Immunoglobulin genes in multiple myeloma: expressed and non-expressed repertoires, heavy and light chain pairings and somatic mutation patterns in a series of 101 cases

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Background and Objectives. The available data on the immunoglobulin gene (*IG*) repertoire in multiple myeloma (MM) derive mainly from heavy chains; considerably less is known about light chains. We assessed in parallel *IGH* and *IGK/IGL* rearrangements in 101 MM patients so as to gain insight into: (i) *IG* repertoires; (ii) antigen impact; (iii) the role of receptor editing.

Design and Methods. Bone marrow aspirates were collected from all cases. *IGH*V-(D)-J and *IGL*V-J rearrangements were amplified by reverse transcriptase polymerase chain reaction (PCR). In all cases, *IGK*V-J rearrangements were analyzed in parallel on cDNA/genomic DNA. *IGK*V-*KDE* and *IGK*J-C-INTRON-*KDE* were also amplified by DNA-PCR. RT-PCR products were directly sequenced.

Results. *IGH*V3 genes predominated; the *IGH*V4-34 gene was used in only one case. Five *IGK*V and five *IGL*V genes accounted for the majority of in-frame, transcribed *IGK*V-J or *IGL*V-J rearrangements. Taking *IGK*V-J, *IGK*V-*KDE* and *IGK*J-C-INTRON-*KDE* rearrangements together, biallelic IGK locus rearrangements were detected in 22/43 κ-MM cases. In λ-MM, 36/42 cases had at least one rearranged *IGK* allele; 8/19 *IGK*V-J rearrangements in λ-MM were in-frame. All in-frame, transcribed *IG*H/*IGK*/*IGL* sequences were mutated; parallel heavy/light chain analysis demonstrated a comparable impact of somatic hypermutation.

Interpretation and Conclusions. Biases in *IG* repertoire did not seem disease-related but followed a similar pattern to that of the normal repertoire. The under-representation of the *IGH*V4-34 gene provides an explanation for the paucity of autoimmune phenomena in MM. Somatic mutation patterns indicate the complementary role of MM *IG*H/*IGK*/*IGL* sequences in antigen recognition. Finally, the frequent inactivation of productive *IGK*V-J joints by secondary rearrangements in MM suggests active receptor editing.

Key words: multiple myeloma, immunoglobulin repertoire, κ deleting element.

Haematologica 2006; 91:781-787

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Multiple myeloma (MM) is a tumor of terminally differentiated plasma cells that home to and expand in the bone marrow. Much speculation exists concerning the exact cell in which malignant transformation occurs. The hypoproliferative nature of MM, with labeling indices in the clonal plasma cells rarely exceeding 1%,[1] has led to the hypothesis that MM is a tumor arising from a transformed precursor cell that proliferates and differentiates, giving rise to the clonal expansion of terminally differentiated plasma cells.[1] *IGH*V repertoire analysis in MM has revealed that malignant plasma cells carry heavily mutated *IGH*V genes, without intraclonal variation, and display a post-switch isotype in almost every case.[2-4] Allele-specific oligonucleotide-based polymerase chain (PCR) studies have demonstrated the existence of pre-switch M/D isotype bearing B-cells, clonally identical to G or A isotype-bearing myeloma cells from the bone marrow of the patients.[5-6] Analysis of enriched B-cell populations from MM patients identified C-μ and C-α transcripts in the peripheral blood clonally related to C-γ transcripts in the bone marrow, with the same number of mutations and no intraclonal variation.[7] Sequence analysis of the clonogenic *IGH*V-D-J transcripts indicated that no further somatic mutations had accumulated from the pre- to the post-switch isotype transition. This result indicates that the cell of origin has already undergone antigen selection while traversing the germinal center. However, it does not justify the conclusion that clonal proliferation and differentiation of the tumor occurs in the absence of antigen selection.[5-9] If the cell of origin of MM has undergone somatic hypermutation and antigen selection before malignant transformation, this experience should be reflected in *IG* heavy and light chain gene sequences. Analysis of *IG* light chain gene rearrangements also provides insight into the role of receptor editing in shaping the expressed repertoire. The structure of the *IGK* locus permits extensive edit-

ing through secondary rearrangements.[10-11] *IGK* loci are often inactivated in κ and mainly in λ light chain-expressing B cells by rearrangement to the κ-deleting element (KDE), which is located 32 to the *IGK*C gene. The KDE is rearranged either to an unrearranged *IGK*V gene upstream of a *IGK*V-J junction or to a recombination signal sequence in the *IGK*J-C intron.[12] Because the *KDE* encodes no protein, rearrangements to *KDE* are non-productive. Given that *KDE* rearrangements appear to occur concurrently with *IGL* locus recombination, they may be predicted to clear the way for λ expression.[10-12]

The available data on *IG* repertoires in MM derive mainly from analyses of IGHV-rearranged genes;[2,13-16] considerably less is known about *IGK*V- or *IGL*V-rearranged genes.[17-20] Furthermore, the available data from parallel analysis of *IGH* and *IGK*/L genes in MM are limited. The only relevant study by Sahota *et al.* comprised 15 cases and demonstrated that the heavy and light chains of the MM clonogenic cell have a complementary role in the process of antigen recognition.[17] Somatic hypermutation patterns reported by Sahota et al. indicated that the MM cell of origin has behaved as a normal germinal center B cell.[17] In the present study, we conducted a parallel analysis of expressed immunoglobulin heavy (*IGH*) and light (*IGK/IGL*) chain rearrangements in a series of 101 MM cases in order to extend our knowledge about *IG* gene repertoires and the impact of antigen selection on rearranged *IGV* genes. Given the importance of the *IGK* locus in receptor editing, we also studied non-expressed *IGK* locus rearrangements in an attempt to gain further insight into potential selection processes affecting MM precursor cells.

Design and Methods

Patients' samples

Bone marrow aspirates were collected from 101 MM patients with plasma cell infiltration of ≥20%. All patients had an identifiable serum monoclonal immunoglobulin of the same light chain type as the major plasma cell population. On the basis of serum immunoelectrophoresis/immunoblot analysis, 49 cases were assigned as κ-expressing MM, while 52 cases were assigned as λ-expressing MM. Our series included patients in whom monoclonal *IG* gene rearrangements were amplified by PCR for at least one *IG* locus. The study was approved by the local Ethics Review Committee of each Institution.

PCR amplification of IG rearrangements and sequence analysis

High-molecular-weight DNA was isolated from bone marrow mononuclear cells with the QIAamp DNA Blood Kit (QIAGEN Inc., Valencia, California, USA). Total cellular RNA isolation and cDNA preparation (after DNAse treatment) were performed as previously described.[21] *IGH*V-D-J and *IGL*V-J rearrangements were amplified by reverse transcription (RT) polymerase chain reaction (PCR) as previously described.[21] In all cases, *IGK*V-J rearrangements were analyzed in parallel by RT-PCR and DNA-PCR. Following our PCR protocol, distinct amplification signals are obtained only in the presence of monoclonal B cells comprising > 10% of the total cell population. This was revealed by dilution experiments in which DNA/cDNA from (i) the Raji cell line or (ii) a case with common acute lymphoblastic leukemia and 100% infiltration of the bone marrow by blast cells was mixed in different dilutions with DNA from normal peripheral blood lymphocytes. Rearrangements of the *IGK* locus to the KDE were also analyzed: both *IGK*V to *KDE* as well as *IGK*J-C-INTRON to *KDE* rearrangements were amplified by DNA-PCR using appropriate primers.[22] Peripheral blood DNA samples from ten healthy individuals and bone marrow DNA samples from two healthy donors were used as negative controls. In all cases, rearranged genes from normal polyclonal lymphocytes did not produce a distinct amplification signal visible on an ethidium bromide-stained agarose gel.

Direct sequencing was performed on the Applied Biosystems ABI 3730 sequencer. Both strands were analyzed. Each sequencing reaction was repeated three times on PCR products of three independent amplification reactions performed at different times. Sequence data were analyzed using the International ImMunoGeneTics information system (*IMGT, http://imgt.cines.fr*)[23-24] and the Basic Local Alignment Search Tool (BLAST) database (*http://www.ncbi.nlm.nih.gov/igblast*). Somatic mutation analysis was conducted following IMGT definitions for FR/CDR lengths.

Database sequence retrieval and analysis

MM sequences from our series were aligned to a comprehensive panel of sequences available from literature and/or retrieved in August 2005 from the IMGT/LIGM-DB sequence database. The compressed file containing the full IMGT flat file release (August 2005) was downloaded and the BioPerl programming package was used to write a Perl script to parse the downloaded IMGT flat file and to extract all human entries in it (25). Stringent criteria were followed so that redundant, poorly annotated, out-of-frame, incomplete sequences or sequences from clonally related cells carrying identical CDR3 were not included in the analysis. The collection of public sequences used for comparisons comprised 4066 sequences from various types of normal B cells (*Supplementary Material, online only*).

Results

IGHV-D-J rearrangements: repertoire analysis and CDR3 formation

A *background* of faint bands arising from polyclonal B cells was occasionally noted, primarily in patients with less infiltration of the bone marrow by malignant plasma cells. However, the *background* was more faint than the pattern observed in normal individuals (possibly due to the suppression of normal immunoglobulin synthesis in MM patients). This facilitated the detection of a predominant RT-PCR band in the MM patients under

study. Nonetheless, the MM *IGH* sequence was not identified in 27 patients. Therefore, *IGH* sequence data were available for 74/101 MM patients of the present series. All *IGH*V-D-J sequences were mutated and rearranged in-frame (*Supplemental Material*). Thirty-two functional *IGH*V genes of the *IGH*V1-6 subgroups were identified: *IGH*V3 genes predominated (31/74 cases; 41.9%). *IGH*V1-69 and *IGH*V5-51 were the most frequent genes, followed by *IGH*V3-30-3 and *IGH*V4-4 (Table 1). Comparison to both the 4066 sequences of the *normal dataset* (*see Patients and Methods and Supplementary Material*) and a subset of 339 sequences from normal IgG$^+$ peripheral blood cells[26] revealed a significantly higher frequency of the *IGH*V2 subgroup (p=0.0008 and p=0.002, respectively).

The median HCDR3 length was 15 amino acids (range 6-28) and, therefore, similar to that of normal B cells.[26-27] A decrease in HCDR3 length was observed in MM rearrangements as the homology to germline reduced. *IGH*D genes were identified in 73/74 HCDR3; *IGH*D3 and *IGH*D2 subgroup genes predominated (Table 2). *IGH*J4 was the most frequent *IGH*J gene (34/74 cases; 45.9%), followed by *IGH*J6 (14/74; 19%), *IGH*J3 (11/74; 14.8%) *IGH*J5 (7/74; 9.5%), *IGH*J2 (5/74; 6.7%) and *IGH*J1 (3/74; 4.1%). Non-templated nucleotides were detected in all junctions.

IGKV-J rearrangements: repertoire analysis and CDR3 formation

κ-expressing multiple myeloma

In κ-MM, 52 *IGK*V-J clonal rearrangements were amplified in 43 cases. Nine cases carried double rearrangements; double in-frame *IGK*V-J transcripts were detected in one case. Sixteen functional *IGK*V genes of the *IGK*V1-4 subgroups were identified in the transcribed, in-frame *IGK*V-J rearrangements (Table 3; *Supplementary Material*). Five *IGK*V genes (*IGK*V4-1, *IGK*V1-33/1D-33, *IGK*V1-39/1D-39, *IGK*V3-20, *IGK*V3-15) accounted for 69% of the in-frame rearrangements. Six different *IGK*V genes were identified in ten out-of-frame and/or non-transcribed *IGK*V-J rearrangements; the *IGK*V4-1 gene was used in 4/10 cases (Table 3; *Supplementary Material*). The median KCDR3 length in both transcribed, in-frame as well as non-transcribed and/or out-of-frame *IGK*V-J rearrangements was nine amino acids (range, 5-11); only 6/52 KCDR3s (11.5%) were longer than nine amino acids. The *IGK*J1-2 genes predominated (36/52 rearrangements; 69.2%), in keeping with the normal repertoire (28-29). Non-templated nucleotides were detected in 26/52 junctions.

λ-expressing multiple myeloma

Nineteen clonal *IGK*V-J rearrangements were amplified in 17 cases; two cases carried two different rearrangements. Ten different germline genes of the *IGK*V1/2/3/4/7 subgroups were identified. *IGK*V4-1 was the most frequent *IGK*V gene, followed by *IGK*V1-33/1D-33 and *IGK*V1-39/1D-39 (Table 3; *Supplementary Material*). Eight out of 19 rearrangements (42%) were in-frame, while 11/19 rearrangements (58%) were out-of-frame. The median KCDR3 length was nine amino acids (range, 5-11); 7/17 KCDR3s (41%) were longer than nine amino

Table 1. *IGHV* gene repertoire in the present series.

Gene	*n (%)*	*Gene*	*n (%)*
*IGHV*1-18	2 (2.70)	*IGHV*3-48	1 (1.35)
*IGHV*1-2	3 (4.05)	*IGHV*3-49	1 (1.35)
*IGHV*1-24	2 (2.70)	*IGHV*3-64	1 (1.35)
*IGHV*1-3	1 (1.35)	*IGHV*3-66	1 (1.35)
*IGHV*1-69	6 (8.11)	*IGHV*3-7	2 (2.70)
*IGHV*2-26	3 (4.05)	*IGHV*3-74	2 (2.70)
*IGHV*2-5	2 (2.70)	*IGHV*3-9	3 (4.05)
*IGHV*2-70	1 (1.35)	*IGHV*4-30-2	1 (1.35)
*IGHV*3-11	2 (2.70)	*IGHV*4-31	1 (1.35)
*IGHV*3-15	3 (4.05)	*IGHV*4-34	1 (1.35)
*IGHV*3-20	1 (1.35)	*IGHV*4-39	4 (5.40)
*IGHV*3-21	3 (4.05)	*IGHV*4-4	5 (6.76)
*IGHV*3-23	2 (2.70)	*IGHV*4-59	3 (4.05)
*IGHV*3-30	3 (4.05)	*IGHV*4-61	1 (1.35)
*IGHV*3-30-3	5 (6.76)	*IGHV*5-51	6 (8.11)
*IGHV*3-33	1 (1.35)	*IGHV*6-1	1 (1.35)

Table 2. *IGHD* gene repertoire in the present series.

IGHD	*n*	*IGHD*	*n*
*IGHD*1-1	1	*IGHD*3-9	1
*IGHD*1-14	1	*IGHD*4-17	5
*IGHD*1-26	2	*IGHD*4-23	4
*IGHD*2-15	4	*IGHD*4-4	1
*IGHD*2-2	4	*IGHD*5-12	2
*IGHD*2-21	3	*IGHD*5-24	1
*IGHD*2-8	5	*IGHD*5-5	2
*IGHD*3-10	8	*IGHD*6-13	4
*IGHD*3-16	4	*IGHD*6-19	6
*IGHD*3-22	5	*IGHD*6-6	1
*IGHD*3-3	8	*IGHD*7-27	1

Table 3. *IGKV* gene repertoire in the present series.

	κ MM total *n (%)*	*κ MM transcribed, in-frame* *n (%)*	*κ MM non-transcribed, out-of-frame* *n (%)*	*λ MM* *n (%)*
*IGKV*1-12	1 (1.92)	1 (2.38)	–	1 (5.26)
*IGKV*1-27	1 (1.92)	1 (2.38)	–	–
*IGKV*1-5	3 (5.77)	3 (7.14)	–	1 (5.26)
*IGKV*1-6	1 (1.92)	1 (2.38)	–	–
*IGKV*1-8	1 (1.92)	–	1 (10.00)	–
*IGKV*1-9	1 (1.92)	1 (2.38)	–	–
*IGKV*1-33/1D-33	7 (13.46)	6 (14.29)	1 (10.00)	3 (15.79)
*IGKV*1-37/1D-37	–	–	–	2 (10.53)
*IGKV*1-39/1D-39	8 (15.38)	6 (14.29)	2 (20.00)	3 (15.79)
*IGKV*1D-43	1 (1.92)	-	1 (10.00)	–
*IGKV*2-24	1 (1.92)	1 (2.38)	–	–
*IGKV*2-30	2 (3.85)	2 (4.76)	–	2 (10.53)
*IGKV*2-28/2D-28	2 (3.85)	2 (4.76)	–	–
*IGKV*2D-29	–	–	–	1 (5.26)
*IGKV*2D-40	1 (1.92)	1 (2.38)	–	–
*IGKV*3-15	4 (7.69)	4 (9.52)	–	–
*IGKV*3-20	6 (11.54)	5 (11.91)	1 (10.00)	1 (5.26)
*IGKV*4-1	12 (23.08)	8 (19.05)	4 (40.00)	4 (21.05)
*IGKV*7-3	–	–	–	1 (5.26)

Table 4. **Distribution of κ and λ multiple myeloma (MM) cases of the present series according to the type of *IGK* locus rearrangements.**

Type of rearrangement	*κ MM*	*λ MM*
No rearrangement	0	6
IGKV-J	19	5
IGKV-KDE	0	3
IGKJ-C-intron-*KDE*	0	10
IGKV-J + *IGKV*-J	7	1
IGKV-J + *IGKV-KDE*	9	1
IGKV-J + *IGKJ*-C-intron-*KDE*	6	4
2 *IGKV*-J + *IGKJ*-C-intron-*KDE*	2	1
IGKV-KDE + *IGKJ*-C-intron-*KDE*	0	6
IGKV-J + *IGKV-KDE* + IGKJ-C-intron-*KDE*	0	5
Total	**43**	**42**

acids. The *IGKJ*1-2 genes were used in 11/19 rearrangements (58%). Non-templated nucleotides were inserted in 12/19 *IGKV*-J junctions.

IGK locus rearrangements to the κ deleting element (KDE)

In κ-MM, 43 cases were examined for non-productive *IGKV-KDE* and *IGKJ*-C-INTRON-*KDE* rearrangements. Nine out of 43 cases (20.9%) carried *IGKV*-KDE rearrangements; *IGKJ*-C-INTRON-*KDE* rearrangements were amplified in 8/43 cases (18.6%). Overall, 17/43 κ-MM cases (39.5%) had PCR evidence for monoallelic *KDE* rearrangements leading to *IGK* alocus inactivation on that particular allele (*see Supplemental Material*). In λ-MM, *IGKV-KDE* and *IGKJ*-C-INTRON-*KDE* rearrangements were amplified in 15/42 cases (35.7%) and 26/42 cases (61.9%), respectively (*see Supplementary Material*). In both κ- and λ-MM, the *IGKV* gene repertoire in *IGKV-KDE* rearrangements was diverse; *IGKV*2-30 was the most frequent gene (3/19 cases with available sequence data).

Multiple rearrangements of the IGK locus

Nineteen out of 43 κ-MM cases (44.2%) analyzed for *IGKV*-J, *IGKV*-KDE and *IGKJ*-C-INTRON-*KDE* rearrangements carried only one *IGK* locus rearrangement in the form of a single *IGKV*-J joint (Table 4; *see Supplementary Material*). PCR evidence for biallelic *IGK* locus rearrangements was obtained in: (i) 17/43 cases (39.5%) carrying a rearrangement to the *KDE* along with an expressed *IGKV*-J rearrangement (Table 4), and (ii) 5/43 cases (11.6%) with double *IGKV*-J rearrangements. In such cases, biallelic *IGK* rearrangements were suggested by *IGKJ* gene repertoire analysis as well as location and mode of recombination (deletional vs. inversional) of the *IGKV* genes identified in both rearrangements. In four κ-MM cases, both *IGKV*-J rearrangements could have occurred sequentially on either one or both IGK alleles. Only six out of 42 (14.3%) λ-MM cases had no PCR evidence for *IGK* locus rearrangements. Eighteen cases carried one rearrangement on the *IGK* locus, while 12 and six cases, respectively, bore two or three *IGK* locus rearrangements (Table 4; *see Supplementary Material*).

Table 5. ***IGLV* gene repertoire in the present series.**

Gene	*n(%)*	*Gene*	*n(%)*
*IGLV*1-40	4 (9.30)	*IGLV*2-23	4 (9.30)
*IGLV*1-41	1 (2.33)	*IGLV*2-8	1 (2.33)
*IGLV*1-44	2 (4.65)	*IGLV*3-1	9 (20.93)
*IGLV*1-47	3 (6.98)	*IGLV*3-10	1 (2.33)
*IGLV*1-51	1 (2.33)	*IGLV*3-21	8 (18.60)
*IGLV*2-11	2 (4.65)	*IGLV*3-25	1 (2.33)
*IGLV*2-14	4 (9.30)	*IGLV*5-45	1 (2.33)
*IGLV*2-18	1 (2.33)		

IGLV-J rearrangements: repertoire and CDR3 formation

Forty-four transcribed *IGLV*-J rearrangements were amplified in 43 λ-MM cases; one case carried an additional, transcribed, out-of-frame *IGLV*-J rearrangement. Five functional *IGLV* genes (*IGLV*3-1, *IGLV*3-21, *IGLV*1-40, *IGLV*2-14, *IGLV*2-23) were used in 29/43 (67.4%) in-frame, transcribed *IGLV*-J rearrangements (Table 5; *see Supplementary Material*). The median LCDR3 length was 11 amino acids (range, 8-12); only 6/43 cases (14%) had a 12 amino acid-long LCDR3. The *IGLJ*2/3 genes were used in 37/43 rearrangements (86%), whereas the *IGLJ*1 gene was used in 6/43 rearrangements (14%). Non-templated nucleotides were inserted in 31/43 *IGLV*-J junctions.

Somatic hypermutation analysis

All *IGH* sequences were mutated with homologies to germline ranging from 83% to 97.6% (average homology: 92.2%). One *IGHV*4-39-expressing case exhibited *IGHV* sequence changes consistent with duplication of three base pairs (bp) at IMGT-CDR2 codon 60. In all cases, the vast majority of HCDR mutations were replacement (R) mutations, contrasting the situation in HFR, in which a more even incidence of R and S (silent) mutations was observed (average R/S ratios in HCDR and HFR were, respectively, 3.12 and 2.05).

All transcribed, in-frame *IGKV*-J rearrangements in κ-MM were mutated with homologies to germline ranging from 84% to 98% (average homology: 93.9%). Most replacement mutations occurred in KCDR1 (clustering over KCDR1 codons 30-32). One *IGKV*2-28/2D-28-expressing case exhibited *IGKV* sequence changes consistent with deletion of three bp at IMGT-CDR1 codon 29. Average R/S ratios in KCDR and KFR were 2.98 and 1.5, respectively. Seven out of ten non-transcribed and/or out-of-frame *IGKV*-J rearrangements in κ-MM had 100% homology to germline. Two out of the remaining three rearrangements were heavily mutated and also carried crippling mutations (one had multiple stop codons and a one-bp deletion in KFR3). In λ-MM, six out of 19 *IGKV*-J rearrangements had less than 100% homology to the germline, of which five were in-frame; three out of six mutated cases had less than 98% homology. One heavily mutated sequence carried a stop codon as well as a one-bp deletion in KFR2. All transcribed, in-frame *IGLV*-J rearrangements

were mutated with homologies to germline ranging from 85.6% to 97.8% (average homology: 93.4%). Most replacement mutations clustered in LCDR1 (LCDR1 codons 30 and 32). Average R/S ratios in LCDR and LFR were 4.37 and 1.75, respectively.

Parallel assessment of *IG*H and *IGK/IGL* mutation status was possible for 30 κ-expressing and 27 λ-expressing MM cases. Five out of 30 κ- and 7/27 λ-MM cases had CDR R/S ratios ≥3.0 along with FR R/S ratios <2.0 in both heavy and light chains. A similar pattern of R/S mutation distribution in CDR vs. FR was observed in exclusively heavy chains in 5/30 κ- and 3/27 λ-MM cases or light chains in 10/30 κ-MM and 6/27 λ-MM cases. Finally, in 10/30 κ- and 11/27 λ-MM cases, the distribution of R and S mutations was relatively uniform over FR and CDR in both heavy and light chains.

Discussion

In the present study, we conducted a parallel analysis of *IG* heavy and light chains in a series of 101 multiple myeloma (MM) cases and also reviewed all sequences available in public databases as of August 2005. Our study revealed predominant rearrangements of *IGHV*3 subgroup genes, in keeping with those in normal B cells.[26,27] A significant over-representation of *IGHV*3 subgroup genes in comparison to other *IGHV* subgroups was recently observed in a series of 84 MM patients analyzed for complete *IGHV*-D-J as well as incomplete *IGHD*-J rearrangements.[16] The increased frequency of the *IGHV*3 subgroup most likely reflects its germline complexity.[30] Since no preference in *IGHD* or *IGHJ* expression was associated with the bias in the *IGHV*3 subgroup in MM cases, potential positive selective influences were related to the *IGHV* region and not to the antigen-binding HCDR3. Several of the most common IGHV genes in our series (eg, *IGHV*3-9, *IGHV*3-30, *IGHV*3-15, *IGHV*4-39, *IGHV*1-2) were used at a frequency which is not disease-related but mirrors the repertoire in normal B cells.[26,27] Compared to normal cells (*Supplementary Material*), other *IGHV* genes were found at increased (eg, *IGHV*1-69, *IGHV*5-51, *IGHV*4-4) or decreased frequency (eg, *IGHV*3-23, *IGHV*4-34) ($p<0.05$ for all comparisons). Nevertheless, *IGHV* repertoire biases in MM were much less pronounced than the biases observed in the IGHV repertoire in chronic lymphocytic leukemia (CLL).[31]

A consistent finding in all MM series[2,14,16] including ours is the very low frequency of the *IGHV*4-34 gene. In contrast, this gene is very frequent in the repertoire of other B-cell malignancies, in particular CLL: as recently reported by our group,[31] the *IGHV*4-34 gene was second overall among all identified *IGHV* genes in a series of 553 CLL patients. The *IGHV*4-34 gene encodes antibodies which are intrinsically autoreactive by virtue of recognition of the I/i blood group antigen.[32,33] The marked under-representation of the *IGHV*4-34 gene in MM provides a plausible molecular explanation for the paucity of autoimmune phenomena in MM as compared to in CLL or other B cell tumors. *IGHD* and *IGHJ* repertoires in the present series were not significantly different from those in normal B cells.[26,27] Similar results have also been reported recently in a study of complete as well as incomplete *IGHD*-J rearrangements in MM (in that study, the frequency of incomplete rearrangements was 60%).[16] Nevertheless, both in our study as well as in the aforementioned study, selected *IGHD* genes were used at increased or decreased frequencies, indicating positive and/or negative selection, respectively.

Comparison of the MM *IGKV* and *IGLV* repertoires found in the present series to published repertoires from normal cells[28,29,34,35] revealed that the MM repertoires are generally similar to those of normal B cells. Therefore, the increased frequency of selected *IGKV* or *IGLV* genes in MM is most likely due to efficient recombination mechanisms rather than antigen selection pressures. The only genes significantly increased in MM vs. normal cells were *IGKV*4-1, *IGKV*1-33/1D-33 and *IGLV*3-1. Comparison of the MM and CLL *IGKV*/*IGLV* repertoires[21] confirmed that the over-representation of the *IGKV*1-33/1D-33 and *IGLV*3-1 genes is *MM-biased*. Interestingly, the *IGKV*1-33/1D-33 and *IGLV*3-1 genes are also frequent in the repertoire of patients with amyloidosis.[36-38]

In normal B cells, secondary rearrangements at the *IGK* loci as part of tolerance-induced receptor editing play an important role in shaping the expressed *IG* repertoire.[10,11] Our analysis included non-expressed *IGK* locus rearrangements in an attempt to gain further insight into potential selection processes affecting myeloma precursor cells. Taking *IGKV*-J, *IGKV*-KDE and *IGKJ*-C-INTRON-KDE rearrangements together, 22/43 κ-MM cases (51.2%) carried biallelic *IGK* locus rearrangements. In keeping with both normal cells[39] and CLL,[40] the vast majority (36/42 cases; 85.7%) of λ-expressing MM cases carried at least monoallelic *IGK* locus rearrangements. This indicates that the light chain rearrangement *hierarchy* in MM clonogenic cells is not inherently different from that of either normal cells or other types of malignant B cells.

Similar to normal λ-expressing B cells,[39] a significant percentage of the amplified *IGKV*-J rearrangements in λ-MM cases of our series (8/19; 42.1%) were in-frame (productive). The inactivation of pre-existing productive rearrangements by secondary *IGK* recombination events could possibly indicate that, in keeping with normal B cells, receptor editing plays an active role in shaping the expressed *IG* repertoire in MM. Similar results have been reported recently by Perfetti *et al*. in a series of 29 λ MM cases.[20]

Collectively, three out of 20 out-of-frame *IGKV*-J rearrangements amplified in either κ- or λ-MM were transcribed. Normally, defective mRNA transcripts are decomposed by special RNA surveillance mechanisms (nonsense-mediated decay), which operate very efficiently in B and T lymphocytes.[41] The low frequency of defective *IG* transcripts reported here indicates operation of active mRNA surveillance mechanisms in MM clonogenic cells.

Our parallel analysis of heavy and light chains in MM extends and confirms previous observations by Sahota *et al*. that a complementary imprint of antigen may be detected in *IGHV* and *IGK/LV* sequences, suggesting an

active role for light chains in antigen recognition.[17] In our study, all transcribed, in-frame I*GH/IGK/IGL* sequences were mutated; average homologies to germline were similar in all groups of sequences. In both κ- and λ-MM, a proportion of non-expressed *IGK*V-J rearrangements had less than 100% homology to germline. Somatic mutations were observed in both in-frame and out-of-frame rearrangements. The existence of mutated, in-frame, non-expressed *IGK*V-J rearrangements might be considered as indirect evidence for secondary rearrangements after the onset of somatic hypermutation, as previously reported in a setting of chronic stimulation of the immune system in autoimmunity or B-cell malignancy.[42-44] Nonetheless, somatic mutation patterns in non-expressed rearrangements of the present series (high R/S mutation ratios in FR, stop codons) could more plausibly be attributed to a *bystander mutagenesis* effect, whereby non-expressed rearrangements are mutated without selection for expression of a functional antigen receptor, as previously reported for both normal and neoplastic cells.[45,46]

In conclusion, the present study confirms that the MM clonogenic cell has been positively selected during the development and reaction of the germinal center. Biases in *IG* repertoire do not seem to be disease-related but generally follow a similar pattern to the normal repertoire, with the striking exception of the *IGHV*4-34 gene which is almost absent in MM. Our results provide strong evidence for the complementary impact of antigen on MM *IGH* and *IGK/IGL* sequences. Furthermore, they indicate the important role of receptor editing in shaping the expressed MM IG repertoire.

AH, KS and CB were responsible for designing the study, interpreting results and writing the manuscript. CL, NS, TS and KH participated in sample collection, molecular analysis of the samples and statistical comparisons. The order in which the names of the authors appear is based on their contribution to the study. All authors critically revised the paper and gave the final approval for its submission. NL, AA and AF are heads of the participating Departments and are cited last.

We wish to thank Prof. Marie-Paule Lefranc and Dr. Veronique Giudicelli (Laboratoire d'Immunogenetique Moleculaire, LIGM, Universite Montpellier II, UPR CNRS) for valuable help with the immunoglobulin gene analysis and many stimulating discussions. The authors declare that they have no potential conflicts of interest.

Manuscript received November 28, 2005. Accepted April 5, 2006.

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