Clonal evolution in Waldenstrom macroglobulinemia highlights functional role of B-cell receptor

Bogoljub Ciric, Virginia VanKeulen, Moses Rodriguez, Robert A. Kyle, Morie A. Gertz, and Larry R. Pease

**Introduction**

Waldenstrom macroglobulinemia (WM) is a malignancy of plasmacytic lymphoid cells accompanied by high concentrations of monoclonal serum immunoglobulin M (IgM). Although in WM the malignant cells are found primarily in bone marrow, 20% to 70% of blood mononuclear cells are malignant B cells. Analyses of the V regions revealed that malignant cells in WM often express somatically mutated IgM. Distribution of replacement (R) and silent (S) mutations suggests that the IgM in WM is derived from cells at a late stage of differentiation that have undergone antigenic selection in germinal centers but failed to coordinate somatic mutation and class switching. Evidence has accumulated indicating malignant B cells display a variety of physiologic activities and requirements possessed by normal cells at similar stages of differentiation. Some of the well-documented examples are the extensive clonal evolution of follicular lymphomas and Burkitt lymphomas.

Although by classical definition WM is monoclonal, the presence of 2 unrelated clones in the same patient has been described. Here we report a case of clonal evolution in WM in which 2 related clones were identified in the blood of a patient. This is the first report of intraclonal heterogeneity in WM. Our results indicate that mutations continued to accumulate after the transformation event, and mutations in the malignant clones were selected by requirements for the preservation of the B-cell receptor (BCR) structure rather than for antigen binding.

**Study design**

**Patient history**

A 62-year-old woman was evaluated at the Mayo Clinic in 1985. She was found to have a monoclonal IgM protein measuring 13.3 g/L (1.33 g/dL).

The patient was monitored until 1993 when her hemoglobin had fallen to 97 g/L (9.7 g/dL). The monoclonal IgM was 32.6 g/L (3.26 g/dL), and 3 g/L (0.3 g/dL) of free light chain was detected in the urine. A bone marrow biopsy demonstrated 90% replacement of hematopoietic elements with small lymphocytes in a diffuse and interstitial pattern, consistent with WM. The patient was placed on chlorambucil for 5 years through 1998. After a period of observation through November 1999, the monoclonal IgM rose to 38 g/L (3.8 g/dL), the platelet count fell to 94 000, and chlorambucil was resumed. The patient’s blood was collected for analysis just before resumption of chlorambucil treatments.

**Characterization of the IgM, sh-IgM.22**

IgM antibody was isolated from patient plasma, and variable fragments (Fv) were produced as described. Fv fragments were sequenced in an automated sequencer (PE Biosystems Procise 492 HT, Foster City, CA), using standard procedures. Complementary DNA (cDNA) was generated by reverse transcriptase–polymerase chain reaction (RT-PCR) of peripheral blood messenger RNA (mRNA), using oligonucleotide primers based on the protein sequence. The cDNA was cloned and sequenced, using standard procedures.

**Results and discussion**

A partial protein sequence of the variable heavy chain (VH) was obtained, including the N-terminal 27 amino acids and the segment spanning amino acids 43 through 113 (Figure 1). Sequencing of the PCR-amplified cDNA (2 cDNA preparations and 6 PCR reactions) revealed a mixture of related VH sequences. Therefore, PCR products were cloned, and the plasmids isolated from 42 bacterial colonies were sequenced. Two VH sequences (A and B) that differ by 2 R and 6 S mutations but with identical CDR3 regions that corresponded to the protein sequence were discovered. Only approximately 10 somatic mutations each. The distribution and type of mutations demonstrate that mutations have continued to accumulate in the malignant clones and that selection has been operating to preserve immunoglobulin structure.

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peptides characteristic of VH IgM-A were identified by amino acid sequence analyses. A total of 242 bacterial colonies containing cDNA bearing the sH-IgM.22 VDJ junctional sequence were identified. With the use of hybridization, 60% were identified as type A and 40% as type B.

The sequences of both sH-IgM.22 VH most closely matched the IGHV3-30/3-30-5*01 germline sequence. The protein sequence obtained contained peptides from each of the encoded VH, indicating that both clones are synthesizing and secreting IgM. Each differed from the protein sequence by a single amino acid. Because the isolation procedure only recovered intact IgM molecules, the identification of both light chains implies also that both heavy chains were present in the serum.

The VH sequences from sH-IgM22 most closely matched the IGLV1-51*01 germline sequence (97% homology). The 2 genes

Figure 1. Sequence of the sH-IgM.22 VH. The sequence is aligned according to the numbering system of human VH sequences in Kabat et al. The sH-IgM.22 VH is a member of VH subgroup III. Underlined amino acids have been confirmed by protein sequencing. Amino acid sequence corresponds to sH-IgM.22 nucleotide sequence. sH-IgM.22 VH type A and type B sequences are represented only with nucleotides that differ from IGHV3-30/3-30-5*01, IGHJ4*02, and IGHD2-21*02 germline sequences. Two amino acid replacements in the protein sequence of SH-IgM.22 VH type B are printed in parentheses.

Figure 2. Sequence of the sH-IgM.22 VA. The sequence is aligned according to the numbering system of human VA sequences in Kabat et al. Vα of sH-IgM.22 is a member of the lambda subgroup I. The underlined amino acids have been confirmed by protein sequencing. Amino acid sequence corresponds to sH-IgM.22 nucleotide sequence. sH-IgM.22 VA type I and II sequences are represented only with nucleotides that differ from IGLV1-51*01 and IGLJ3*01 germline sequences. Two amino acid replacements in the protein sequence of SH-IgM.22 VA type B are printed in parentheses. Reference for germline sequences on both figures are IMGT.
differ from their common ancestor by a single nucleotide change (Figure 2). Because only the 2 mutations differentiating the 2 clones could be placed after the transformation event, no conclusions could be drawn regarding the selective forces on the light chain.

It has been established that in vivo BCR expression is needed for B-cell development and maintenance. Progression through the pre-B-cell stage of differentiation depends on signals from the BCR and its precursor. This phase of B-cell development is antigen independent, which means that successful assembly and expression of the BCR itself delivers a survival signal. A mature B cell, even in the absence of antigen stimulation, must retain continuous BCR expression to avoid apoptotic death.16-20

The idea that many B-cell lymphomas need expression of the BCR and potentially antigen stimulation to maintain viability is also supported by numerous observations.21-23 This requirement does not apply to all types of B-cell malignancies (eg, multiple myelomas). Our data suggest a preservation of BCR structure in WM, which implies that the presence of BCR is necessary to generate a survival signal in these malignant cells.

References


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