

Immunoglobulin gene rearrangements and the pathogenesis of multiple myeloma

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The ability to rearrange the germ-line DNA to generate antibody diversity is an essential prerequisite for the production of a functional repertoire. While this is essential to prevent infections, it also represents the “Achilles heel” of the B-cell lineage, occasionally leading to malignant transformation of these cells by translocation of protooncogenes into the immunoglobulin (Ig) loci. However, in evolutionary terms this is a small price to

pay for a functional immune system. The study of the configuration and rearrangements of the Ig gene loci has contributed extensively to our understanding of the natural history of development of myeloma. In addition to this, the analysis of Ig gene rearrangements in B-cell neoplasms provides information about the clonal origin of the disease, prognosis, as well as providing a clinical useful tool for clonality detection and minimal residual

disease monitoring. Herein, we review the data currently available on both Ig gene rearrangements and protein patterns seen in myeloma with the aim of illustrating how this knowledge has contributed to our understanding of the pathobiology of myeloma. (Blood. 2007;110:3112-3121)

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Introduction

During early B-cell differentiation in the bone marrow (BM) the variable (V), diversity (D), and joining (J) gene segments of the immunoglobulin (Ig) genes are rearranged in an ordered fashion to generate the primary Ig repertoire. Ig heavy chain gene (*IGH*) rearrangement precedes Ig light chain gene rearrangement, and D_H to J_H joining precedes V_H to DJ_H joining.^{1,2} These rearrangements are mediated by a tightly regulated enzymatic machinery involving several different proteins operating at the DNA level, which are controlled by the recombination signal sequences (RSSs) flanking each gene segment (Figure 1).^{3,4} The process of allelic exclusion ensures that once a functional VJ_H rearrangement has been achieved, the other *IGH* allele is generally excluded from further recombination attempts.⁵ Following successful *IGH* recombination, the Ig light chain loci proceed to rearrange; initial attempts occur at the Ig kappa locus (*IGK*), and if a functional *IGK* rearrangement is not achieved the Ig lambda locus (*IGL*) undergoes recombination.^{6,7} Usually, rearrangements of the *IGL* locus are accompanied by deletion of the nonfunctional *IGK* rearrangements (Figure 2).^{7,8}

Assembly of a functional IgH-IgL protein complex on the cell surface, the so-called B-cell receptor (BCR), allows B cells to escape apoptosis and proceed with maturation.⁹ Immature yet immunocompetent B cells expressing surface Ig exit the BM environment and move to the secondary lymphoid organs. In the lymph node B cells pass through the mantle zone to reach the germinal center (GC), where affinity maturation occurs, following encounter with their cognate antigen.¹⁰ This process involves close interaction between B cells, antigen-processing dendritic cells, and T cells, allowing B cells carrying antigen-specific receptors on their surface to survive and proliferate. At this stage, 2 major molecular events occur: somatic hypermutation (SHM) and class switch recombination (CSR).^{11–14} SHM introduces point mutations in the rearranged Ig genes, producing high-affinity antibodies that recognize and optimally bind to foreign antigens. After initial

contact with antigen, low-affinity IgM is produced, and CSR is the mechanism that changes that isotype to IgG-, IgA-, or IgE-generating specific antibodies with different functional characteristics (Figure 1).

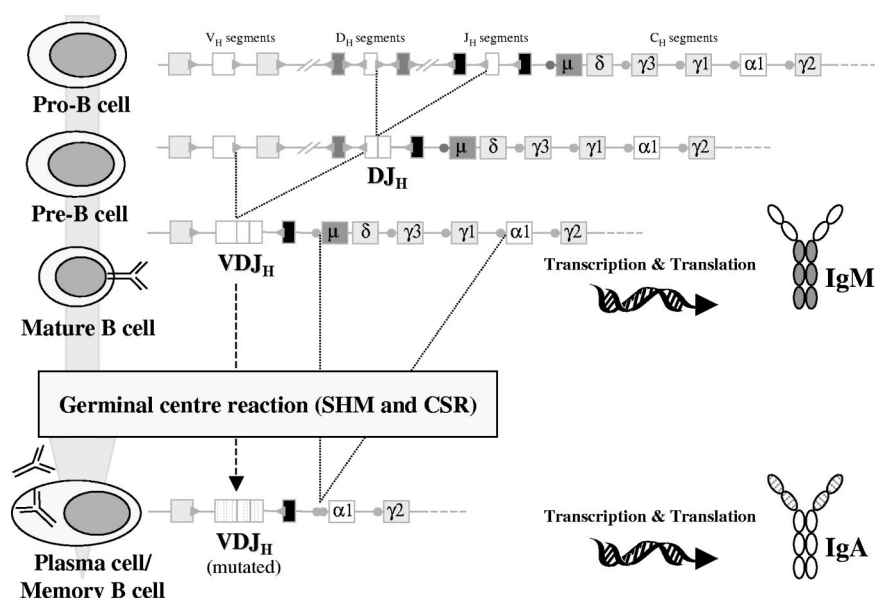
As a result of having undergone the processes of SHM and CSR, the Ig genes in plasma cells (PCs) from myeloma patients are characterized by heavily mutated V_H regions with no intraclonal variation and carry isotype-switched *IGH* genes (IgG or IgA).¹⁵ A significant number of myelomas (50% to 70%) also carry translocations targeting the switch regions of the *IGH* genes located at chromosome 14q32.^{16,17} These aberrant rearrangements juxtapose oncogenes into the proximity of the powerful *IGH* enhancers, driving abnormal expression of the translocated oncogenes. These characteristics together with the known biased usage for certain V and D gene segments establish a molecular archaeology for myeloma in which positive and negative selection processes shape the Ig repertoire prior to the acquisition of the malignant phenotype.^{18–20}

Paraprotein patterns in myeloma

Heavy chain expression patterns

Recently, the International Myeloma Working Group has collected more than 10 000 cases as part of the International Staging System (ISS) study.²¹ In this series 60% of cases had an IgG paraprotein, 24% had IgA, 11% produced only a light chain, 3% had IgD, and 2% had biclonal or another isotype.²¹ It is also possible to distinguish 4 different subclasses of IgG and 2 IgA subclasses. Pooling the published data the following distribution can be seen: 68% IgG1, 17% IgG2, 11% IgG3, and 4% IgG4 for the IgG group

Figure 1. Schematic diagram of *IGH* gene rearrangement, CSR, and SHM during B-cell development. D_H to J_H recombination occurs at the pro-B stage, producing an incomplete DJ_H rearrangement. This is followed by V_H to DJ_H recombination at the pre-B stage that will give rise to a complete VDJ_H rearrangement. If this VDJ_H is functional, the cell will develop into a mature B-cell-expressing surface IgM (IgM^+). These cells can enter the GCs where SHM and CSR occur, thus becoming V_H -mutated, class-switched (eg, IgA) memory B or plasma cells. Circles preceding the C_H gene segments (except δ) represent the switch regions. Dotted lines on the V_H , D_H , and J_H segments and the variable region of the Ig protein represent hypermutated genes after the SHM process.



and 93% IgA1 and 7% IgA2 for the IgA cases.^{22,23} These percentages largely reflect the proportions of Ig produced per day in normal individuals and, overall, they correlate with previous findings by different groups.^{24,25}

Myeloma has been considered to be a disease of postswitch cells, but it is now well established that preswitched IgM and IgD myelomas exist, though they are uncommon (0.5% and 2%, respectively, in the Mayo Clinic series).²⁴ Similarly, only 39 IgE myelomas have been described to date. Despite their low frequency, IgE, IgD, IgM, and nonsecretory myelomas are of

special interest due to their associations and biologic implications. A collection of 33 IgE, IgD, IgM, and nonsecretory myelomas showed an overrepresentation of t(11;14)(q13;q32) compared with IgG/IgA myelomas, with 83% of cases carrying the translocation.²⁶

Nonsecretory and light chain-only myelomas are seen in 1% to 3% and 10% to 20% of the total, respectively.^{21,24,27} These data have to be interpreted with caution because new techniques, such as the serum-free light chain assay, can actually detect portions of the Ig molecule in nonsecretory myelomas. Furthermore, to

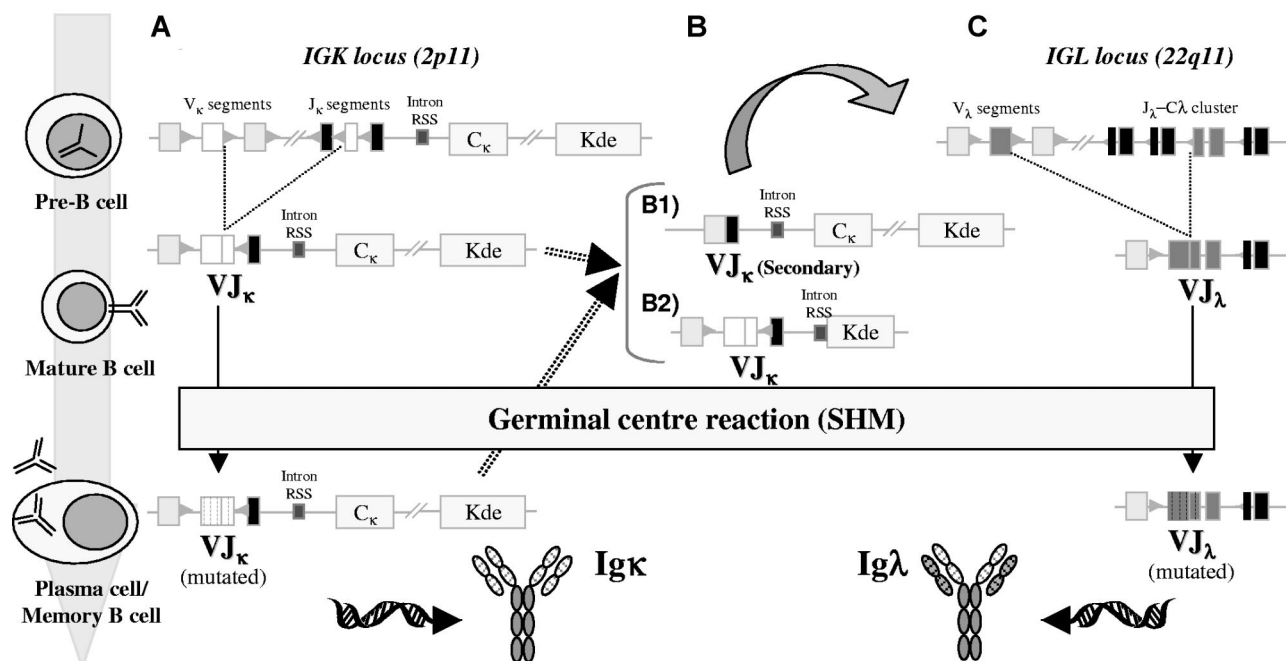


Figure 2. Schematic diagram of the ordered *IGK* and *IGL* gene rearrangement and SHM during B-cell development. At the pre-B cell stage, after *IGH* rearrangements have been completed and a μ heavy chain is produced, recombination at the *IGK* locus occurs between a V_K and a J_K segment. If such rearrangement is functional, the cell can enter the GCs and undergo SHM, with the end result a mutated IgK^+ memory B or plasma cell (A). However, if the *IGK* rearrangements at the pre-B stage produce nonfunctional IgK chains, additional rearrangements can occur in both *IGK* alleles, either before entering the GC or during the GC reaction (B). We can distinguish between 2 different types of rearrangements. First, if 5' V_K and 3' J_K segments remain still available, secondary VJ_K rearrangements can occur, rendering a new (functional) VJ_K rearrangement (B1). Alternatively, deletional rearrangements via Kde can occur, with excision of the C_K region as shown or with deletion of the entire J_K - C_K region through direct V_K to Kde recombination (B2). Such deletions are normally accompanied by rearrangement of the *IGL* locus in 22q11 to rescue B cells from apoptosis, which can then enter the GCs undergoing affinity maturation via the SHM process (C).

conclusively define a case of true nonsecretory myeloma, the absence of a paraprotein must be maintained, with M protein developing in up to 24% of patients during follow-up.²⁴ Consequently, true nonsecretory myeloma may account for less than 1% of all myelomas.

Light chain expression patterns

Neoplastic PCs produce either κ or λ Ig light chain at a similar ratio to that normally produced in healthy individuals, 63% IgL- κ and 37% IgL- λ .²⁴ Although such distribution is thought to be similar for the different myeloma subtypes, a bias toward Ig λ expression has been noted in IgD-only myeloma. This could theoretically result as a consequence of receptor revision.²⁸ Three IgD λ myelomas have been analyzed in detail, and no indication of receptor revision was found.²⁹ The V κ -J κ rearrangements identified were all out-of-frame and without somatic mutations and, in support of this, a study on normal B cells reported similar observations.³⁰ Therefore, it remains to be established why IgD myeloma has a bias for Ig λ expression or why C μ deletion, which seems to be the basis of the IgD-only phenotype, occurs preferentially in Ig λ ⁺ B cells.

Biclonal/dual IgH expression

Based on protein electrophoresis data, up to 2% of myelomas can secrete 2 different IgH isotypes or subclasses, with most being IgG⁺/IgA⁺ (53%) or IgG⁺/IgM⁺ (26%).³¹ The existence of true biclonal myeloma based on the presence of more than 1 IgH subtype must be carefully reviewed because both serum M components may share the same clonal origin with an identical variable region rather than being 2 independent clones. Thus, at the RNA level, clonotypic C μ transcripts can be found in the BM of 68% of patients with IgG⁺ myeloma, consistent with the idea that these cells could be the clonogenic origin of the tumor clone.^{32,33} Similarly, in IgM⁺ myelomas C γ transcripts with a clonogenic CDR3 region can be identified, consistent with the cell of origin being able to undergo the CSR process.³⁴ Unfortunately, myelomas secreting 2 M components as detected by protein electrophoresis have not been studied in enough detail to allow us to comment on their clonal relationship. In this sense, they could also represent truly independent myeloma clones, with ongoing CSR detectable in subclones derived from the same myeloma progenitor or, alternatively, lack of allelic exclusion within the same myeloma cell.³⁵

Biclonal Ig κ /Ig λ expression

In contrast to cases showing 2 different IgH subtypes, cases characterized by 2 different IgL peaks can be considered as truly biclonal, because no molecular mechanism for changing light chain expression within the same myeloma clone has been described so far. Biclonal myeloma cases expressing both kappa and lambda are not exceptional, although its frequency has been estimated to be only around 1% to 2%.^{21,36} This low frequency has led to difficulty

in interpreting the clinical characteristics of these patients; some authors having found no difference in the clinical characteristics and prognosis,³¹ while others suggest a poor response to therapy.³⁷ Two cases expressing cytoplasmic Ig κ and Ig λ but secreting only IgG λ have been described by ourselves (M.v.d.B., unpublished results, June 2002), suggesting that it is possible to produce both light chain isotypes. This observation can be explained by the fact that the Ig κ light chain was unable to assemble with the Ig heavy chain and illustrates how a functional IgH-IgL assembly may be one of the mechanisms leading to allelic exclusion and monospecificity.

IGH gene rearrangements in myeloma

The *IGH* locus contains 27 D_H segments, 6 J_H segments, and up to 44 functional V_H gene segments.³⁸ Rearranged VDJ_H genes represent the variable domain of the IgH molecules (Figure 1), with the V-D-J joining area—or complementarity-determining region (CDR)—constituting the hypervariable domain that determines the Ig specificity for a particular antigen.²

Patterns of IGH rearrangements

Most malignant PCs secrete mature antibodies (class switched and hypermutated) coded by a functional VDJ_H rearrangement.^{1,39} Southern blot analysis of *IGH* rearrangements in mature B-cell malignancies shows the presence of J_H rearrangements in virtually all samples, with up to 80% containing biallelic rearrangements.⁴⁰ It has recently been shown by polymerase chain reaction (PCR) that up to 60% of myeloma samples contain a single DJ_H rearrangement on the nonproductively rearranged allele. The remaining biallelic samples usually contain 2 VDJ_H rearrangements, 1 of which is nonfunctional.^{20,41} These data are consistent with the theoretical and observed frequencies of VDJ_H and DJ_H rearrangements in normal B cells, which again supports the regulated model of allelic exclusion during B-cell development (reviewed by Jung et al⁴²).

V_H, D_H, and J_H gene segment usage

Preferential usage of Ig gene segments has been shown during the different stages of normal B-cell maturation.^{43,44} The V_H3 family contains more members than any other and, consequently, it comprises most V_H gene segments found in myeloma, followed by the V_H4 and V_H1 families (Table 1).^{18-20,45,46} V_H3-30 has been consistently found to be preferentially rearranged in myeloma, followed by V_H5-51, V_H1-69, and V_H3-23 (Table 2).^{18,20,46} V_H3-30 and V_H3-23 are the most frequently used gene segments both in normal and malignant B-cell populations,⁴⁷⁻⁵⁰ suggesting that their preferential usage is not a myeloma-specific feature but reflects the frequency seen in normal VDJ_H recombination. Probably the most

Table 1. V_H family usage in *IGH* rearrangements in multiple myeloma

	VH1, no. (%)	VH2, no. (%)	VH3, no. (%)	VH4, no. (%)	VH5, no. (%)	VH6, no. (%)	No. of patients
Baker et al, 1994 ⁴⁵	5 (17.9)	1 (3.6)	15 (53.6)	6 (21.4)	0 (0)	1 (3.6)	28
Rettig et al, 1996 ¹⁸	13 (18.1)	4 (5.6)	34 (47.2)	14 (19.4)	6 (8.3)	1 (1.4)	72
Kiyoi et al, 1996 ¹⁹	4 (17.4)	1 (4.3)	10 (43.5)	4 (17.4)	4 (17.4)	0 (0.0)	23
Gonzalez et al, 2005 ²⁰	6 (8.2)	5 (6.8)	42 (57.5)	15 (20.6)	5 (6.8)	0 (0.0)	73
Hadzidimitriou et al, 2006 ⁴⁶	14 (18.9)	6 (8.1)	31 (41.9)	16 (21.6)	6 (8.1)	1 (1.4)	74
Total	42 (15.6)	17 (6.3)	132 (48.9)	55 (20.4)	21 (7.8)	3 (1.1)	270

Table 2. Preferential VH gene segment usage in *IGH* rearrangements in MM

	Hadzidimitriou et al, 2006 ⁴⁶	Gonzalez et al, 2005 ²⁰	Rettig et al, 1996 ¹⁸	No. * (%)
3-30†	8	8	6	22 (10)
5-51	6	4	2	12 (5.5)
3-23	2	4	5	11 (5)
1-69	6	1	4	11 (5)
3-15	3	5	2	10 (4.6)
3-09	3	2	4	9 (4.1)
4-59	3	4	2	9 (4.1)
3-11	2	4	1	7 (3.2)
Total per series	33	32	26	91 (41.6)

*Total = 219.

†Includes the polymorphic VH segment 3-30.3.

interesting finding in this area of research is the complete absence of V_H4-34 in the functional VDJ_H repertoire of myeloma cells,^{18,51} although it can be found in nonfunctional or extensively hypermutated *IGH* rearrangements from myeloma samples.²⁰ The fact that V_H4-34-producing cells are prevented from differentiating into antibody-producing plasma cells in healthy individuals (although not in patients with systemic lupus erythematosus)⁵² means that the lack of V_H4-34 in the functional repertoire of myeloma cells is not a disease-specific feature but a normal B-cell differentiation mechanism of negative selection. It is well known that V_H4-34 encodes antibodies that are intrinsically autoreactive, and this finding has also been proposed as an explanation for the paucity of autoimmune phenomena in myeloma. Moreover, V_H4-34 is extensively represented in the normal B-cell repertoire^{49,52-55} and in B-cell malignancies derived from all preplasma cell stages of differentiation.^{56,57} Taken together, these data indicate that myeloma cells arise from a B cell in a late stage of differentiation in which negative selection of autoreactive clones has already occurred, as opposed to other B-cell malignancies, where V_H4-34 is overrepresented.

D_H3 and D_H2 families are overrepresented in both the VDJ_H and DJ_H rearrangements in myeloma,^{19,20,46} a finding most likely reflecting normal frequencies of *IGH* rearrangements at the pro-B cell stage. However, some particular segments like D_H1-07 are found in DJ_H rearrangements at normal frequencies whereas they are completely excluded from the VDJ_H repertoire.^{20,46} These data again suggest that particular gene segments can be negatively selected after VDJ_H recombination in precursor cells. Taking into account all of the above findings, the obvious conclusion is that environmentally encountered antigen plays at least some part in the etiology of myeloma and/or normal PC differentiation.

Somatic hypermutation

All VDJ_H rearrangements in myeloma are hypermutated, containing higher mutation rates than any other B-cell malignancy, with an average rate of 9%, with up to 23% mutations being seen in some samples.^{41,51,58,59} Several studies have indicated that the pattern of SHM in *IGH* rearrangements can be accounted for by antigen selection according to a binomial distribution model.⁶⁰ However, about 40% of myeloma patients do not display such pattern in the *IGH* genes.

As opposed to VDJ_H rearrangements, most incomplete DJ_H rearrangements in myeloma are characterized by the absence of SHM.⁴¹ This finding has been applied in clinical strategies to improve detection rates aimed at demonstrating clonality and for PCR-based minimal residual disease (MRD) follow-up studies.^{61,62} Nonetheless, about 10% of DJ_H rearrangements show some evi-

dence of mutation, containing less than 98% homology to the closest germ-line gene.⁴¹ This finding may have biologic significance or simply reflect the presence of previously unknown polymorphisms. However, the nature of this phenomenon is currently uncertain.

Molecular explanation of IgH-negative myeloma

Lack of IgH protein production in nonsecretory myeloma can theoretically be caused by abnormalities at various levels (ie, at the genomic level, at the transcriptional level, at the translational level, or through rapid degradation of newly synthesized IgH protein). In a series of 9 IgH-negative myeloma cases and cell lines investigated to address this issue, lack of *IGH* transcription was suggested as the major cause.⁶³ However, in a study of 12 primary IgH-negative myeloma cases in which the *IGH* locus was analyzed in detail, gene rearrangement defects at the DNA level were confirmed as the principal cause. In 1 case a biallelic deletion of the J_H region was found, whereas in 5 others only incomplete DJ_H rearrangements were observed, mostly associated with deletion of the other allele. This finding of exclusive DJ_H rearrangements in combination with mutated *IGK* genes can only be explained in terms of normal B-cell development through deletion of the functional *IGH* allele. Also, the use of C_H probes showed that rearrangements of the J_H and C_H regions were not concordant in 5 cases, probably due to illegitimate CSR resulting in chromosomal translocation into the *IGH* locus affecting the other allele.⁶⁴ Consistent with this, using fluorescence in situ hybridization (FISH) most myeloma samples and cell lines lacking IgH production have been found to contain chromosome translocations.^{65,66} Therefore, the available data suggest that aberrations at the DNA level are the major cause of nonsecretory myeloma.

IGK and *IGL* gene rearrangements

Both the *IGK* and *IGL* loci contain a large set of V and several J gene segments but, unlike the *IGH* locus, there are no D gene segments (Figure 2). The human *IGK* light chain locus comprises many V_K gene segments, grouped into 7 families, and 5 J_K gene segments.⁶⁷ In addition, the kappa-deleting element (Kde), located near an RSS several kilobases downstream of the J_K-C_K region, can rearrange to V_K gene segments (V_K-Kde) or to an isolated RSS in the J_K-C_K intron (intron-Kde).⁶⁸⁻⁷⁰ Both types of Kde gene rearrangements lead to inactivation of the *IGK* allele, through deletion of the entire J_K-C_K area or the C_K region only, respectively (Figure 2B). The *IGL* locus contains many functional and pseudo V_λ gene segments, grouped into 10 families and organized in

Table 3. *IGK* gene segment usage in Igκ⁺ myeloma

Study	Vκ family usage					Jκ gene segment usage				
	Vκ1	Vκ2	Vκ3	Vκ4	Vκ5-7	Jκ1	Jκ2	Jκ3	Jκ4	Jκ5
Cannell et al, 1994 ⁷⁴	2/3	—	—	1/3	—	ND	ND	ND	ND	ND
Wagner et al, 1994 ⁷⁵	3/4	—	1/4	—	—	1/4	1/4	—	2/4	—
Kosmas et al, 1996 ⁷⁶	9/11	—	—	2/11	—	ND	ND	ND	ND	ND
Sahota et al, 1997 ⁵⁹	5/9	—	4/9	—	—	2/9	2/9	1/9	3/9	1/9
Kiyoi et al, 1998 ⁷⁸	8/17	3/17	4/17	1/17	1/17*	5/17	2/17	3/17	3/17	4/17
Hadzidimitriou et al, 2006 ⁴⁶	19/42	6/42	9/42	8/42	—	ND	ND	ND	ND	ND
Total	46/86	9/59	18/72	12/73	1/17	8/30	5/30	4/30	8/30	5/30

Values are number/total number of cases.

— indicates no single case found; ND, not determined.

*Vκ5 family usage.

3 large separate clusters, as well as 7 Jλ-Cλ regions, of which only the J-Cλ1, J-Cλ2, J-Cλ3, and J-Cλ7 regions are functional.^{71,72}

It can be anticipated that the *IGK* and *IGL* gene rearrangement patterns in Igκ⁺ and Igλ⁺ myeloma are similar to those found in other mature B-cell malignancies.^{46,59} Thus, Igκ⁺ myeloma cells harbor functional Vκ-Jκ gene rearrangements and hardly any *IGL* gene rearrangements, whereas Igλ⁺ myeloma cases show functional Vλ-Jλ gene rearrangements. Deletional rearrangements in the *IGK* locus are found on the nonexpressed second allele in Igκ⁺ myeloma cases but are even more frequent in Igλ⁺ cases, reflecting the end phase of unsuccessful recombination of the *IGK* locus prior to *IGL* rearrangement (Figure 2B). In a series of 84 myeloma patients analyzed by Southern blotting, 57% of cases showed monoclonal Kde rearrangements to the Vκ/intron-RSS segments (21% monoallelic and 36% biallelic rearrangements, respectively). All patients lacking this *IGK* deletion and those with monoallelic deletion showed an Igκ monoclonal component in serum and/or urine, while all cases with biallelic Kde rearrangements showed an Igλ monoclonal component (36%).⁷³

Gene segment usage and somatic hypermutation

Several reports have produced detailed analysis of Vκ and Vλ gene segment usage in myeloma. Most of the studies show a clear preference for Vκ1 family usage (53% of the total), followed by Vκ3 (25%) (Table 3).^{46,59,74-78} These percentages are in line with Vκ family usage in normal B cells and other B-cell lymphoproliferative disorders, largely reflecting the distribution of Vκ gene segments in the germ-line pool. In addition, a few Vκ1 gene segments seem to be used very often; these include the Vκ1-33/1D-33, Vκ1-39/1D-39, and Vκ1-6 gene segments.^{59,76,78} Also, the Vκ3-20 segment, believed to be indicative of autoimmunity, was seen relatively often in 3 studies.^{46,59,78} This finding would seem to contrast to that seen at the *IGH* locus, where no evidence of VDJ_H

rearrangements associated with autoantibodies is seen. However, the number of cases in the collective studies is too small to provide evidence for the involvement of particular (self-) antigens or epitopes in the etiology of myeloma. The studies dealing with Vλ usage in Igλ myeloma cases show largely equal Vλ1 (27%), Vλ2 (29%), and Vλ3 (41%) family usage (Table 4).^{46,59,77,78} Within these families, no clear preference for individual gene segments was observed.

Ample evidence exists for SHM in Vκ and Vλ gene segments of myeloma cases.^{59,74-78} However, the degree of somatic mutation can be variable between individual cases, with IgD/λ myeloma cases being renowned for extensive mutation in their V_H and Vλ regions.^{28,29} Similar to VDJ_H rearrangements, there are no indications of intraclonal heterogeneity of the Vκ or Vλ gene segments, implying that there is no ongoing SHM in myeloma cells. Clustering of replacement mutations has been found in the CDR regions of both Vκ and Vλ gene segments. Probability calculations according to Chang and Casali⁶⁰ using the Replacement/Silent mutation distribution revealed evidence for antigen selection in 8 of 17 (47%) myeloma cases in the studies of Kosmas et al^{76,77} and 4 of 15 (27%) in the study of Sahota et al.⁵⁹ In the latter, it was demonstrated that if significant clustering of mutation was present, it was in either V_H or V_L but not both. Notably, sequence changes in the *IGH* and *IGL* CDR3, which are not considered in the binomial model, can also drastically alter antigen binding, leading to an underestimation of those Ig gene rearrangements that have been selected for by antigen.^{50,59,79,80} Taking together these data and the data from the *IGH* genes, it can be concluded that most myeloma clones have been selected for by antigen.

Jκ and Jλ gene segment usage and *IGL* isotype

Theoretically, the differential usage of Jκ segments has no implications for the Igκ isotype, because all Vκ-Jκ gene rearrangements

Table 4. *IGL* gene segment usage in Igλ⁺ myeloma

Study	Vλ family usage				Jλ gene segment usage				
	Vλ1	Vλ2	Vλ3	Vλ4-10	Jλ1	Jλ2	Jλ3	Jλ4-6	Jλ7
Sahota, 1997 ⁵⁹	2/6	2/6	2/6	—	1/6	3/6	—	—	2/6
Kosmas, 1998 ⁷⁷	2/6	1/6	2/6	1/6*	ND	ND	ND	ND	ND
Kiyoi, 1998 ⁷⁸	4/16	5/16	6/16	1/16†	5/16	11/16‡	11/16‡	—	—
Hadzidimitriou, 2006 ⁴⁶	11/43	12/43	19/43	1/43§	6/43	37/43‡	37/43‡	—	—
Total	19/71	20/71	29/71	3/65	6/22	14/22	14/22	0/22	2/22

Values are number/total number of cases.

— indicates no single case found; ND, not determined.

*Vλ6 family usage.

†Vλ7 family usage.

‡No discrimination was made between Jλ2 and Jλ3.

§Vλ5 family usage.

will eventually use the same C κ segment. Accordingly, published data have shown the 5 different J κ gene segments used at similar frequencies in Ig κ^+ myeloma, with a slight predominance of J κ 1 and J κ 4 gene segments (Table 3).^{59,75,78} In the case of Ig λ , however, J λ usage has direct implications for the C λ isotype used, as a result of the J λ segments being located directly upstream of the respective C λ segments. Several studies of Ig λ^+ myelomas by Southern blotting and PCR have shown that J-C λ 2 and, to a lesser extent, J-C λ 3 are found more frequently (Table 4).^{59,78,81} This J λ 2 and J λ 3 usage in myeloma cases is similar to other B-cell malignancies. Remarkably, however, myeloma cases show predominance of J λ 2 over J λ 3 usage, whereas an inverse pattern is seen in other types of B-cell malignancies.⁸¹

Molecular explanations for Ig light chain negativity and aberrations in myeloma

Compared with IgH, the lack of Ig light chains has been studied much less extensively. Of the 3 true nonproducer cases studied in a series of 12 IgH-negative myelomas, no functional Ig light chain rearrangements could be amplified in 1 case.⁶⁴ In the other 2 cases an in-frame *IGK* and biallelic *IGL* gene rearrangements could be found, suggesting that genomic aberrations may not be the major factor in Ig light chain negativity. A clue toward the unusual posttranslational modification of Ig light chains has come from a report describing a light chain disease case with a truncated Ig κ chain due to unusual cleavage by signal peptidase.⁸² Although deletions in the V gene segments of the light chain loci have been shown to be a cause for truncated Ig light chain proteins in some cases of heavy chain disease (reviewed by Cogne et al⁸³), this has not been shown to happen in myeloma, probably due to the low total number of cases so far studied.

IGH translocations and class switch recombination

Translocations involving the *IGH* locus have been reported to be the most common genetic lesion in myeloma, including t(11;14), 15% to 21%; t(4;14), 10% to 14%; t(16;14), less than 5%; and t(6;14), less than 5% of all cases.⁸⁴⁻⁸⁶ Each *IGH* translocation is associated with the deregulation of a specific gene (or genes) mediating ectopic expression via the influence of the powerful *IGH* enhancers. The t(11;14)(q13;q32) leads to the overexpression of cyclin D1,⁸⁷ although it has been proposed that another gene at 11q13, *MYEOV*, can also be deregulated via this translocation.⁸⁸ The karyotypically cryptic t(4;14)(p16;q32) involves *FGFR3* and *WHSC1*, and it is well characterized at the molecular level.⁸⁹ *WHSC1* contains a SET domain and is overexpressed in t(4;14) myelomas in the form of an *IGH-WHSC1* hybrid fusion transcript.⁸⁹ Interestingly, the presence of *IGH-WHSC1* transcripts without expression of *FGFR3* is observed in a significant number (25% to 33%) of t(4;14) cases.^{90,91} This has been shown to occur through deletions of the whole der14 or the translocated *FGFR3* gene, although different mechanisms have also been seen in some cases.^{90,92} The incidence of such cases (one third of all t(4;14) myelomas) and the fact that *FGFR3* gene deletion is present in more than 80% of the myeloma cells in these cases suggests that it is a very early event soon after the translocation, making *FGFR3* overexpression dispensable for myeloma progression/evolution. Finally, t(6;14)(p21;q32) deregulates cyclin D3 and has been reported in about 4% of the patients, whereas t(14;16)(q32;q23)

deregulates c-maf and is present in about 5% of the patients. Deregulation of *MYC* by t(8;14)(8q24;q32) is more of a secondary event associated with tumor progression—being more commonly seen in cell lines—and is characterized by a different structure at the underlying breakpoints.⁹³⁻⁹⁵

In myeloma, the breakpoints on 14q32 are localized downstream of the VDJ region, in the more centromeric switch (S) regions situated upstream of all the constant genes except δ . Physiological CSR occurs between 2 S regions producing a hybrid S region; by convention, there is breakage of DNA in S μ and a downstream S α or S γ region (Figure 1). Errors in the process of breakage and reassembling the double-stranded DNA during CSR may cause DNA from another chromosome to become involved, resulting in a translocation event, so-called aberrant or illegitimate CSR. Given that most myeloma cells secrete a functional IgG or IgA molecule, any illegitimate CSR must therefore occur on the other (nonfunctional) allele. Furthermore, FISH analyses have shown that 14q32 translocations are normally present in most of the myeloma clone, indicating that they are one of the initiating events in myeloma pathogenesis.

Altogether, these data point toward defects during the physiological CSR process being responsible for the translocation events seen in most myeloma cases and that these translocations into the *IGH* locus provide one of the initial immortalizing events in the pathogenesis of myeloma. However, late acquisition of translocations may occur during progression of the disease (eg, t(8;14)), although in these cases the structure of the breakpoints seems to be different than in primary translocations (reviewed by Gabrea et al⁹⁶) and begs the question about the underlying mechanisms involved in these translocations.

Ontogeny of multiple myeloma

Any hypothesis about the etiology of myeloma should be able to explain the molecular characteristics found in myeloma cells that we have described so far: (a) evidence of antigen-driven selection with restricted VH segment usage and highly hypermutated Ig heavy and light chain genes with no intracлонаl variation; (b) class-switched functional Ig molecules (mainly IgG or IgA) in most cases, with detectable pre-switch (C μ) clonotypic transcripts in some cases; and (c) aberrant Ig translocations in 40% to 70% of samples.

One such hypothesis is illustrated in Figure 3 and is further described here: during the GC reaction, mature B cells are selected by foreign antigens and undergo the SHM and CSR processes to provide a functional IgG or IgA plasma cell that subsequently migrates to the BM. At this stage, cells carrying Ig molecules specific for certain antigens (ie, VH4-34–positive cells) are excluded from becoming PCs. If illegitimate CSR occurs during the GC reaction while the cell can still undergo maturation to a memory B or plasma cell, it may exit the lymph node with an acquired ability to survive and proliferate as a consequence of oncogene deregulation. Alternatively, translocations might occur in the Ig light chain loci in 2p11 or 22q11 during the process of receptor editing.⁹³ The acquired survival/proliferative ability would allow this premalignant clone of PCs to accumulate secondary hits, which will eventually occur in and deregulate critical genes, leading to the emergence of a malignant myeloma clone in the BM. The lack of intracлонаl variation in the Ig genes of myeloma cells is consistent with this idea. A feature of this hypothesis is that the final malignant transformation events occur within the BM after the cell

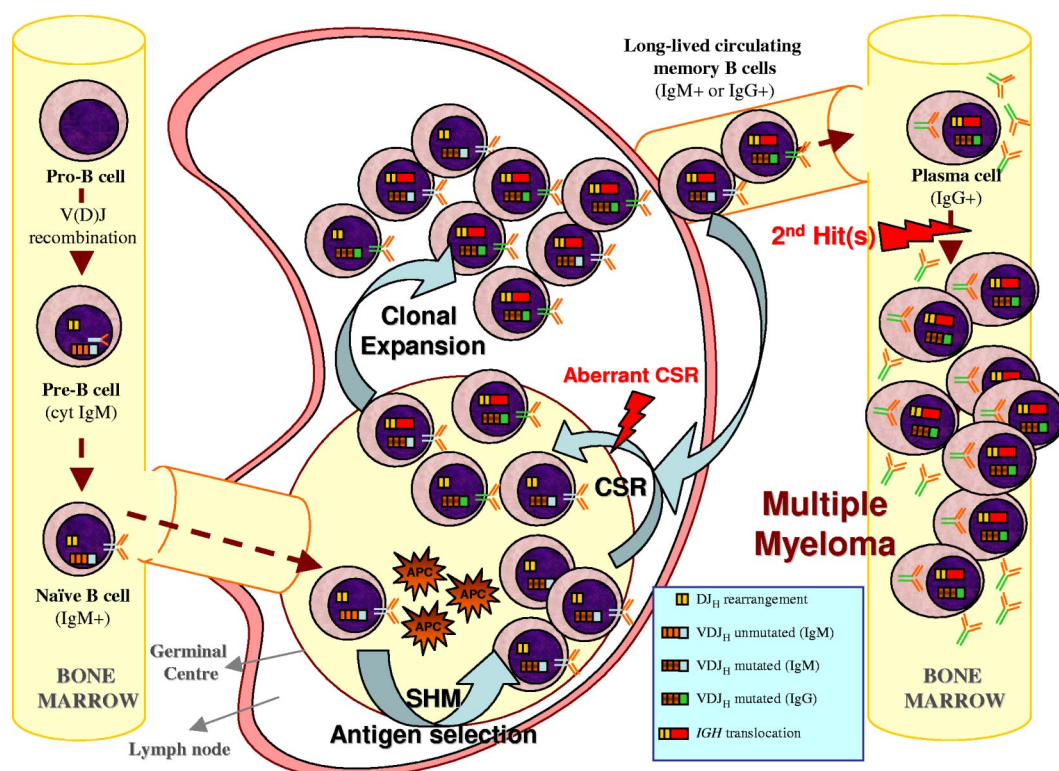


Figure 3. Multiple myeloma ontogeny's hypothesis exemplified by a 14q translocation-positive case. A naïve B cell harboring an incomplete DJ_H and a functional VDJ_H rearrangement enters the GC where it undergoes SHM and antigen selection (eg, selection against VH4-34 *IGH* rearrangements), followed by CSR. During the process of physiological CSR, double-strand breaks on the switch regions of the nonfunctional allele (DJ_H in the example) can be resolved by joining with a different chromosome resulting in a 14q32 translocation. If this illegitimate recombination occurs prior to legitimate CSR on the functional *IGH* allele, a mixture of different subpopulations (eg, IgM⁺ and IgG⁺ or IgA⁺) will have a survival or proliferative advantage due to the translocation, which will make them long-lived memory B or plasma cells without becoming fully malignant. After one of these subpopulations leaves the GC to become a plasma cell homing to the BM (normally IgG⁺ or IgA⁺), secondary genetic hits may occur that render such cells malignant plasma cells.

has abandoned the GC microenvironment. This hypothesis is similar to ideas put forward to explain the findings in follicular and mantle cell lymphoma, where Ig translocations are suggested to happen at the pro-B cell stage without altering the differentiation process to mature B cells.⁹⁷ In fact, the t(14;18) is frequently detected in healthy individuals without evidence of developing follicular lymphoma.⁹⁸

Switch translocations in myeloma occur preferentially in the nonfunctional allele, because only a small proportion of myeloma cases lack paraprotein expression. This suggests that at the time of the translocation, the cells are still dependent on BCR signaling, which, in turn, implies the need for a functional Ig molecule to be present at the cell surface. The presence and the molecular characteristics of the translocations in the switch regions of the *IGH* genes in myeloma suggest that they occur during the process of physiological CSR within the GC. On the basis of these and other observations, it can be concluded that translocation events in myeloma constitute early alterations being responsible for tumor initiation without being responsible for complete tumorigenic transformation. It is obvious, however, from gene expression and genomic studies that these translocations define subgroups of the disease with highly distinct gene signatures and outcomes.^{85,86,99}

The idea that myeloma-specific translocations are insufficient to produce a fully malignant phenotype is also supported by the observation that the frequency of translocations in monoclonal gammopathy of undetermined significance (MGUS) and myeloma is similar, with only a small number of MGUS patients progressing to myeloma. A possible exception is the low frequency of MGUS cases with a t(4;14), which seem to

progress more rapidly to myeloma. In this context, the t(4;14) could be associated with a higher rate of genomic instability that might facilitate the acquisition of new genomic alterations leading more rapidly to myeloma.

The hypothesis put forward herein is also consistent with the experimental observations of clonotypic IgM⁺ VDJ_H rearrangements in IgG or IgA myeloma patients. The explanation is that there are residual clonotypic “preswitch” cells—which may or may not contain the Ig translocation—that have differentiated into long-lived memory B cells or PCs without becoming fully malignant (Figure 3). These cells are predicted to be dependent on BCR signaling, and the consistent and ongoing interaction of these cells with the foreign antigen they are specific for may lead to clonal expansion and recirculation of these cells through the peripheral blood and BM, allowing their detection by PCR methods. Further experimental observation that would strengthen and support these ideas would be the detection of translocations involving the switch region of the *IGH* genes in a small percentage of memory B and nonmalignant PCs in myeloma patients as well as in PCs from healthy individuals. However, to the best of our knowledge, such studies are yet to be published.

Conclusions and future directions

Studying the “molecular archaeology” of myeloma using Ig gene rearrangement and mutation status has been particularly useful because of the lack of other tools with which to examine this process. In particular, analysis of the MGUS/myeloma transition

has been particularly informative. The presence of ongoing mutation within the PCs in some MGUS cases strongly suggests that the precursor cell in MGUS is continually exposed to the process of SHM and, by inference, must exist at a level still related to the GC.¹⁰⁰ Nonetheless, it does not exclude the possibility that cells acquire mutations in their Ig regions via other routes, as yet not fully defined. What is certain, however, is that in myeloma this apparent intraclonal diversity is absent and, on relapse, most myeloma samples secrete the same paraprotein and carry the same primary chromosomal aberrations as at diagnosis, consistent with the outgrowth of a single clone of PCs located within the BM. In this sense, this PC would seem to be the logical target for treatment, and eradicating this cell should be the focus of therapeutic approaches. However, this does not fully exclude the possibility that late relapses could be caused by a myeloma "stem cell" with a different phenotype than the myeloma PC, for which new treatment strategies may prove to be more effective.

The study of Ig gene rearrangements can also help us to understand the impact of treatment on the malignant clone. The clonal Ig gene rearrangements provide specific targets to monitor the tumor cells. In this context, PCR is a sensitive tool to detect clonal rearrangements at these loci, and the lack of SHM in most DJ_H rearrangements increases the rate of clonality detection by PCR and offers excellent targets for monitoring MRD by consensus real-time quantitative-PCR, thus overcoming the limitations of designing specific probes for each individual patient, which is significantly more expensive and time consuming.^{41,61,62}

Both patients and clinicians alike are keen to understand what causes myeloma, and while classical epidemiologic approaches have established some candidate factors, there is still a lot to learn about the etiology of this disease. Studies of Ig gene rearrangements can provide insights into this process and perhaps allow us to better focus classical and molecular epidemiologic studies. In this respect, Ig gene segment usage in myeloma appears to reflect some degree of both positive and negative selection by environmentally encountered antigen. These data, together with the presence of a high degree of SHM in *IGH* and *IGK/IGL* functional rearrangements and characteristic translocations targeting the switch regions of the *IGH* locus in most of the myeloma cases, indicate that processes involving the Ig genes, such as BCR signaling, CSR, and SHM, play a fundamental role in early myeloma pathogenesis.

Authorship

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