

Biased Ig λ expression in hypermutated IgD multiple myelomas does not result from receptor revision

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Normal IgM⁻IgD⁺ CD38⁺ B cells and IgM⁻IgD⁺ multiple myelomas (MM) are characterized by C μ deletion, biased Ig λ expression and hypermutated IgV regions. The predominant Ig λ usage has been proposed as resulting from secondary Ig gene rearrangements during extensive clonal expansion in the germinal center environment. Here, four cases of IgD λ MM were studied to address the question of light chain receptor revision in a 'single cell' model. Detailed analyses of both *IGK* and *IGL* alleles of each case were performed by Southern blotting, (RT-) PCR, and sequencing. The expressed IgV genes were extensively mutated and C μ deletion was confirmed in two cases. In addition, in the four MM a total of six non-functional deletional *IGK* rearrangements were identified, which proved to be unmutated. We conclude that IgD myelomas indeed originate from (post) germinal center B cells in which, in spite of the fact that they are hypermutated, there is no evidence of receptor revision.

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Introduction

A minority of normal human B cells and rare B cell malignancies express IgD in the absence of IgM. It has been reported that in the human tonsil, 2–5% of the CD38⁺ germinal center B cells and 6–20% of the plasma cells are IgM⁻IgD⁺.^{1,2} In the peripheral blood, sIgM⁻IgD⁺ CD27⁺ memory B cells have been detected with a frequency of less than 1%.³ Among the B cell malignancies, approximately 1% of multiple myelomas (MM) and 10% of hairy cell leukemias display the IgD-only phenotype.⁴ The lack of IgM in all these cell populations is explained by unusual C μ -C δ class 'switch' recombination mediated by sequences in the J_H-C μ and C μ -C δ introns, ie either homologous recombination between two direct 442 bp repeats (σ_{μ} and Σ_{μ})^{5,6} or non-homologous, so-called S μ - $\sigma\delta$ recombination,⁴ both with deletion of the S μ -C μ region and precluding further isotype switching. The IgM⁻IgD⁺ B cell populations mentioned share two additional molecular features. First, IgM⁻IgD⁺ B cells generally carry a very high load of somatic mutations, most likely as a result of extensive clonal expansion: whereas the frequency of nucleotide substitutions in the V_H genes of normal (post)-germinal center B cells ranges between 2 and 6%,^{3,7} the reported frequencies for both the normal and neoplastic single IgD-positive B cells are on average two- to three-fold higher.^{1–3,5,6,8} Second, the various normal IgM⁻IgD⁺ B cells almost exclusively express Ig λ (>99%).¹ Also in IgM⁻IgD⁺ MM, high frequencies of Ig λ light chain expression have been reported, ranging between 60 and 90%.^{9–14}

It has been hypothesized that the biased Ig λ expression of IgM⁻IgD⁺ B cells results from secondary Ig gene rearrangements at the light chain loci in the germinal center environment (ie receptor revision), elicited by unfavorable somatic mutations that cause loss of Ig expression or disturbed pairing of Ig heavy and light chains.² Ig light chain gene rearrangements generally occur in an ordered sequence, starting at the *IGK* locus followed by *IGK* deletion and subsequent *IGL* rearrangements.¹⁵ Therefore, secondary rearrangements are expected to result in skewing towards *IGL* gene usage.

Evidence for receptor revision as a rescue mechanism in mature human B cells is as yet scarce. Interestingly, occurrence of V_H replacements has recently been claimed in IgM⁻IgD⁺ CD38⁺ tonsillar B cells and in synovial-tissue B cells.^{16,17} Previous reports, however, could not draw firm conclusions on the issue of receptor revision of Ig light chain genes since this requires analysis of all four Ig light chain alleles, ie of both *IGK* and both *IGL* alleles, of individual cells. To address the question of Ig light chain receptor revision in a 'single cell' model, detailed studies on the *IGH*, *IGK* and *IGL* loci of four cases of IgD λ MMs were performed using Southern blot (SB), PCR and sequence analyses.

Materials and methods

IGH, *IGK* and *IGL* genes were analysed by SB, PCR and sequencing. Fifteen μ g of genomic DNA was digested with *Bam*HI and *Bgl*II, size fractionated in a 0.7% agarose gel and transferred to a nylon membrane. The *Bam*HI filter was successively hybridized with the IGHMU and IGHJ6 probes for detection of *IGH* rearrangements and C μ deletion. For detection of the IgM-IgD recombination by PCR primers upstream of σ_{μ} and downstream of Σ_{μ} were used (adapted from Arpin *et al*).⁵ The *Bgl*II filter was hybridized with *IGK* and *IGL* probes for determination of the *IGK*, and *IGL* gene configurations.^{15,18} Family-specific V_H, V κ , and V λ primers were used in combination with J_H, J κ and J λ primers (genomic PCR) or in combination with C δ , C κ or C λ primers (RT-PCR).^{15,19} Clonal (RT-)PCR products were either directly sequenced or cloned in pGEM-T Easy vector followed by sequencing of four to six individual clones.

Results and discussion

The configuration of the *IGH*, *IGK* and *IGL* genes in four IgD λ MMs were studied by SB, PCR and sequencing (Table 1). MM-1 contained a single in-frame *IGH* gene rearrangement with deletion of the C μ region caused by a σ_{μ} to Σ_{μ} recombination. In MM-2 and MM-4, no clonal bands could be identified at the DNA level by SB, most likely due to a limited tumor load in the bone marrow samples. However, by RT-PCR and sequencing, single clonal in-frame V_H-C δ rearrangements were identified. MM-3 contained a biallelic *IGH* gene

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Table 1 Summarized data of Ig gene configurations, gene segment usage, and somatic mutations in four IgDλ MM

MM	Cμ deletion	Ig locus	SB analysis ^a	(RT-)PCR and sequencing (frame) ^b	Observed somatic mutations								
					Total	%	CDR			FR			p _t
							R	S	R/S	R	S	R/S	
1	Yes	IGH	R/G	V _H 3-23/D _H 3-16/J _H 2(in)	31	10.5	7	3	2.3	13	8	1.6	0.032
		IGK	D/G	V _κ 1-33/J _κ 2(out)+intronRSS-Kde Vλ2-14/Jλ2(in)	0	0	—	—	—	—	—	—	—
		IGL	R/G	ni	8	4.7	1	1	1	5	1	5	nd
2	ni ^e	IGH	— ^d	V _H 3-11/D _H 5-24/J _H 6(in)	89	31.5	16	5	3.2	48	20	2.4	0.075
		IGK	—	V _κ 4-1/Kde	0	0	—	—	—	—	—	—	—
		IGL	—	Vλ2-18/Jλ1(in)	18	5.8	6	3	2.0	9	0	—	nd
3	Yes	IGH	R/R	V _H 4-31/D _H 5-18/J _H 2(in)	60	20.4	15	2	7.5	27	16	1.7	0.026
		IGK	D/D	V _κ 2-29/J _κ 4(out)+intronRSS-Kde V _κ 1-43/J _κ 5(out)+intronRSS-Kde Vλ2-23/Jλ3(in)	0	0	—	—	—	—	—	—	—
		IGL	R/G	ni	23	12	4	1	4.0	12	6	2.0	nd
4	ni ^e	IGH	— ^d	V _H 5-51/D _H 5-12/J _H 6(in)	31	10.5	7	4	1.8	12	8	1.5	0.010
		IGK	—	V _κ 3D-20/J _κ 4(out)+intronRSS-Kde V _κ 2-24/Kde	0	0	—	—	—	—	—	—	—
		IGL	—	ni	0	0	—	—	—	—	—	—	—

^aG, germline configuration; R, rearranged allele; D, deletion of J_κ and/or C_κ region via a recombination involving the kappa deleting element (Kde).

^bIMGT nomenclature (<http://imgt.cines.fr:8104>) was used for assigning the V (D) and J gene segments.

^cR, replacement mutation; S, silent mutation.

^dDue to a low tumor load, no rearranged bands could be detected via Southern blot analysis.

^eni, not identified; nd, not determined.

^fP, probability that the scarcity of R mutations in the FR resulted from chance only (see Chang and Casali).²²

rearrangement with a deletion of the Cμ region on one allele, which was in accordance with identification of clonal in-frame V_H-Cδ transcripts, but no V_H-Cμ transcripts by RT-PCR. No second complete V_H-J_H rearrangement was identified by PCR analysis in MM-3 (Table 1). In myelomas 1, 2 and 3 in-frame IGL gene rearrangements were also identified.

All the functional V_H and Vλ genes were indeed exceptionally hypermutated. The highest number of somatic mutations was found in the V_H region of MM-2, ie 89 mutations. MM-1, MM-3 and MM-4 carried 31, 60 and 31 mutations in their V_H genes, respectively (Figure 1). The average mutation frequency in the V_H regions of the four MM was thus 18%, whereas the reported average mutation frequency of the V_H region in non-IgD MM is 8%.^{20,21} Also the Vλ regions showed a slightly higher average mutation load (7.5%) than reported for the light chains of non-IgD MM (6%).²¹ In MM-1, 3 and 4, the ratios of replacement (R) vs silent (S) mutations in the framework regions (FR) was approximately 1.5, which is significantly lower than would be expected if the mutations had occurred randomly in the absence of selection.²² However, in MM-2, with the extremely high mutation load of 31.5%, the R/S ratio was 2.4, suggesting that selectional forces to protect the FR regions and thus to maintain the integrity of the overall Ig structure had been less stringent. Still, this MM, like the other three cases, was found to express the IgDλ protein. Interestingly, a relatively high number of replacement mutations in the FRs has also been noted previously in the hypermutated IgD-only germinal center cells.¹

The IGH sequences of the four cases were analyzed for the presence of hybrid V_H gene segments, indicative of V_H

replacement processes.¹⁶ However, we obtained no evidence for this type of receptor revision at the IGH locus.

Analysis of the IGK genes showed that all rearranged IGK alleles involved the kappa deleting element (Kde). This concerned four intronRSS-Kde rearrangements with preserved out-of-frame V_κ-J_κ rearrangements upstream (MM-1, 3 and 4). We detected no somatic mutations in the V-κ gene segments of any of these four non-functional V_κ-J_κ rearrangements. In MM-2 and the second allele of MM-4, V_κ-Kde rearrangements were found which deleted the complete J_κ-C_κ region. Also IgDκ MM, although not present in our limited panel, may contain, next to a functional IGK gene rearrangement, a V_κ-J_κ rearrangement at the second allele either or not followed by an intronRSS-Kde rearrangement. Such an Ig gene configuration may in a similar fashion be informative with respect to the topic of receptor revision.

Analysis of the IGL genes showed that the three identified IGL gene rearrangements used three different Jλ gene segments. This implies that the single IgD expression was not associated with a specific Igλ isotype.

The detailed information obtained on the IGK and IGL loci indicated that in at least two cases (MM-1 and MM-3) the cells had not expressed another Ig light chain prior to the identified in-frame Igλ chains, since all V_κ-J_κ rearrangements found upstream of the intronRSS-Kde recombinations were out-of-frame and not somatically mutated. This is formally not proven for MM-2 and MM-4 as the V_κ-Kde rearrangements precluded analysis of the possibly pre-existing V_κ-J_κ rearrangements.

In conclusion, our data indicate that IgDλ MM are most

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MM-1
GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG TCC CTG AGA
--C --- --a --a --T C-- --c ---
CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC AGC TAT GGC ATG AGC TGG GTC CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAA --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CAG GCT CCA GGG AAG GGG CTG GAG TGG GTC TCA GCT ATT AGT GGT AGT GGT GGT AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CDR1
ACA TAC TAC GCA GAC TCC GTG AAG GGC GGG TTC ACC ATC TCC AGA GAC AAT TCC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AT- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCC GTA TAT TAC
--- --- --a G-a --- --- --C --- --- --- --- --- --- --- --- --- --- --- ---
TGT GCG AAA
--- --- ---

MM-2
CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTC AAG CCT GGA GGG TCC CTG AGA
--- --- --Ga --- TC- --- --g --- --- --- --a -C- --- CC- GT- --t C-- C-- --- -C-
CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT GAC TAC TAC ATG AGC TGG ATC CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CA- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CAG GCT CCA GGG AAG GGG CTG GAG TGG GTT TCA TAC ATT AGT AGT AGT GGT AGT ACC
--- --- T-g T-- --a G-- --a --- C-- -AC C-- G-g -C- C-a G-- -TG GC- --a --- GG-
CDR2
ATA TAC TAC GCA GAC TCT GTG AAG GGC CGA TTC ACC ATC TCC AGG GAC AAC GCC AAG
G-G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
--Tc A-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAC TCA CTG TAT CTC CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCC GTG TAT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GTC t-- --- --- G-C --A TTG GCA -C- --g --- --- --- --- --- --- --- --- ---
TGT GCG AGA
--- --- ---

MM-3
CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCA CAG ACC CTG TCC
-T- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CTC ACC TGC ACT GTC TCT GGT GGC TCC ATC AGC AGT GGT GGT TAC TAC TGG AGC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
G-- TT- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATC CGC CAG CAC CCA GGG AAG GGC CTG GAG TGG ATT GGG TAC ATC TAT TAC AGT GGG
T-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CDR1
AGC ACC TAC TAC AAC CCG TCC CTC AAG AGT CGA GTT ACC ATA TCA GTA GAC ACG TCT
--- --- G-- C-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
C-c T-- --c --c --c --- --- --- --- --- --- --- --- --- --- --- --- ---
AAG AAC CAG TTC TCC CTG AAG CTG AGC TCT GTG ACT GCC GCG GAC ACG GCC GTG TAT
GGA --A --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CGc --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TAC TGT GCG AGA
C-- --- --- t- C-G

MM-4
GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A-- --g --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATC TCC TGT AAG GGT TCT GGA TAC AGC TTT ACC AGC TAC TGG ATC GGC TGG GTG CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CAG ATG CCC GGG AAA GGC CTG GAG TGG ATG GGG ATC ATC TAT CCT GGT GAC TCT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TC- --a T- --g --- --- --- --- --- --- --- --- --- --- --- --- ---
CDR2
ACC AGA TAC AGC CCG TCC TTC CAA GGC CAG GTC ACC ATC TCA GCC GAC AAG TCC ATC
-Gt --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AGC ACC GCC TAC CTG CAG TGG AGC AGC CTG AAG GCC TCG GAC ACC GCC ATG TAT TAC
-At --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
--a --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TGT GCG AGA CAT
--- --- --- --g --

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Figure 1 Sequences of the V_H regions of the four IgDλ MM patients (lower line) compared to the most homologous germline V_H gene segments (upper line) according to VBASE (<http://www.dnaplot.de>). The most homologous sequences were V3–23/DP-47 (V_H3–23, according to IMGT, see Table 1) for MM-1, V3–11/DP-35 (V_H3–11) for MM-2, V4.33/DP-65 (V_H4–31) for MM-3, and V5–51/DP-73 (V_H5–51) for MM-4. Replacement mutations are indicated by capitals; silent mutations are indicated by lower cases.

likely derived from IgM⁺IgD⁺ Igλ-positive B cells that were switched to IgD-only by Cμ deletion. In spite of the fact that the IgV regions were clearly hypermutated, proving that the progenitor cells must have undergone extensive clonal expansion in the germinal centers, we find no evidence of receptor revision at the Ig heavy or Ig light chain loci. It thus remains to be established why IgD, in the absence of IgM, is mainly found in association with Igλ or alternatively why Cμ deletion

occurs preferentially in Igλ⁺ B cells and whether the extraordinary level of somatic hypermutation has a causative role in the induction of this unusual Cμ deletion.

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References

- Liu YJ, de Bouteiller O, Arpin C, Briere F, Galibert L, Ho S, Martinez-Valdez H, Banchemareau J, Lebecque S. Normal human IgD+IgM- germinal center B cells can express up to 80 mutations in the variable region of their IgD transcripts. *Immunity* 1996; **4**: 603–613.
- Arpin C, de Bouteiller O, Razanajaona D, Fugier-Vivier I, Briere F, Banchemareau J, Lebecque S, Liu YJ. The normal counterpart of IgD myeloma cells in germinal center displays extensively mutated IgVH gene, Cm-Cd switch, and lambda light chain expression. *J Exp Med* 1998; **187**: 1169–1178.
- Klein U, Rajewsky K, Küppers R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J Exp Med* 1998; **188**: 1679–1689.
- Kluin PM, Kayano H, Zani VJ, Kluin-Nelemans HC, Tucker PW, Satterwhite E, Dyer MJ. IgD class switching: identification of a novel recombination site in neoplastic and normal B cells. *Eur J Immunol* 1995; **25**: 3504–3508.
- Yasui H, Akahori Y, Hirano M, Yamada K, Kurosawa Y. Class switch from m to d is mediated by homologous recombination between S_m and S_m sequences in human immunoglobulin gene loci. *Eur J Immunol* 1989; **19**: 1399–1403.
- White MB, Word CJ, Humphries CG, Blattner FR, Tucker PW. Immunoglobulin D switching can occur through homologous recombination in human B cells. *Mol Cell Biol* 1990; **10**: 3690–3699.
- Klein U, Küppers R, Rajewsky K. Evidence for a large compartment of IgM-expressing memory B cells in humans. *Blood* 1997; **89**: 1288–1298.
- Preud'homme J, Petit I, Barra A, Morel Jean-Claude Lecron F, Lelievre E. Structural and functional properties of membrane and secreted IgD. *Mol Immunol* 2000; **37**: 871–887.
- Hobbs JR, Corbett AA. Younger age of presentation and extraosseous tumour in IgD myelomatosis. *Br Med J* 1969; **1**: 412–414.
- Fine JM, Rivat C, Lambin P, Ropartz C. Monoclonal IgD. A comparative study of 60 sera with IgD 'M' component. *Biomedicine* 1974; **21**: 119–125.
- Jancelewicz Z, Takatsuki K, Sugai S, Pruzanski W. IgD multiple myeloma. Review of 133 cases. *Arch Intern Med* 1975; **135**: 87–93.
- Fibbe WE, Jansen J. Prognostic factors in IgD myeloma: a study of 21 cases. *Scand J Haematol* 1984; **33**: 471–475.
- Shimamoto Y, Anami Y, Yamaguchi M. A new risk grouping for IgD myeloma based on analysis of 165 Japanese patients. *Eur J Haematol* 1991; **47**: 262–267.
- Blade J, Lust JA, Kyle RA. Immunoglobulin D multiple myeloma: presenting features, response to therapy, and survival in a series of 53 cases. *J Clin Oncol* 1994; **12**: 2398–2404.
- Van der Burg M, Tümkaya T, Boerma M, De Bruin-Versteeg S, Langerak AW, Van Dongen JJM. Ordered recombination of immunoglobulin light chain genes occurs at the IGK locus but seems less strict at the IGL locus. *Blood* 2001; **97**: 1001–1008.
- Wilson PC, Wilson K, Liu YJ, Banchemareau J, Pascual V, Capra JD. Receptor revision of immunoglobulin heavy chain variable region

- genes in normal human B lymphocytes. *J Exp Med* 2000; **191**: 1881–1894.
- 17 Itoh K, Meffre E, Albesiano E, Farber A, Dines D, Stein P, Asnis SE, Furie RA, Jain RI, Chiorazzi N. Immunoglobulin heavy chain variable region gene replacement as a mechanism for receptor revision in rheumatoid arthritis synovial tissue B lymphocytes. *J Exp Med* 2000; **192**: 1151–1164.
- 18 Tümkaya T, Van der Burg M, Garcia Sanz R, Gonzalez Diaz M, Langerak AW, San Miguel JF, Van Dongen JJM. Immunoglobulin lambda isotype rearrangements in B cell malignancies. *Leukemia* 2001; **15**: 121–127.
- 19 Aarts WM, Willemze R, Bende RJ, Meijer CJ, Pals ST, van Noesel CJ. VH gene analysis of primary cutaneous B-cell lymphomas: evidence for ongoing somatic hypermutation and isotype switching. *Blood* 1998; **92**: 3857–3864.
- 20 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* 1995; **155**: 2487–2497.
- 21 Sahota SS, Leo R, Hamblin TJ, Stevenson FK. Myeloma VL and VH gene sequences reveal a complementary imprint of antigen selection in tumor cells. *Blood* 1997; **89**: 219–226.
- 22 Chang B, Casali P. The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. *Immunol Today* 1994; **15**: 367–373.