Typical Waldenstrom macroglobulinemia is derived from a B-cell arrested after cessation of somatic mutation but prior to isotype switch events

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There exists a wide spectrum of IgM-secreting B-cell tumors with different clinical behavior. Knowledge of the V_H gene status can reveal their origin and clonal history. For Waldenstrom macroglobulinemia (WM), a distinct subtype of lymphoplasmacytic lymphoma, early data on limited sequences showed evidence for somatic mutation. A recent report of one case demonstrated intraclonal mutational activity occurring after transformation, a characteristic of germinal center lymphomas. To extend the investigation, we have analyzed 7 cases of WM. V_H genes were somatically mutated with no evidence of intraclonal variation in all cases. In contrast to IgM-secreting multiple myeloma, there was no evidence for isotype switch transcripts in any of the cases. These data support the concept that typical WM is derived from a B cell that has undergone somatic mutation prior to transformation, at a point where isotype switch events have not been initiated. (Blood. 2002;100:1505-1507)

Introduction

According to recent World Health Organization (WHO) proposals, typical Waldenstrom macroglobulinemia (WM) is a specific clinical syndrome commonly associated with a low-grade lymphoplasmacytic lymphoma (LPL).[1] It has a characteristic monoclonal serum IgM of more than 5g/L.[2] WM involves primarily the bone marrow and possibly other lymphoid tissues.[1,3] Tumor morphology shows small lymphocytes with a variable degree of plasma cell maturation, an immunophenotype of $CD19^+CD20^+CD5^-CD10^-cIg^+sIgM^{++}$, and distinct histopathology.[1,3,4] These considerations distinguish it from other B-cell neoplasias with a serum monoclonal IgM, including B-chronic lymphocytic leukemia (B-CLL), splenic marginal zone lymphoma (SMZL), mucosa-associated lymphoid tissue (MALT) lymphoma, follicular lymphoma (FL), and mantle cell lymphoma.[1,3]

Variable region (V) gene analysis is now providing a robust means of identifying tumor origins in B-cell tumors,[5] because neoplastic transformation imprints the clonal history of the B cell in these genes. It has become feasible to classify B-cell tumors in relation to the germinal center (GC), a site of somatic mutation.[6] V gene somatic mutation status defines tumors as those that never enter the GC, those that remain in the GC, and those that transit the GC and exit.[5] Tumors located in the GC display intraclonal heterogeneity of V gene sequence, reflecting continual targeting by the mutation mechanism following neoplastic arrest.

Analysis of isotype switch events via V gene probes provides another tier of maturational status in tumor cells.[5] The presence of tumor-derived isotype-switched transcripts can reveal whether tumor cells have activated switch mechanisms, at least at the RNA level. It is an assay of at least a log-scale greater sensitivity than Southern blotting to assess isotype switch.[7]

In the case of WM, previous V gene analysis had suggested origins from a somatically mutated B cell. These data consisted of V_H analysis from complementarity-determining region 3 to framework region 4 (CDR3-FR4),[8] and a few V_H sequences from Epstein-Barr virus–transformed lines from WM patients secreting IgM with cold agglutinin activity.[9] V_κ gene use had also been analyzed in some cases.[10] Intraclonal variation in WM remained unresolved, because analysis of the CDR3-FR4 regions excluded other domains in each V_H gene.[8] Recently, Ciric et al[11] reported a single WM case where V_H gene sequence analysis revealed intraclonal heterogeneity in tumor cells, suggesting a continual influence on tumor cells of the mutator mechanism in the GC environment.

To resolve the stage of arrest in WM, we have analyzed the V_H gene status of 7 patients with typical WM. In all cases, tumor V_H genes revealed somatic mutation and a complete lack of intraclonal variation, and no evidence for any isotype-switched transcripts in 6 of 6 cases. These results suggest tumor origins in WM from an IgM-secreting B cell that transforms before the onset of isotype switch, with somatic mutation silenced.

Study design

Patients

Unselected patients from the hematology clinic were studied. Diagnosis of typical WM (cases WM1-7) was made using the WHO/Revised European and American Classification of Lymphoid Neoplasm (REAL) criteria.[1,3] Monoclonal serum IgM was raised, more than 30 g/L in 6 of 7 patients and 18 g/L in the other patient, with no evidence of other monoclonal

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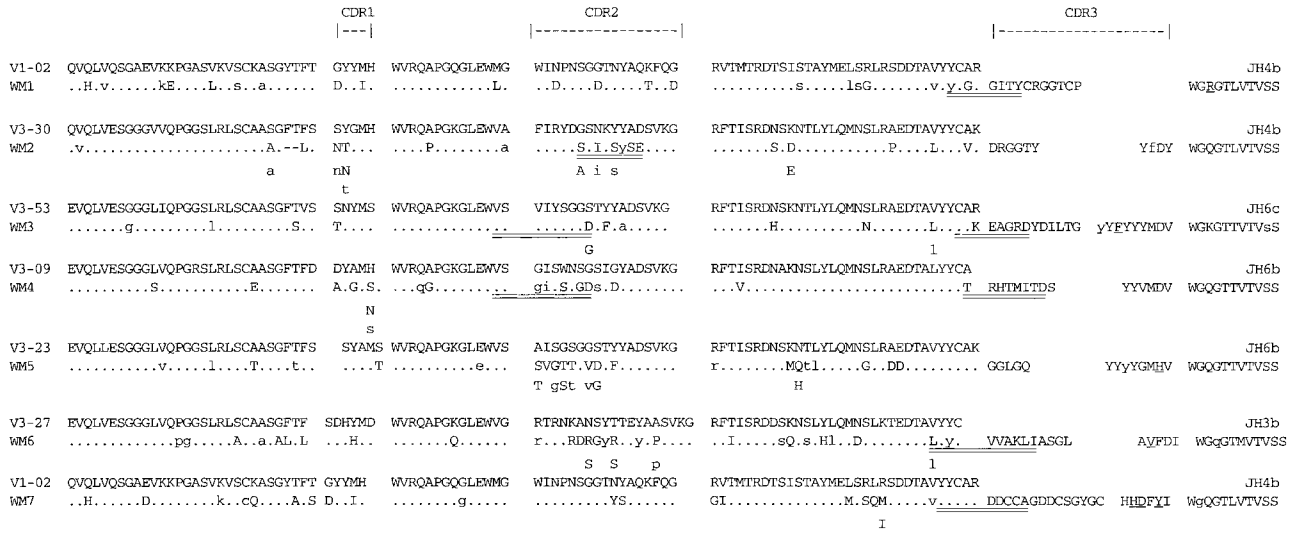

Figure 1. Deduced amino acid sequences of tumor-derived V_H genes from WM cases, indicating extent of somatic mutation and primer design for alternative transcripts. Comparisons for WM (WM1-7) are made with the closest germline V_H gene: uppercase, replacement (R) mutation (underlined in J_H); lower case, silent (S) mutation (EMBL accession nos. AJ488538-44). Each mutation was defined by nucleotide exchanges in a single codon, with successive mutations leading in some cases to 2 or 3 distinct R or S events. These are shown as vertically aligned amino acid changes at specific sites. Nucleotide mutations in the last codons of tumor V_H genes were assessed only if followed by at least a 2-base pair homology to the germline gene. Tumor V_H sequence specific 5′ primers to assess switched transcripts are double-underlined in individual sequences.

immunoglobulins in all patients. Bone marrow aspirate and trephine in each case indicated infiltration with lymphocytes, lymphoplasmacytoid cells, and plasma cells. Immunophenotype was typically $CD19^+CD20^+cIgM^+$ $sIgM^{++}CD5^-$. In 3 of 3 available cases, tumor cells were $IgM^+IgD^{+/-}$-IgG^-IgA^-. Case WM1 underwent splenectomy with histology typical of LPL; case WM5 had lymphadenopathy, and case WM6 had liver involvement. Four patients' cases had no detectable node/spleen involvement. In summary, none of these patients' cases exhibited any immunophenotypic and histopathologic evidence of other B-cell neoplasms known to produce a serum monoclonal IgM.

Cell preparation and V_H gene analysis

Heparinized bone marrow aspirates were taken and mononuclear cells (MNCs) were obtained by Ficoll-gradient centrifugation. Total RNA (5-10 μg) was extracted from MNCs and used for complementary DNA synthesis as decribed.[12] In one case (WM5), genomic DNA was used for analysis. Amplification of V_H genes and sequence analysis followed published methods.[12-14] Identification of isotype switch events involved reverse transcription–polymerase chain reaction (RT-PCR) identification of tumor-derived V_HD-J_H linked to individual C_H isotypes (Cγ,α,ε). The nested PCR strategy, with a sensitivity of about 10^{-4} to 10^{-5} has been described previously.[12,15] Individual tumor V_H-derived primers used are underlined in Figure 1.

Results and discussion

Potentially functional, in-frame tumor-derived V_H sequences (5 V_H3, 2 V_H1) were readily identified in each case by a signature CDR3 sequence in multiple clones from separate PCRs (Table 1 and Figure 1).

Each WM tumor V_H gene revealed extensive somatic mutations, indicating exposure of the cell of origin to somatic hypermutation.[6] This confirms previous observations of V gene status in WM, suggesting a common feature.[8-11] Analysis of the pattern of somatic mutations for antigen imprint by clustering algorithms[16,17] is now considered flawed, first because analysis of CDRs is compromised by the existence of hot-spots in nonfunctional V(D)J rearrangements.[18] Second, an overall low R/S ratio of less than 1.7 in FRs, although predictive of structural conservation,[18] is clearly insufficient: 4 of 7 WM patients (Table 1) did not meet this requirement, despite the fact that the immunoglobulin is being expressed.

Significantly, there was no evidence for any intraclonal variation in V_H sequence in any of the WM cases, within 6 to 16 (mean, 9) clones sequenced in each case (Table 1). Our findings establish a postfollicular tumor origin for typical WM, now confirmed very recently in 3 WM cases lacking intraclonal variation.[19] This is a characteristic feature of LPL[20] and IgM-secreting and typical multiple myeloma.[12,14] In contrast, FL, MALT lymphoma, and some SMZL and monoclonal gammopathies of undetermined significance cases display intraclonal variation in tumor V_H sequences, analyzed in comparable numbers of clones, suggesting an earlier stage of arrest.[5,14,21,22]

To further delineate the stage of arrest, tumor-associated isotype switch events were examined. No tumor-derived isotype switch variants could be identified in 6 of 6 WM cases, using a strategy that has

Table 1. Analysis of WM tumor–derived V_H genes

Patient	V_H family	Germline gene	Homology, %	R/S mutation FR	R/S mutation CDR	J_H	Tumor-derived clones/clones screened	Tumor clones sequenced
WM1	V_H 1	V1-02	92.8	6/9 (0.7)	6/0 (—)	J_H 4b	7/10	7
WM2	V_H 3	V3-30	91.0	9/3 (3.0)	9/5 (1.8)	J_H 4b	6/8	6
WM3	V_H 3	V3-53	95.5	5/3 (1.7)	4/1 (4.0)	J_H 6c	8/10	6
WM4	V_H 3	V3-9	93.9	5/1 (5.0)	8/4 (2.0)	J_H 6b	9/9	9
WM5	V_H 3	V3-23	89.4	7/7 (1.0)	12/3 (4.0)	J_H 6b	17/18	16
WM6	V_H 3	V3-72	88.4	10/8 (1.3)	9/4 (2.3)	J_H 3b	11/11	11
WM7	V_H 1	V1-02	93.2	12/4 (3.0)	4/0 (—)	J_H 4b	8/24	8

successfully identified such transcripts in a number of other B-cell tumors in small cohort studies.[5,12,15] This suggests an origin for WM from an IgM^+ mature B cell that transforms before the isotype switch stage and indicates that tumor cells do not activate switch mechanisms during tumor maintenance. Our findings are supported by emerging preliminary evidence that WM cells do not undergo switch recombination when assessed by Southern blotting.[23]

Malignant B cells arise at distinct stages of maturation and the patterns of V gene sequences can reveal events likely to be occurring during normal B-cell maturation. Tumors also occupy multiple sites and may be able to respond to environmental signals in those sites. The fact that IgM-secreting tumors commonly localize to the bone marrow fits with the observation that normal IgM^+ memory B cells can be found there.[24] Indeed, these cells retain the capacity to mature to IgM-secreting cells[24] and support the concept that they may undergo isotype switching in that site. In WM there is maturation to plasma cells, but neoplastic arrest evidently occurs prior to isotype switch, with ongoing somatic mutation silenced. Absence of somatic mutation in the bone marrow is also suggested by IgM-secreting myeloma.[12] In contrast to WM, these myeloma cases harbor isotype variant transcripts within the tumor clone,[12] indicating that they have activated the switch mechanism. In myeloma, illegitimate switching has been implicated as a causative mechanism in transformation.[7] Preliminary evidence from interphase fluorescent in situ hybridization analysis in WM suggests that the 14q32 locus is free of aberrant translocations,[23] abrogating such potentially neoplastic mechanisms in WM. Our cases of WM have consistent features, but exceptions clearly can occur,[11] and serve to remind us of the heterogeneity within our current tumor categories.

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References

1. Jaffe ES, Harris NL, Stein H, et al. WHO Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001.
2. Morel P, Monconduit M, Jacomy P, et al. Prognostic factors in Waldenstrom macroglobulinemia: a report on 232 patients with the description of a new scoring system and its validation on 253 other patients. Blood. 2000;96:852-858.
3. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the international lymphoma study group. Blood. 1994;84:1361-1392.
4. Owen RG, Johnson SA, Morgan GJ. Waldenstrom's macroglobulinemia: laboratory diagnosis and treatment. Hematol Oncol. 2000;18:41-49.
5. Stevenson FK, Sahota SS, Ottensmeier CH, Zhu D, Forconi F, Hamblin TJ. The occurrence and significance of V gene mutations in B cell-derived human malignancy. Adv Cancer Res. 2001;83:81-116.
6. Berek C. The development of B cells and the B-cell repertoire in the microenvironment of the germinal center. Immunol Rev. 1992;126:5-19.
7. Bergsagel PL, Chesi M, Nardini E, et al. Promiscuous translocations into immunoglobulin heavy chain switch regions in multiple myeloma. Proc Natl Acad Sci U S A. 1996;93:13931-13936.
8. Aoki H, Takishita M, Kosaka M, Saito S. Frequent somatic mutations in D and/or J_H segments of Ig gene in Waldenstrom's macroglobulinemia and chronic lymphocytic leukemia (CLL) with Richter's syndrome but not in common CLL. Blood. 1995;85:1913-1919.
9. Pascual V, Victor K, Lelsz D, et al. Nucleotide sequence analysis of two IgM cold agglutinins: evidence that the V_H4-21 gene segment is responsible for the major cross-reactive idiotype. J Immunol. 1991;146:4385-4391.
10. Wagner SD, Martinelli V, Luzzato L. Similar patterns of $V\kappa$ gene usage but different degrees of somatic mutation in hairy cell leukemia, prolymphocytic leukemia, Waldenstrom's macroglobulinemia, and myeloma. Blood. 1994;83:3647-3653.
11. Ciric B, VanKeulen V, Rodriguez M, Kyle RA, Gertz MA, Pease LR. Clonal evolution in Waldenstrom's macroglobulinemia highlights functional role of B-cell receptor. Blood. 2001;97:321-323.
12. Sahota SS, Garand R, Mahroof R, et al. V_H gene analysis of IgM-secreting myeloma indicates an origin from a memory cell undergoing isotype switch events. Blood. 1999;94:1070-1076.
13. Hawkins RE, Zhu D, Ovecka M, et al. Idiotypic vaccination against human B-cell lymphoma: rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines. Blood. 1994;83:3279-3288.
14. Sahota SS, Leo R, Hamblin TJ, Stevenson FK. Ig V_H mutational patterns indicate different tumor cell status in human myeloma and monoclonal gammopathy of undetermined significance. Blood. 1996;87:746-755.
15. Forconi F, Sahota SS, Raspadori D, Mockridge CI, Lauria F, Stevenson FK. Tumor cells of hairy cell leukemia express multiple clonally related immunoglobulin isotypes via RNA splicing. Blood. 2001;98:1174-1181.
16. Chang B, Casali P. The CDR1 sequence of a major proportion of human germline Ig V_H genes are inherently susceptible to amino acid replacement. Immunol Today. 1994;15:367-373.
17. Lossos IS, Tibshirani R, Narasimham B, Levy R. The inference of antigen selection on Ig genes. J Immunol. 2000;165:5122-5126.
18. Dorner T, Foster SJ, Brezinschek HP, Lipsky PE. Analysis of the targetting of the hypermutational machinery and the impact of subsequent selection on the distribution of nucleotide changes in human VHDJH rearrangements. Immunol Rev. 1998;162:161-171.
19. Shiokawa S, Suehiro Y, Uike N, Muta K, Nishimura J. Sequence and expression of μ and δ transcripts in patients with Waldenstrom's macroglobulinemia. Am J Hematol. 2001;68:139-143.
20. Sahota SS, Garand R, Bataille R, Smith AJ, Stevenson FK. V_H gene analysis of clonally related IgM and IgG from human lymphoplasmacytoid B-cell tumors with chronic lymphocytic leukemia features and high serum monoclonal IgG. Blood. 1998;91:238-243.
21. Bahler DW, Miklos JA, Swerdlow SH. Ongoing Ig gene hypermutation in salivary gland mucosa-associated lymphoid tissue-type lymphomas. Blood. 1997;89:3335-3344.
22. Dunn-Walters DK, Boursier L, Spencer J, Isaacson PG. Analysis of immunoglobulin genes in splenic marginal zone lymphoma suggests ongoing mutation. Hum Pathol. 1998;29:585-593.
23. Schop RFJ, Kuehl WM, Van Wier SA, et al. Genomic aberrations in Waldenstrom's macroglobulinemia clonal cells detected by FISH [abstact]. Blood. 2001;98:154a.
24. Paramithiotis E, Cooper MD. Memory B lymphocytes migrate to bone marrow in humans. Proc Natl Acad Sci U S A. 1997;94:208-212.