[ABSTRACT WM1.3]

IMMUNOGLOBULIN GENE REARRANGEMENTS IN WALDENSTRÖM'S MACROGLOBULINEMIA

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The characterization of VDJH rearrangements as well as related processes such as somatic hypermutation (SHM) and class switch recombination (CSR) have largely contributed to gain insights in the pathogenetical development of B-cell Lymphoproliferative disorders (LPD), because the differentiation process follows a strict hierarchical order in generating the Ig repertoire.1,2 Gene segment usage, CDR3 composition and somatic hypermutation rates have been described for some B-cell malignancies and B-cell subtypes, where usage of particular VH and DH families and gene segments is often biased.3,4 In B cell chronic lymphocytic leukemia (B-CLL) the presence of somatic hypermutation (SHM) in the IgH genes is correlated with a more favorable prognosis compared to unmutated cases.4 In addition, the pattern of SHM and CDR3 composition has been associated with particular VH families and gene segments in B-CLL4 and multiple myeloma (MM)3 and incomplete rearrangements are frequently found in precursor B-cell acute lymphoblastic leukemia, MM, and Hairy Cell Leukemia. These characteristics are not completely known in Waldenström Macroglobulinemia (WM) because the low frequency of the disease hampers such analyses. We have recently characterized complete VDJH and incomplete DJH rearrangements in 81 IgM monoclonal gammopathies: 44 symptomatic WM, 27 asymptomatic WM and 10 IgM Monoclonal Gammopathies of Uncertain Significance (IgM-MGUS). Monoclonal VDJH rearrangements could be amplified in 90% of patients, with no differences between the three sub-entities. VH selection was biased in WM, since the most frequently used family and single segment were VH3 and VH3-23 (74% and 25%, respectively), which markedly differs from the repertoire in normal B-cells and MM. Interestingly the VH3-23 segment was never selected in Ig-MGUS (0% vs. 28%, p=0.05). In addition, the VH4-34, which is frequently selected normal circulating B cells,5 B-CLL,4 and others such as B-ALL and diffuse large B-cell lymphoma, was never selected in our WM cases and only in one IgM-MGUS. This concurs with the hypothesis that VH4-34, a gene segment frequently associated with autoimmune diseases, is prevented from the normal PC repertoire and neoplastic functional B-LPD such as MM.4 In the same line, the VH1-69, V3-07 and VH3-21, which are overrepresented in B-CLL, were selected by 0%, 0% and 4% of our IgM monoclonal gammopathies. In addition, the highly frequent selection of the VH3-23 seems to be specific of WM. This differential repertoire selection respect to CLL is on the other side very similar to that observed in MM. Accordingly, these findings reinforced the similarities between WM and MM in a moment in which mRNA expression studies were emphasizing the closeness between WM and BCLL. 6 This suggests that the origin WM remains between both diseases in the B-cell differentiation. As far as the DH and JH distribution of the VDJH segments are concerned, they did not differ from B-lymphocytes in healthy individuals or other B-cell neoplasias. In addition, monoclonal incomplete DJH rearrangements were detected in 48% of our IgM

monoclonal disorders. Interestingly, only one case of IgM-MGUS displayed an incomplete DJH rearrangement, in opposition to the symptomatic or asymptomatic WM cases (10% vs. 54%, p=0.01). Somatic hypermutation with >2% deviation from the germline was seen in 89% of all cases, without significant differences between different symptomatic, asymptomatic and MGUS cases. However, using the number of mutations as continuous variable, symptomatic cases demonstrated a higher grade of somatic hypermutation (SHM), since the median percentage of changes was 6.61%, 76.40% and 9.38%, although differences did not achieve statistical significance (K-W, p=0,277). Such differences were mainly attributed to the VH segment usage, since VH3-23 segments, which were never used in MGUS, displayed a higher grade of SHM than the remaining segments (10.9±2.9 vs. 6.8±3.7, p<0.001). These findings did not relate with any specific clinical characteristics, since all clinical parameters at diagnosis were similar between patients with high or low SHM rate. The only exception was the time to the therapeutic requirement, which was shorter in unmutated patients, although differences were not statistically significant and this unfavorable parameter did not have any impact on the overall survival when the study was closed. The lack of clinical relevance of the unmutated cases in this series reinforces again the dissimilarities between B-CLL and WM, since the presence of unmutated VDJH rearrangements could be the most important prognostic parameter in B-CLL. Regarding DJH rearrangements, all were unmutated, which would make them an eventual attractive target for minimal residual disease investigation. In our study, mRNA transcripts could be evaluated in 21 WM and 7 IgM-MGUS patients. IgM clonotypic transcripts were observed in all cases, while IgD was observed in 83%. Interestingly, non-clinical isotypes (IgA and/or IgG) were seen in three WM (14%) and one IgM-MGUS (14%) patients. This requires a recombination that has been assumed to be impossible in WM.7,8 However, clonotypic transcripts encoding post-switch isotypes have been observed *in vitro* in WM and IgM-MGUS cells cultured with CD40L/IL-40.2 In our series we show that this process is possible *in vivo*; in addition, one of the three cases was able to produce a fully monoclonal IgG protein together with the original IgM.9 Very recently, this phenomenon has been shown to be possible in selected cells of all cases of WM.10 The clinical relevance of this finding remains to be explored since in our series it did not associate with any specific clinical characteristic. In conclusion, characterization of IgH rearrangements in an extensive series of IgM related disorders allow documenting some WM and IgM-MGUS dissimilarities which could suggest a distinct differentiation process between them. In addition, this characterization allows the identification of differences and similarities with other B-cell Lymphoproliferative disorders that can help to more precisely arrange the tumor target cell of WM along the B-cell differentiation process.

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