-----	SYGMH WVRQAPGKGLEWVA	VISYDGSNKYYADSVKG -----g-Q-MV Y--S--NT-y-----	RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR -----RS--F-----G---v-----	DPG LDYWQQGLTVSVSS JH4b

Fig 4. Deduced amino acid sequences of the V_H regions of tumor cells from patients with myeloma. Comparisons are made with the closest germline V_H genes. Uppercase, replacement mutations; lowercase, silent mutations. Replacement mutations in J_H are underlined. Patient identification numbers are indicated. V_H sequences from other patients are published.^{4,37}

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.²⁴

The current results have focused on functional V_L 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_κ and V_λ sequences. These confirm the high level of somatic hypermutation, with the level of 5.8% mutation for V_L being comparable with that of 8.2% for V_H.⁵ There is also a lack of intraclonal heterogeneity in V_L of all patients, again confirming findings in V_H, and supporting the concept that the final event in malignant transformation has occurred at a postfollicular stage.^{3,4,14} In contrast, the V_H 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_H gene sequence identical to the isotype-switched plasma cell.^{12,13,38} 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.^{11,17,33} 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_H 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_L 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_L.

In this study, significant clustering of mutations in CDRs of V_L was seen in 4 of 15 sequences. In contrast, a preliminary report of a study of V_κ sequences in 9 cases of myeloma has indicated that clustering of replacement mutations in CDRs occurred in all cases, but more details of the analysis are required.³⁹ In our cases, comparison with V_H sequence in the same cell showed that clustering was in either V_H or V_L, but not both. This suggests that a role for antigen might be more common than estimated from V_H alone, reaching 67% in our study. There have been insufficient studies of normal human B cells to know if this is a typical finding. Investigation of the classical murine response to the hapten phenyl oxazolone has shown that affinity maturation is accompanied by somatic mutations in the CDRs of both V_H and V_κ.⁴⁰ This is likely to be the case in human antibodies, and might suggest that a role for antigen is more common than estimated from V_H alone. For myeloma, the findings support the idea that the cell of origin has undergone the process of conventional antigen selection. However, there remains a minority of sequences which appear to have no clustering in either V-region. Even this pattern does not rule

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_H and V_L.

ACKNOWLEDGMENT

We thank Dr D.G. Oscier for providing patient material and for helpful comments.

REFERENCES

1. Kubagawa, H, Vogler LB, Capra JD, Conrad ME, Lawton AR, Cooper MD: Use of individually specific (idiotype) antibodies to trace the oncogenic event to its earliest point in B-cell differentiation. *J Exp Med* 150:792, 1979
2. Grogan TM, Durie BGM, Lomen C, Spier C, Wirt DP, Nagle R, Wilson GS, Richter L, Vela E, Maxey V, McDaniel K, Rangel C: Delineation of a novel pre-B cell component in plasma cell myeloma: Immunohistochemical, immunophenotypic, genotypic, cytologic, cell culture and kinetic features. *Blood* 70:932, 1987
3. Bakkus MHC, Heirman C, van Riet I, van Camp B, Thielemans K: Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intraclonal variation. *Blood* 80:2326, 1992
4. Sahota S, Hamblin T, Oscier DG, Stevenson FK: Assessment of the role of clonogenic B lymphocytes in the pathogenesis of multiple myeloma. *Leukemia* 8:1285, 1994
5. Vescio RA, Cao J, Hong CH, Lee JC, Wu CH, der Danielian M, Wu V, Newman R, Lichtenstein AK, Berenson JR: Myeloma Ig heavy chain V region sequences reveal prior antigenic selection and marked somatic mutation but no intraclonal diversity. *J Immunol* 155:2487, 1995
6. Kabat EA, Wu TT, Perry HM, Gottesman KS, Foeller C: Sequences of Proteins of Immunological Interest (ed 5). Washington, DC, US Department of Health and Human Services, National Institutes of Health, 1991
7. Pascual V, Victor K, Spellerberg M, Hamblin TJ, Stevenson FK, Capra JD: V_H restriction among human cold agglutinins. The V_H4-21 gene segment is required to encode anti-I and anti-i specificities. *J Immunol* 149:2337, 1992
8. Rettig MB, Vescio RA, Cao J, Wu CH, Lee JC, Han E, Der-Danielian M, Newman R, Hong C, Lichtenstein AK, Berenson JR: V_H gene usage in multiple myeloma: Complete absence of the V_H4.21 (V_H4-34) gene. *Blood* 87:2846, 1996
9. Bahler DW, Levy R: Clonal evolution of a follicular lymphoma: Evidence for antigen selection. *Proc Natl Acad Sci USA* 89:6770, 1992
10. Zhu D, Hawkins RE, Hamblin TJ, Stevenson FK: Clonal history of a human follicular lymphoma as revealed in the immunoglobulin variable region genes. *Br J Haematol* 86:505, 1994
11. Berek C: The development of B cells and the B cell repertoire in the microenvironment of the germinal centre. *Immunol Rev* 126:5, 1992
12. Corradini P, Boccadoro M, Voena C, Pileri A: Evidence for a bone marrow B cell transcribing malignant plasma cell VDJ joined to a C_μ sequence in IgG and IgA secreting multiple myelomas. *J Exp Med* 178:1091, 1993
13. Billadeau D, Ahmann G, Greipp P, van Ness B: The bone marrow of multiple myeloma patients contains B cell populations at different stages of differentiation that are clonally related to the malignant plasma cell. *J Exp Med* 178:1023, 1993
14. Berenson JR, Vescio RA, Hong CH, Cao J, Kim A, Lee CC, Schiller G, Berenson RJ, Lichtenstein AK: Multiple myeloma clones are derived from a cell late in B lymphoid development. *Curr Topics Microbiol Immunol* 194:25, 1995
15. Kirkham PM, Schroeder HW Jr: Antibody structure and the evolution of immunoglobulin V gene segments. *Semin Immunol* 6:347, 1994
16. Kocks C, Rajewsky K: Stable expression and somatic hypermutation of antibody V regions in B cell development pathways. *Annu Rev Immunol* 7:537, 1989
17. Chang B, Casali P: The CDR1 sequences of a major proportion of human germline Ig V_H genes are inherently susceptible to amino acid replacement. *Immunol Today* 15:367, 1994
18. Tello D, Goldbaum FA, Mariuzza RA, Ysern X, Schwartz FP, Poljak RJ: Three-dimensional structure and thermodynamics of antigen binding by anti-lysozyme antibodies. *Biochem Soc Trans* 21:943, 1993
19. Leo R, Boeker M, Peest D, Hein R, Bartl R, Gessner JE, Selbach G, Wacker G, Deicher H: Multiparameter analyses of normal and malignant human plasma cells: CD38⁺⁺, CD56⁺, CD54⁺, C1g is the common phenotype of myeloma cells. *Ann Hematol* 64:132, 1992
20. Hawkins RE, Zhu D, Ovecka M, Winter G, Hamblin TJ, Long A, Stevenson FK: Idiotypic vaccination against human B-cell lymphoma. Rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines. *Blood* 83:3279, 1994
21. Cook GP, Tomlinson IM: The human immunoglobulin V_H repertoire. *Immunol Today* 16:237, 1995
22. Solomon A: Light chains of immunoglobulins: Structural-genetic correlates. *Blood* 68:603, 1986
23. Cuisinier AM, Fumoux D, Moinier D, Boubli L, Guigou V, Milili M, Schiff C, Fougereau M, Tonnel C: Rapid expansion of human immunoglobulin repertoire (V_H, V_κ, V_λ) expressed in early fetal bone marrow. *New Biologist* 2:689, 1990
24. Cannell PK, Amlot P, Attard M, Hoffbrand AV, Foroni L: Variable κ gene rearrangement in lymphoproliferative disorders and analysis of V_κ gene usage, VJ joining and somatic mutation. *Leukemia* 8:1139, 1994
25. Kipps TJ, Fong S, Tomhave E, Chen PP, Goldfein R, Carson D: High frequency expression of a conserved κ light-chain variable-region gene in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 84:2916, 1987
26. Zachau HG: The human immunoglobulin κ genes, in Honjo T, Alt FW (eds): *Immunoglobulin Genes*. San Diego, CA, Academic, 1995, p 173
27. Williams SC, Winter G: Cloning and sequencing of human immunoglobulin V_λ gene segments. *Eur J Immunol* 23:1456, 1993
28. Klein R, Jaenichen R, Zachau HG: Expressed human immunoglobulin κ genes and their hypermutation. *Eur J Immunol* 23:3248, 1993
29. Sahota SS, Leo R, Hamblin TJ, Stevenson FK: Immunoglobulin V_H gene mutational patterns indicate different tumor cell status in human myeloma and MGUS. *Blood* 87:746, 1996
30. Matsuda F, Shin EK, Nagaoka H, Matsumara R, Haino M, Fukita Y, Taka-ishi S, Imai T, Riley JH, Anand R, Soeda E, Honjo T: Structure and physical map of 64 variable segments in the 3' 0.8-megabase region of the human immunoglobulin heavy chain locus. *Nat Genet* 3:88, 1993
31. Sasso EH, van Dijk KW, Milner EC: Prevalence and polymorphism of human V_H3 genes. *J Immunol* 145:2751, 1990
32. Gray D: Immunological memory. *Annu Rev Immunol* 11:49, 1993
33. Schlomchik MJ, Aucoin AH, Pisetsky DS, Weigert MG: Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc Natl Acad Sci USA* 84:9150, 1987

34. Ralph QM, Brisco MJ, Joshua DE, Brown R, Gibson J, Morley AA: Advancement of multiple myeloma from diagnosis through plateau phase to progression does not involve a new B-cell clone: Evidence from the Ig heavy chain gene. *Blood* 82:202, 1993
35. Zachau HG: The immunoglobulin κ locus-or-what has been learned from looking closely at one-tenth of a percent of the human genome. *Gene* 135:167, 1993
36. Cox JPL, Tomlinson IM, Winter G: A directory of human germ-line V κ segments reveals a strong bias in their usage. *Eur J Immunol* 24:827, 1994
37. Wagner SD, Martinelli V, Luzzatto L: Similar patterns of V κ gene usage but different degrees of somatic mutation in hairy cell leukemia, prolymphocytic leukemia, Waldenstrom's macroglobulinemia, and myeloma. *Blood* 83:3647, 1994
38. Bakkus MHC, van Riet I, van Camp B, Thielemans K: Evidence that the clonogenic cell in multiple myeloma originates from a pre-switched but somatically mutated B cell. *Br J Haematol* 87:68, 1994
39. Kosmas C, Viniou N, Stamatopoulos K, Courtenay-Luck N, Papadaki T, Paterakis G, Anagnostou D, Yataganas X, Loukopoulos D: Analysis of κ light chain variable region in multiple myeloma. *Blood* 86:732a, 1995 (abstr, suppl 1)
40. Milstein C: From the structure of antibodies to the diversification of the immune response. Nobel Lecture. *Scand J Immunol* 37:385, 1993