Multiple myeloma shows no intra-disease clustering of immunoglobulin heavy chain genes

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ABSTRACT

Background

Characterization of the immunoglobulin gene repertoire has improved our understanding of the immunopathogenesis of lymphoid tumors. Early B-lymphocyte precursors of multiple myeloma are known to exist and might be susceptible to antigenic drive.

Design and Methods

To verify this hypothesis, we collected a database of 345 fully readable multiple myeloma immunoglobulin sequences. We characterized the immunoglobulin repertoire, analyzed the somatic hypermutation load, and investigated for stereotyped receptor clusters.

Results

Compared to the normal immunoglobulin repertoire, multiple myeloma displayed only modest differences involving only a few genes, showing that the myeloma immunoglobulin repertoire is the least skewed among mature B-cell tumors. Median somatic hypermutation load was 7.8%; median length of complementarity determining-region 3 was 15.5 amino acids. Clustering analysis showed the absence of myeloma specific clusters and no similarity with published chronic lymphocytic leukemia or lymphoma subsets.

Conclusions

Analysis of multiple myeloma immunoglobulin repertoire does not support a pathogenetic role for antigen selection in this tumor.

Key words: multiple myeloma, immunoglobulin, antigen selection, heavy chain gene.

Citation: Ferrero S, Capello D, Svaldi M, Boi M, Gatti D, Drandi D, Rossi D, Barbiero S, Mantoan B, Mantella E, Zanni M, Ghione P, Larocca A, Passera R, Bertoni F, Gattei V, Forconi F, Laurenti L, Del Poeta G, Marasca R, Cortelazzo S, Gaidano G, Palumbo A, Boccadoro M and Ladetto M. Multiple myeloma shows no intra-disease clustering of immunoglobulin heavy chain genes. Haematologica 2012;97(6):849-853. doi:10.3324/haematol.2011.052852

Acknowledgments: the authors would like to thank Antonella Fiorillo and Tiziana Marangon for excellent secretarial support. Thanks to CV for her scientific and personal support.

Funding: this work was supported by Compagnia di San Paolo (Turin, Italy); Ministero Italiano Università e Ricerca (MIUR), (code: 7.07.02.60 AE01); Ministero del Lavoro, della Salute e delle Politiche Sociali, Rome, Italy; Progetto di Ricerca Finalizzata 2008 (co-financed by provincia autonoma di Bolzano/ Bozen Italy), (code: 7.07.08.60 P51); Progetto di Ricerca Finalizzata 2009 (co-financed by provincia autonoma di Bolzano/Bozen Italy), (code: RF-2009-1469205) Progetto Ricerca Finalizzata 2008 IRCCS CROB Rionero CUP J65J0800009000, (code: 7.07.08.60 P49); Progetto di Ricerca Finalizzata Regione Piemonte 2009; Fondazione Italiana Linfomi, Alessandria, Turin Italy; Regione Piemonte, Turin, Italy; Fondazione Neoplasie del sangue (FO. NE. SA), Turin, Italy; Fondazione per la Ricerca e la Cura sui Linfomi, Lugano, Switzerland.

Manuscript received on August 4, 2011. Revised version arrived on November 17, 2011. Manuscript accepted December 12, 2011.

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The online version of this article has a Supplementary Appendix.

Introduction

Characterization of the immunoglobulin receptor has provided useful insights into the pathogenesis and natural history of lymphoid neoplasms.[1-7] In particular, large databases of immunoglobulin heavy chain gene (IGH) sequences have been analyzed to demonstrate the role of antigen stimulation as a key environmental driver of malignant transformation. The recognition of stereotyped clusters of immunoglobulin receptors in chronic lymphocytic leukemia (CLL) provides strong evidence to support the role of antigen-driven mechanisms in the pathogenesis of this neoplasm.[8-12] Furthermore, recent reports in mantle cell lymphoma (MCL)[13] and marginal zone lymphoma (MZL)[14] have led to speculation that stereotyped clusters of immunoglobulin receptors might represent a common phenomenon across most, if not all, mature B-cell tumors.

Although terminally-differentiated malignant plasma cells (PC) do not express immunoglobulin receptors, it is known that myelomagenesis is a complex multi-step process that might involve earlier B-cell precursors.[15] In particular, the class switch recombination mechanisms involved in multiple myeloma (MM) related chromosomal translocations suggest that early events in the development of MM occur during germinal center (GC) maturation.[16] Furthermore, the presence of pre-switch B cells idiotypically related to malignant PC has been reported.[17] Therefore, there is a clear rationale to evaluate the MM IGH repertoire and to search for stereotyped clusters of immunoglobulin receptors on appropriately sized patient series. This will allow us to investigate further into the role of antigen-driven stimulation in B-lymphoid tumorigenesis and whether antigen stimulation might play a role in early myelomagenesis. Published databases of IGH MM sequences[1,6,7] each include less than 80 evaluable patients and are, therefore, too small to perform extensive cluster analysis of immunoglobulin receptors. We have combined a large number of MM IGH sequences obtained at our institutions for minimal residual disease (MRD) evaluation[18] with all the sequences reported in the literature, leading to a panel of 345 fully evaluable MM IGH sequences. This database was then used to comprehensively investigate the characteristics of the IGH rearrangement in MM.

Design and Methods

Collection of immunoglobulin sequences

The original database collected for this study consisted of 406 MM IGH sequences. A total of 237 MM IGH sequences were retrieved from EMBL, NCBI and IMGT/LIGM-DB public databases, as already reported (Literature Series, LS),[10,11,14] while 169 were directly sequenced at our institutions for MRD purposes (Institutional Series, IS). All patients provided informed consent in accordance with the requirements of the local Institutional Review Board (IRB) and with the Declaration of Helsinki. The study was approved by the IRB Commissione Regionale per le Sperimentazioni Cliniche, Turin, Italy. Methods for IGH sequencing have been described elsewhere.[19] Redundant, poorly annotated, out-of-frame, or clonally related sequences were excluded from the analysis, as previously reported.[10,11,14] Therefore, the final collection included 345 evaluable IGH MM sequences (MM total, each sequence corresponding to one single patient), 214 from LS (*Online Supplementary Table S1*) and 131 from IS (*Online Supplementary Table S2*). For clustering analysis, a total of 28,376 non-MM IGH sequences were retrieved from public databases (n=26,618)[10,11,14] and from our multi-laboratory database (n=1,758). The non-MM cohort intentionally included several different entities to allow comparison with various types of B cells (*Online Supplementary Table S3*). In particular, a subanalysis was also performed on 461 sequences from PC from healthy donors.

Sequence analysis and data mining

All sequences were aligned to the IMGT directory for the identification of IGH rearrangements, following established criteria.[10,11,20] *IGHV*, *IGHD*, and *IGHJ* gene usage, somatic hypermutation (SHM) load, and the complementarity determining region 3 on heavy chain gene (HCDR3) length were recorded for each sequence. Comparisons in terms of *IGHV* usage were made between the MM series and the largest published series of *IGHV* sequences from healthy donors.[10] Only smaller comparative series from healthy donors were available to compare *IGHD-J* usage, mutational load, and HCDR3 length distribution.[10, 21-26]

HCDR3-driven clustering

Clustering analysis was performed using ClustalX 2.0, as previously described.[10,27] Stereotyped HCDR3 sequences were those characterized by an aminoacidic identity of 60% or over, according to the criteria of Messmer and Stamatopoulos.[10,28] Subsets not previously described and including only two sequences ('provisional') were only considered if they fulfilled the following additional criteria:[11,12,14] i) use of *IGHV* germline genes of the same clan; ii) use of the same *IGHD* and *IGHJ* germline genes; iii) use of the same *IGHD* segment reading frame; and iv) identical HCDR3 length.

Statistical analysis

Patients' characteristics were tested using Fisher's exact test for discrete variables and the Mann-Whitney test for continuous variables. All reported *P* values were obtained by a two-sided exact method, at the conventional 5% significance level. Data were analyzed as of April 2011 using SPSS 19.0.0 software.

Results and Discussion

We analyzed the largest database of IGH sequences from MM patients, consisting of 345 fully evaluable MM sequences partly derived from our institutional database (38%) and partly retrieved from published databases (62%). Usage of *IGHV-D-J* genes is shown in Figure 1 and *Online Supplementary Figures S1 and S2*, and described in detail in the *Online Supplementary Table S4*.

Overall, the most frequently reported *IGHV* families in MM were *IGHV3* (53.9%), *IGHV4* (18.6%) and *IGHV1* (12.5%). The most frequently reported *IGHV* genes were *IGHV3-30-3* (9.3%), *IGHV3-23* (8.7%) and *IGHV1-69* (5.8%), together representing 23.8% of the entire *IGHV* repertoire; this is much less than that observed in CLL (30%), MCL (40%) and MZL (45%) (Figure 2).[10,13,14] The most frequently reported *IGHD* genes were *IGHD3-10* (11.3%), *IGHD3-22* (8.1%) and *IGHD3-3* (8.1%), while the *IGHJ4* gene by itself accounted for more than half of the patients' *IGHJ* repertoire (54.8%), in line with post-GC B-cell *IGHJ* usage.[30]

The IS *IGHV-D-J* repertoire was essentially in line with LS and previously published smaller MM repertoires.[1,6,7] The few modest differences (*Online Supplementary Tables S4 and S5*) might be due to technical or interpretative bias-

es in the context of the highly hypermutated repertoire of MM, particularly when dealing with older studies. In contrast, the complete absence of *IGHV3-21* in a North-American MM series[1] might be the result of technical differences in IGH sequencing methods or the expression of some yet unexplained geographical variation, already reported for this gene in CLL.[31]

Despite a global picture representing a nearly physiological IGH repertoire in MM, we were able to demonstrate the presence of modest over and underrepresentation of some genes compared to normal *IGHV* repertoire.[10] In particular, we observed overrepresentation of *IGHV1-69*, *IGHV2-5*, *IGHV2-70*, *IGHV3-21*, *IGHV3-30-3*, *IGHV3-43*, *IGHV5-51* and *IGHV6-1* genes, and underrepresentation of *IGHV1-18*, *IGHV1-8*, *IGHV3-30*, *IGHV3-53* and *IGHV4-34* genes (*Online Supplementary Figure S3*). When the comparison was restricted only to a panel of 461 PC sequences derived from healthy donors,[10] the skewing of the MM IGHV repertoire was even less pronounced, resulting only in an overrepresentation of *IGHV2-70*, *IGHV3-21* and *IGHV3-30-3* genes and in an underrepresentation of *IGHV1-2*, *IGHV1-8*, *IGHV3-30* and *IGHV4-34* (*Online Supplementary Figure S4*). On the other hand, there were no significant differences in *IGHD* and *IGHJ* usage in MM when compared to the normal *IGHD-IGHJ* repertoire from both unselected B cells from healthy donors[10, 21-26] and from selected healthy PC,[10] with the exception of the underrepresentation of *IGHJ6* (14.5% *vs.*

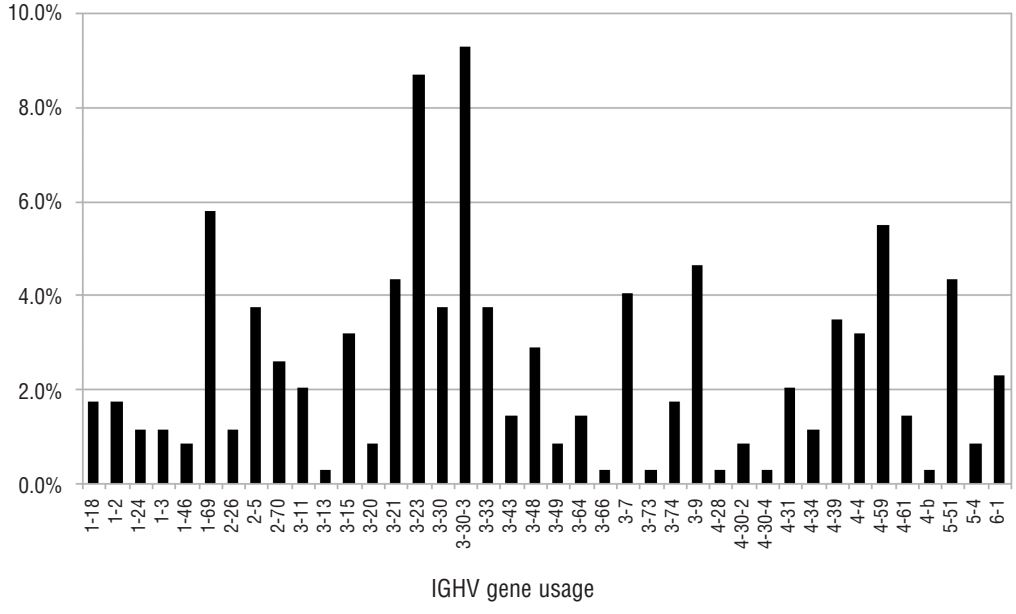

Figure 1. ***IGHV*** **gene usage of multiple myeloma total series. MM: multiple myeloma; *IGHV*: immunoglobulin heavy chain variable region.**

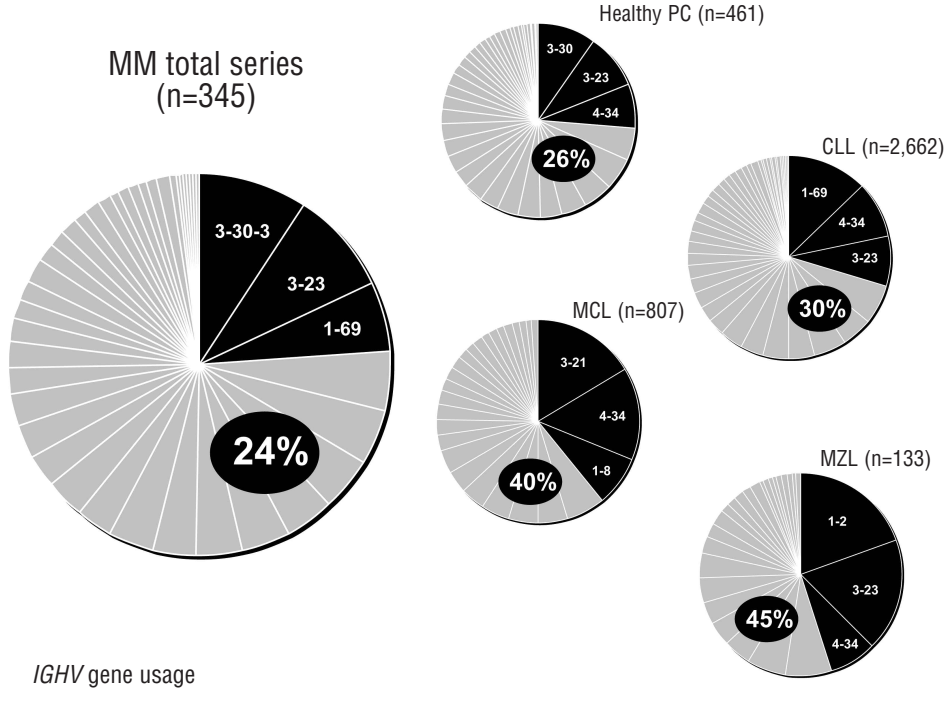

Figure 2. ***IGHV*** **gene usage in multiple myeloma, other B-cell malignancies and healthy plasma cells. In black the contribution of the three most frequent genes in MM, other B-cell malignancies and healthy plasma cells to the totality of their *IGHV* repertoire. *IGHV*: immunoglobulin-heavy chain variable region; MM: multiple myeloma; PC: plasma cells; CLL: chronic lymphocytic leukemia; MCL: mantle cell lymphoma; MZL: marginal zone lymphoma. For PC, MCL and MZL databases, see [10,13,14] while CLL database has been retrieved from: *Darzentas N, et al.*[29]**

24.6% in healthy B-cell published repertoires; $P<0.001$; *data not shown*) which agrees with *IGHJ* gene distribution in post-GC B cells.[30] These modest skewings observed in MM might potentially reflect the expression of processes different to antigen selection. Finally, our study confirmed previous observations of a lower incidence of IGHV4-34 in MM compared to healthy B cells and PC IGHV repertoire, in accordance with the paucity of autoimmune phenomena in myeloma.[1,6,7]

Our series also confirms the high rate of SHM in MM and this agrees with previous studies,[1,6,7] supporting the hypothesis that the transforming events leading to full-blown myeloma occur in a post-GC memory B cell. There were 4 out of 345 (1.2%) MM sequences with a germline identity of 98% or over and only one patient showed IGH genes in full germline configuration (100% identity). Available biological and clinical data for these patients are described in the *Online Supplementary Table S6*. In terms of SHM rate, most MM patients showed a homogeneous and normal distribution with a median value of 7.8% (range 0-24.6%, SD 3.7%). This is similar to that observed in healthy PC series in which, nevertheless, the frequency of unmutated sequences is higher, possibly due to the presence of a small proportion of IgM secreting PC (Figure 3A). These features are, therefore, radically different from those of CLL and other B-cell tumors for which we observe a more heterogeneous distribution pattern reflecting major pathogenetic and clinical differences.[3,4,13,14] Lengths of HCDR3 AA sequences in MM were normally distributed with a mean value of 15.5 AA (range 6-29, SD 3.89), in accordance with published series of healthy PC (Figure 3B).

An extensive analysis of the HCDR3 region failed to find any evidence of stereotyped receptor clusters in MM. Our sample size for this analysis was more than adequate since original reports documenting the presence of HCDR3 clustering in CLL, MCL and MZL included 346, 807, and 133 patients, respectively.[9,13,14] As shown in the *Online Supplementary Table S7*, even among the best observed matches, critical features hampered an effective intra-MM clustering, such as different *IGHV-D-J* usage and different HCDR3 lengths. Furthermore, no clusters were found irrespective of *IGHV-D-J* usage, even considering only HCDR3 similarities.[10]

When MM sequences were compared to a large panel of 28,376 non-MM IGH sequences, only a minority of MM sequences (n=4, 1.2%) clustered with sequences from other lymphoid tumors or non-neoplastic B cells. In particular, no MM sequences clustered with previously described CLL or non-Hodgkin's lymphoma (NHL) subsets.[10,11,13,14] Only one 'mixed' MM-NHL and three MM-normal or reactive B-cell provisional clusters were observed, each including only two sequences (*Online Supplementary Table S8*). These four non-MM partner sequences belong to distinctly different conditions which are difficult to reconcile into a common pathogenetic mechanism. These might, therefore, represent a kind of 'background signal' in the context of a highly diversified IGH repertoire. Nevertheless, 341 of 345 (98.8%) MM sequences did not belong to any cluster and could be defined as heterologous.

In conclusion, our results clearly indicate that MM has the least skewed IGH repertoire among mature B-cell tumors. This shows, along with the absence of clusters of stereotyped IGH receptors, that the MM IGH repertoire does not contain prominent markers of specific antigen selection, in contrast to what is observed in other lymphoproliferative diseases. However, these conclusions need to be reconciled with previously reported evidence demonstrating the existence of MM-related B-cell clones that, according to their differentiation phase, should retain full antigen responsivity.[17] One may speculate that pre-MM B-cell clones might respond to an extremely diversified antigenic challenge, leading to a very modest skewing of the normal repertoire. Alternatively, early events in MM pathogenesis might provide adequate proliferative and survival advantage to pre-malignant MM-related clones allowing them to bypass the need for antigenic stimuli. Finally, pre-malignant MM-related clones might frequently and stochastically arise during physiological immune responses, while secondary events leading to full-blown MM are driven by non-immune mechanisms occurring in later disease phases. IGH repertoire studies such as ours are clearly not able to formally exclude a role of antigen selection during the complex pathway leading to overt MM. Nevertheless, analysis of the largest MM IGH repertoire does not provide any evidence to support the view that the concept of antigen-driven tumorigenesis could be transferred beyond the field of CLL and NHL. Nor is there any evidence in favor of a critical role for antigen-responsive B-cell precursors in the pathogenesis of MM.

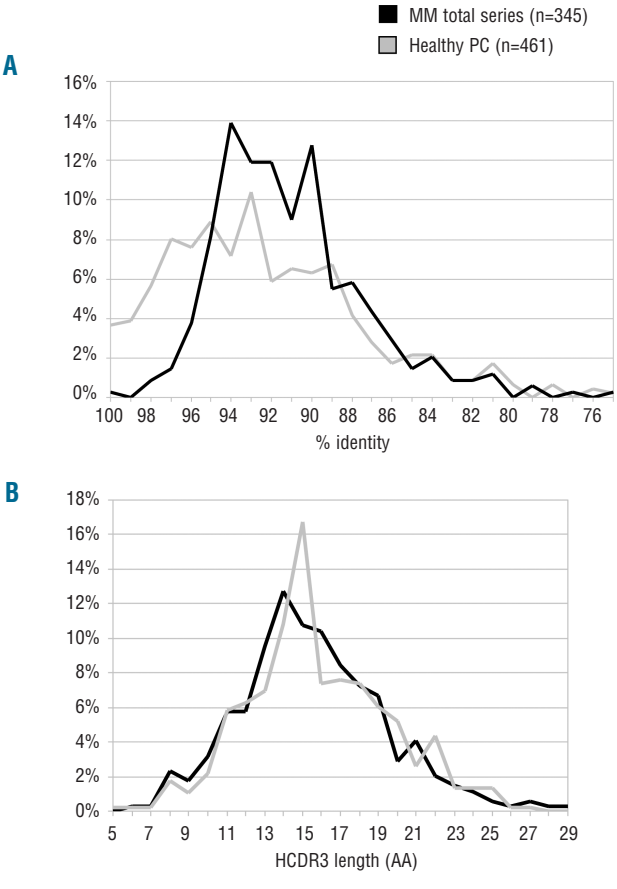

Figure 3. Somatic hypermutation load (A) and HCDR3 length distributions (B) of multiple myeloma total series compared to published series of healthy plasma cells. HCDR3: heavy chain complementarity determining region 3; AA: amino acidic; MM: multiple myeloma (black line); PC: plasma cells (gray line).[10]

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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