Molecular heterogeneity in MCL defined by the use of specific V_H genes and the frequency of somatic mutations

Francisca I. Camacho, Patrocinio Algara, Antonia Rodríguez, Elena Ruíz-Ballesteros, Manuela Mollejo, Nerea Martínez, José A. Martínez-Climent, Marcos González, Marisol Mateo, Alessia Caleo, Margarita Sánchez-Beato, J. Menárguez, Javier García-Conde, Francesc Solé, Elías Campo, and Miguel A. Piris

This study explores whether the presence of somatic mutations or a biased use of *IgV$_H$* genes were associated with the clinical features in a series of 96 patients with mantle cell lymphoma (MCL). The cases were studied by seminested polymerase chain reaction using primers from the FR1 and J_H regions. There was an unexpectedly high frequency of somatic mutations, with 29 of 103 sequences showing more than 2% of mutations. Biased usage of specific *V$_H$* segments was also found; the most widely used genes in this series were *V$_H$3-21* (10 cases), *V$_H$3-23* (9 cases), *V$_H$4-34* (11 cases), and *V$_H$4-59* (9 cases). *V$_H$* mutation frequency, taking into account different thresholds, did not distinguish different overall survival probabilities. Nevertheless, a more frequent use of *V$_H$3-21* or *V$_H$4-59* (8 of 18) was observed in the group of long-term survivors (18 cases > 5 years; *P* < .01). None of these long-term survivors presented the *V$_H$3-23* gene rearrangement. As in other lymphoproliferative disorders, the expression of CD38 or p53 or both was associated with a poorer survival probability. This nonrandom usage of *IgV$_H$* segments suggests that specific antigens may play a pathogenically relevant role in the genesis or progression of subsets of MCL cases and may help in distinguishing a significant group of MCL long-term survivors. (Blood. 2003;101: 4042-4046)

Introduction

The presence of somatically acquired *IgV$_H$* mutations is considered a consequence of the exposure of B cells to the microenvironment of the germinal center. Thus, it has been exploited as a distinctive feature of benign B cells and malignant lymphomas. The analysis of *IgV$_H$* mutations in B-cell lymphomas has revealed an unsuspected heterogeneity in some small B-cell lymphomas, such as B-chronic lymphocytic leukemia (B-CLL)[1,2] and splenic marginal zone lymphoma (SMZL).[3,4] In both conditions, approximately half of the cases bear *IgV$_H$* mutations in at least 2% of the sequence. This relationship is associated with lower clinical aggressiveness and extended survival. Additionally, a biased use of V_H genes has been demonstrated in B-CLL and SMZL, with an increase in the frequency of *V$_H$1-69* and *V$_H$ 1-2*, respectively, thus suggesting that these lymphoproliferative process types could be related to specific subsets of B lymphocytes, primed for their growth by autoantigens or superantigens.[1,5]

Mantle cell lymphoma (MCL) is a B-cell lymphoma characterized by the translocation t(11;14)(q13;q32) that results in overexpression of cyclin D1 protein. MCL represents 3% to 10% of non-Hodgkin lymphoma (NHL) and has a median survival of 3 to 5 years. Despite this relatively short survival, subsets of patients with MCL show a more favorable clinical course with a relatively long period of stable disease. Additionally, MCL cases are cytologically heterogeneous; a subset of aggressive MCL cases has been identified that has a blastoid cytology and is associated with frequent inactivation of p53 and p16 genes.[6-8]

Although it has long been assumed that MCL cells bear unmutated IgV_H genes,[9-11] recent investigations have revealed that somatic mutations in the immunoglobulin genes are present in a significant fraction of MCL cases.[12] Here we have analyzed a series of 96 MCL cases, a number large enough to reveal even slight but significant associations between the presence of somatic mutation, the use of specific IgV_H genes, and the morphologic or clinical variability of MCL cases.

Patients and methods

Patients and tissue samples

We studied DNA samples extracted from frozen tissue blocks (71 cases) and peripheral blood (25 cases) from 96 patients with newly diagnosed MCL. Cases were consecutively diagnosed in the centers participating in this study: Hospital Clínico, Valencia; Hospital Clinic, Barcelona; Hospital

From the Centro Nacional de Investigaciones Oncológicas (CNIO), Molecular Pathology Program, Madrid, Spain; Department of Genetics and Pathology, Hospital Virgen de la Salud, Toledo, Spain; Department of Hematology and Medical Oncology, Hospital Clínico, University of Valencia, Valencia, Spain; Department of Hematology, Hospital Clínico, Salamanca, Spain; Laboratory of Citología Hematológica, Department of Pathology, Hospital del Mar, Barcelona, Spain; Department of Pathology, Hospital Clinic, Barcelona, Spain; and Department of Pathology, Hospital Gregorio Marañón, Madrid, Spain.

Submitted November 15, 2002; accepted December 23, 2002. Prepublished online as *Blood* First Edition Paper, January 2, 2003; DOI 10.1182/blood-2002-11-3456.

Supported by grants from the Comunidad Autónoma de Madrid (CAM 08.1/0011/2001.1), the Ministerio de Sanidad y Consumo (FIS 01-0035), and the Comunidad Autónoma de Castilla-La Mancha (02031-00), Spain. F.I.C. is supported by a grant from the Madrid City Council and the CNIO.

F.I.C. and P.A. contributed equally to this work.

Reprints: Miguel A. Piris, Molecular Pathology Program, Centro Nacional de Investigaciones Oncológicas, C/ Melchor Fernández Almagro 3, E-28029 Madrid, Spain; e-mail: mapiris@cnio.es.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Clínico, Salamanca; Hospital del Mar, Barcelona; Hospital Virgen de la Salud, Toledo; and Tumor Bank of Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain. Diagnostic criteria were based on those described in the World Health Organization classification, including, in addition to a morphology consistent with the diagnosis, the demonstration of cyclin D1 expression for the cases diagnosed in paraffin-embedded tissue samples, or the presence of t(11;14) in the cases diagnosed in peripheral blood (PB) and bone marrow (BM).

Patient medical records were reviewed to determine age, sex, localization, and stage of disease at diagnosis, splenomegaly at diagnosis, International Prognostic Index (IPI), and disease course. Cases were considered as primary gastrointestinal or Waldeyer ring types when the disease was restricted to these organs. Approval was obtained from the institutional review board of Hospital Universitario La Paz for this study. When necessary, informed consent was provided according to the Declaration of Helsinki.

Immunohistochemistry

All paraffin-embedded samples were subjected to routine hematoxylin-eosin and immunohistochemical study in sections. Immunohistochemical analysis was performed on formalin-fixed or B-5 paraffin-embedded tissue. After incubation with the primary antibody, immunodetection was performed with biotinylated antimouse immunoglobulins, followed by peroxidase-labeled streptavidin (LSAB-DAKO, Copenhagen, Denmark) and with diaminobenzidine chromogen as substrate. All immunostaining was performed using the TechMate 500 (DAKO) automatic immunostaining device. The labeling system and antibodies (CD20, CD5, CD27, CD38, IgD, cyclin D1, p53, and Ki-67) were obtained from DAKO.

IgV$_H$ study

Rearranged *IgV$_H$* genes were amplified using a seminested polymerase chain reaction (PCR) method, as described previously.[13] In the first round of PCR, a mixture of 6 framework 1 (FR1) V_H family-specific primers and 2 consensus primers for the J_H gene were used. The second round of PCR was performed in 6 separate reactions with 1 of the 6 V_H FR1 primers and J_H internal primers.

Briefly, 200 ng DNA was amplified in a volume of 50 μL with 1 × PCR buffer, 200 μM dNTPs (deoxyribonucleoside triphosphate), 2.5 mM $MgCl_2$, 250 nM each primer, and 1 U Ampli*Taq* gold. In the second round of amplification, the same concentrations of reagent were used, except for $MgCl_2$, which was 1.5 mM. Then, 1 μL of the first-round PCR product was added to the seminested reaction as a template. The PCR conditions have been described previously.[3]

Direct sequencing was performed on both strands using the same primers as in the amplification. The direct sequencing procedure was performed using an ABI PRISM 310 or 3700 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany), following the manufacturer's procedure. Mutations were identified by comparison with the germline sequence (Ig BLAST at http://www.ncbi.nlm.nih.gov/igblast and V BASE at http://www.mrc-cpe.cam.ac.uk/vbase-ok sequence directories).

To determine whether the number of replacement (R) and silent (S) amino acid substitutions identified were indicative of antigen selection, the Chang and Casali method was used.[14]

Statistical analysis

Survival analyses were performed using the Kaplan-Meier method. Statistical significance of associations between individual variables and overall survival was determined using the log-rank test. The Cox univariate proportional hazard analysis was also performed independently for each variable to estimate relative risk [Exp (B)], and the associated χ^2 value for assessing significance. $P < .05$ was considered significant. Survival probability was analyzed using a Cox multivariate analysis, including all the variables with a $P < .1$. All statistical analyses were carried out using SPSS for Windows (Chicago, IL).

Results

Clinical features

Ninety-six patients meeting the criteria for MCL diagnosis were enrolled, 72 men and 24 women. The median age was 66 years (range, 32-86 years). Eighty-seven of them were at stages III or IV at diagnosis; the IPI group distribution was 20 high-risk (4 or 5), patients, 54 intermediate risk (2 or 3), and 20 low-risk (0 or 1) cases. Twenty-six patients presented splenomegaly at the time of diagnosis and only 5 of them were considered as purely splenic forms. Six cases were considered to be the primary gastrointestinal form of MCL, another 6 as the primary Waldeyer ring form, and an additional case presented initial involvement of both territories. Infiltration of the PB or BM (or both) was observed in 51 cases. The median follow-up period for the entire series was 25 months (range, 0-133 months).

V$_H$ gene usage in MCL

In this series of 96 MCL cases, we have amplified and sequenced 103 clonal *IgV$_H$* rearrangements. There were 7 cases with 2 different rearrangements. Among the 103 clonal V_H gene sequences, 100 were potentially functional and 3 were rendered nonfunctional by out-of-frame rearrangement (stop codon). In 4 of 7 cases, 2 apparently productive V_H gene rearrangements were obtained, which probably represents a lack of allelic exclusion, similar to that described in B-CLL.[15]

The similarity of the V_H genes to the closest germline gene segment is shown in Table 1. The most frequent V_H family was *V$_H$3* (46% of the sequences), followed by *V$_H$4* (29%), *V$_H$1* (20%), *V$_H$5* (3%), and *V$_H$2* (2%). The frequencies of the used V_H families differ from those usually present in peripheral and lymph node lymphocytes in healthy individuals, mainly as a consequence of the higher frequency of *V$_H$4* family genes observed here.

The 103 rearranged V_H gene sequences used by these 96 cases have a striking bias toward using specific genes, such as *V$_H$3-21* (11.8%), *V$_H$4-34* (10.8%), *V$_H$3-23* (8.8%), *V$_H$4-59* (8.8%), and *V$_H$4-39* (7.8%), by comparison with the relative frequencies in peripheral blood lymphocytes (PBLs). There was no difference in the usage of V_H families or segments among the cases analyzed by tissue biopsy or PB.

Mutational analysis

The frequency of mutations was higher than previously described, with around one fourth (29 of 103) of *IgV$_H$* rearranged genes showing more than 2% of mutations. There was a group of 13 rearranged *IgV$_H$* genes with a high mutational index (defined as > 5% of mutations). The range of percentage of mutations was from 0.42% to 16%, with a mean of 1.89% (Table 2).

There was no significant relationship between the mutational index and the V_H family, although studying the relationship

Table 1. *V$_H$* families observed in the 103 MCL sequences

	V$_H$1	*V$_H$2*	*V$_H$3*	*V$_H$4*	*V$_H$5*	*V$_H$6*	*V$_H$7*
Functional members	10	3	20	7	1	1	1
MCL sequences, %*	20	2	46	29	3	0	0
CD5 + IgM + PBLs,[22] %	19	2	56	18	1	1	0
PBLs,[23] %	11	3	52	19	10	0	—

The frequency is compared with that described in PBLs (χ^2 test). — indicates data not shown.

*$P < .05$.

Table 2. *IgV$_H$* mutational frequency considering the productive rearrangement in 96 MCL cases, in relation to the main clinical features at presentation

		Frequency of *IgV$_H$* somatic mutation		
	0%	More than 0%	More than 2%	More than 5%
Total	29	67	28	13
Splenomegaly at diagnosis	5/26	21/26	11/26	5/26
Splenic primary MCL	1/5	4/5	3/5	3/5*
PB/BM infiltration	13/51	38/51	17/51	9/51
I/II CS	4/9	5/9	1/9	1/9
Waldeyer	1/7	6/7	0/7†	0/7
Intestinal	0/7	7/7	3/7	0/7
Diffuse/nodular/MZ pattern	6/13/3	20/18/6	8/6/3	2/3/1
Blastoid	5/14	9/14	6/14	3/14
i-GC Cyclin D1$^+$ cells	5/20	14/20	6/20	3/20

PB indicates peripheral blood; BM, bone marrow; CS, clinical stage; MZ, marginal zone; i-GC, intragerminal center cyclin D1$^+$ cells.

*$P < .05$.

†$P = .069$.

between the mutational index and the use of specific V_H genes reveals statistically significant differences ($P < .001$). Thus, all *V_H3-21* (12 of 12) sequences were unmutated (> 98% homology) and had on average 0.30 mutations, whereas 5 of 9 *V_H3-23* sequences and 3 of 3 *V_H3-48* rearrangements showed mutations, with respective mean numbers of mutations of 3.28 and 5.82 (Table 3).

The mutational index was higher (> 5%) for cases whose clinical staging revealed BM or PB infiltration. Additionally, cases with extranodal involvement at diagnosis were shown to have a low mutational load (< 5%). Cases considered as primary splenic forms of MCL (5 cases) carried a high mutational index (3 of 5 cases > 5%; $P < .05$).

Mutational index was independent of architectural pattern (diffuse/nodular/mantle zone) and cytology (classic/blastoid). A specific search was performed to examine the hypothetical association of high somatic mutation with the presence of cyclin D1$^+$ tumor cells in the germinal center microenvironment. These intragerminal center cyclin D1$^+$ cells were observed in 20 of 56 cases, although there was no significant relationship with the mutational index (Table 2).

V_H3-21 cases. All 12 *V_H3-21* sequences had a lower mutational index (mean, 0.30) than the remaining MCL cases. Ten cases with *V_H3-21* as the only rearrangement (mean mutational index, 0.18) also had a relatively low frequency of BM and PB infiltration. These *V_H3-21* cases exhibited a better survival probability, 4 of 10 remaining alive 5 years from diagnosis (Figure 1).

V_H3-23 cases. Mutation frequency was distinctly higher in the 9 cases with *V_H3-23* (mean mutational index, 3.28; 5 cases > 2% mutations). Patients in this group were characterized by a more aggressive clinical course, all of them having died within 5 years of the diagnosis (Figure 1). A relatively high frequency of splenomegaly (5 of 9) at diagnosis was observed in this group.

V_H4-59 cases. A low mutation frequency (mean, 1.53; 1 case > 2% mutations), combined with a relatively high frequency of splenomegaly and PB/BM infiltration at diagnosis, was observed in this group. Cases using *V_H4-59* show a trend for a better survival probability (Figure 1), with 4 of 9 alive at 5 years after diagnosis.

Antigen selection

The distribution of replacement (R) and silent (S) mutations was analyzed by considering all possible mutations, as described by Chang and Casali[14] in 26 mutated cases (> 2% of mutations). One of the 26 cases presented a double V_H rearrangement, both mutated, and it was only considered the in-frame sequence. No statistically significant evidence for antigen selection was observed in 16 sequences. In the other 10 cases, there was evidence for negative selection, whereby fewer replacement mutations than expected were seen in the FR regions, indicating pressure to maintain the germinal configuration.

Other immunohistochemical and morphologic results

Other markers analyzed in this series were CD27 (14 of 55 positive), CD5 (58 of 76 positive), CD38 (50 of 67 positive), p53 (13 of 71 positive), IgD (54 of 65 positive), and Ki67 (42 low, 27 high). Blastoid cytology was present in 14 of 86 cases.

Overall survival predictors

The capacity of all these variables to predict overall survival probability was evaluated (Table 4). V_H mutation frequency, taking into account the different thresholds, did not distinguish different overall survival probabilities among the patients.

Parameters found to be significant predictors of survival probability were the IPI, splenic involvement at diagnosis, tetraploidy, proliferation index (Ki67), and the expression of CD38 or

Table 3. V$_H$ gene usage and relationship with mutation frequency and clinical data at diagnosis

V_H gene	Frequency in CD5$^+$ IgM$^+$ PBLs[22,23], %	MCL cases	Mutation more than 2%	Mean no. of mutations	Splenomegaly at diagnosis	PB/BM infiltration	I/II CS	Waldeyer	Intestinal	Survival longer than 5 y
V_H1-08	—	7	1	0.70	0	2	2	1	1	1
V_H1-18	3.5	3	0	0.60	0	2	1	2	0	0
V_H3-07	5.6	6	3	2.00	1	3	0	0	0	1
V_H3-09	—	6	1	0.67	4	3	1	0	0	1
V_H3-21	—	10	0	0.18	1	3	1	0	0	4
V_H3-23	13.9	9	5	3.28	5	6	0	0	2	0
V_H3-48	—	3	3	5.82	1	1	0	0	0	1
V_H4-34	3.5	11	3	1.22	3	6	1	2	0	3
V_H4-39	2.8	7	3	2.84	1	4	0	0	0	1
V_H4-59	6.3	9	1	1.53	4	7	0	0	1	4
Others	—	25	8	3.03	6	14	2	2	3	2
Total	NA	96	28	1.94	26	51	8	7	7	18
Significance	NA	NA	$P < .001$		NS	NS	NS	NS	NS	NS

Most frequent *IgV$_H$* genes in relation to mutational index and several clinically relevant features. Only productive rearrangements have been considered in those 7 cases with double rearrangements. CS indicates clinical stage; NA, not available; NS, not significant; —, data not shown.

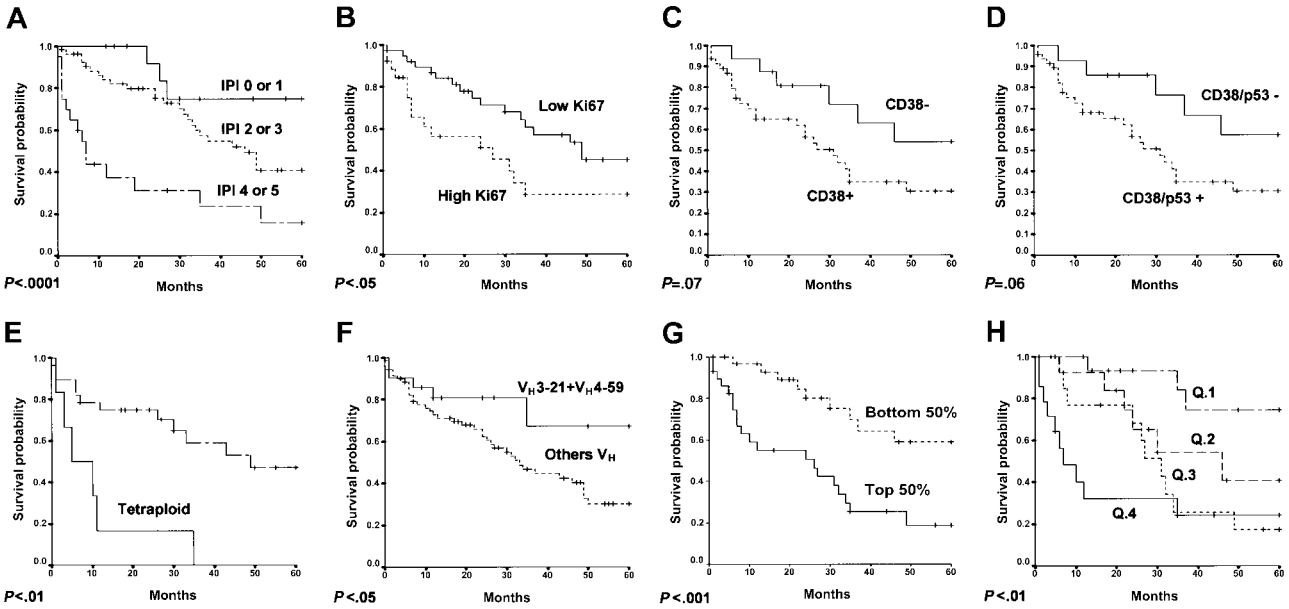

Figure 1. Survival curves of MCL cases. These curves are (A) IPI, (B) Ki67, (C) CD38, (D) CD38 or p53, (E) tetraploidy, (F) use of *V_H3-21* or *V_H4-59*, and (G) Kaplan-Meier estimation of overall survival according to the assigned probability in the multivariate analysis. Cases were divided into 2 groups, each comprising the bottom and the top 50% of the cases. (H) Kaplan-Meier estimation of overall survival according to the assigned probability in the multivariate analysis. Cases are divided into quartiles.

p53. A trend was also observed for higher survival probability to be associated with the use of *V_H3-21* ($P = .061$; Exp (B) = 0.290) and a nonsignificant tendency with *V_H4-59*, whereas a trend was observed for an unfavorable prognosis of the use of *V_H3-23* ($P = .066$; Exp (B) = 2.47). Combining cases using *V_H3-21* and *V_H4-59* in a single group, the survival analysis revealed significantly longer survival for this group (Figure 1).

The series included a small group (18 cases) of long-term survivors (> 5 years). A review of the characteristics of these patients revealed significant differences in V_H gene usage. Almost half (8 of 18) showed expression of either *V_H3-21* or *V_H4-59*, compared with 14.9% in the group with less than 5-year survival ($P < .01$). None of these long-term survivors presented the *V_H3-23* gene rearrangement. Long-term survivors also have a lower frequency of CD38 expression (45.5% versus 80.8%; $P < .05$) and CD38 or p53 expression (45.5% versus 84.3%; $P < .05$).

Finally a Cox multivariate analysis showed that both the use of *V_H3-21* or *V_H4-59* and the expression of CD38 or p53 predicted the overall survival probability independently of the IPI (Table 5). The patients were ranked according to their score, estimated by Cox multivariate analysis, and divided into two groups of identical size (the bottom 50% and the top 50%) and into quartiles (Q). The bottom 50% and the top 50% corresponded to low and high estimated risk groups, respectively; Q1, Q2, Q3 and Q4 included low, intermediate-low, intermediate-high and high estimated risk cases, respectively. Their survival probability was then estimated by the Kaplan-Meier method (Figure 1).

Table 4. Survival studies using log-rank test and Cox univariate analysis of the main variables studied

Variables	*P*, log-rank test	Exp (B)-Cox	95.0% CI for Exp (B) Low	High
Age older than 60 y	NS	—	—	—
Sex (F vs M)	NS	—	—	—
Stage III-IV	NS	—	—	—
IPI (4-5)	< .001	3.346	1.749	6.402
Spleen	< .05	1.958	1.034	3.709
Waldeyer	NS	—	—	—
Gastrointestinal	NS	—	—	—
Leukemic	NS	—	—	—
Tetraploidy	< .01	5.362	1.862	15.439
Blastoid	NS	—	—	—
Architectural pattern	NS	—	—	—
Reactive GC	NS	—	—	—
CD5	NS	—	—	—
IgD	NS	—	—	—
MIB-1	< .05	2.055	1.0207	4.1390
CD38	.076	2.178	0.893	5.310
p53	NS	—	—	—
CD38-p53	.062	2.411	0.921	6.310
% ID less than 100	NS	—	—	—
% ID less than 98	NS	—	—	—
% ID less than 95	NS	—	—	—
V_H USAGE	NS	—	—	—
V_H3-23	.066	2.47	0.952	6.407
V_H3-21	.061	0.290	0.070	1.200
V_H4-34	NS	—	—	—
V_H4-59	NS	—	—	—
V_H3-21 or *V_H4-59*	< .05	0.366	0.144	0.930

F indicates female; M, male; GC, germinal center; ID (identity), the homology of V_H genes to the germline sequences; CI, confidence interval; NS, not significant; —, data not needed.

Discussion

This study analyzes the incidence and distribution of somatic mutations in V_H genes in a series of 96 cases diagnosed as MCL. To

Table 5. Survival studies using Cox multivariate analysis of the IPI (4-5), CD38 and/or p53, and *V_H3-21* or *V_H4-59* variables

Variables	*P*	Exp (B)-Cox	95.0% CI for Exp (B) Low	High
IPI (4-5)	< .0001	7.555	3.056	18.681
CD38-p53	.011	3.925	1.374	11.211
V_H3-21 or *V_H4-59*	.023	0.229	0.064	0.819

guarantee the homogeneity of the series, we used restrictive inclusion criteria. The results show that 27% of the sequences presented less than 98% homology with the germinal sequence. Mutation frequency appears not be distributed at random, but is related with the use of specific IgV_H genes and, to a lesser but still significant extent, with particular anatomic localizations.

The percentage of mutated cases in this series is slightly higher than that previously reported (20% in the series of 51 cases studied by Thorselius and coworkers),[12] although this may depend on the smaller size of the latter series.

V_H3-21 usage in MCL appears to be associated with a low mutational index and a relatively favorable clinical course. Most of these cases seem to have a disease that is mainly restricted to lymph nodes. This contrasts with the observations performed in B-CLL, where the use of *V_H3-21* is associated with a high mutational index and a more aggressive clinical course.[2] This contradiction seems to support the interpretation that mutation frequency is not dependent on the usage of a specific IgV_H gene, but is perhaps related to some unknown antigens that are of probable significance in the pathogenesis of this MCL subset.

The most favorable outcome of the *V_H3-21* cases is shared by the *V_H4-59* cases, quite the opposite of what is observed in *V_H3-23* cases. Thus, *V_H3-23* cases, with their higher mutational index, show a trend toward more aggressive behavior. Frequency of BM or leukemic infiltration was significantly higher in these groups of cases harboring the *V_H3-23* gene.

Other prognostic factors found here are tetraploidy and the association of p53 and CD38. Previous observations have already demonstrated that karyotypic complexity has a strong impact on prognosis in MCL,[16,17] and the presence of tetraploidy here is probably associated with the blastoid cytology, which usually pursues a more aggressive course.[18] A paradoxical observation in this series is that blastoid cytology is not related with more aggressive behavior, whereas p53 expression, increased growth fraction, and tetraploidy, all of which are usually associated with blastoid cytology, are associated with this poorer outcome. This implies that a reconsideration of the diagnostic criteria for the blastoid variant of MCL could provide a more accurate clinical meaning of this diagnosis.

CD38 expression appears also to predict shorter survival in this series, mainly when analyzed jointly with p53, thereby mimicking the findings in B-CLL.[19-21]

The data obtained in this study also allow recognition of a group of MCL patients with relatively long-term survival ($>$ 5 years), which seems to depend on an association between the use of specific V_H genes and the lack of adverse prognostic markers. Despite the adverse outcome for MCL patients, repeated observations indicate the existence of a distinct group of cases that seem to display a relatively long period of stable disease. Molecular heterogeneity of MCL, taking into greater account the usage of specific genes than the frequency of mutations, goes some way to explain this clinical variability.

This nonrandom usage of IgV_H segments suggests that specific antigens may play a pathogenically relevant role in the genesis or progression of subsets of MCL cases.

Acknowledgments

We thank Dr M. Pollán for her valuable help with the statistical analysis, and Drs P. Domínguez, M. Medina, F. Bosch, E. Flores, M. A. Martínez, B. Espinet, M. J. Terol, A. Ferrández, and A. Teruel from the Hospitals of Alcorcón (Madrid), Virgen de la Merced (Osuna), Hospital Clinic of Barcelona, Hospital General Universitario Gregorio Marañón (Madrid), Hospital 12 de Octubre (Madrid), Hospital del Mar (Barcelona), and Hospital Clínico Universitario (Valencia), Spain, for kindly providing the clinical data for the cases included in this series.

References

1. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848-1854.
2. Tobin G, Thunberg U, Johnson A, et al. Somatically mutated Ig V(H)3-21 genes characterize a new subset of chronic lymphocytic leukemia. Blood. 2002;99:2262-2264.
3. Algara P, Mateo MS, Sanchez-Beato M, et al. Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. Blood. 2002;99:1299-1304.
4. Bahler DW, Pindzola JA, Swerdlow SH. Splenic marginal zone lymphomas appear to originate from different B cell types. Am J Pathol. 2002; 161:81-88.
5. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest. 1998;102:1515-1525.
6. Hernandez L, Fest T, Cazorla M, et al. p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. Blood. 1996;87:3351-3359.
7. Greiner TC, Moynihan MJ, Chan WC, et al. p53 mutations in mantle cell lymphoma are associated with variant cytology and predict a poor prognosis. Blood. 1996;87:4302-4310.
8. Pinyol M, Hernandez L, Cazorla M, et al. Deletions and loss of expression of p16INK4a and p21Waf1 genes are associated with aggressive variants of mantle cell lymphomas. Blood. 1997; 89:272-280.
9. Du MQ, Diss TC, Xu CF, Wotherspoon AC, Isaacson PG, Pan LX. Ongoing immunoglobulin gene mutations in mantle cell lymphomas. Br J Haematol. 1997;96:124-131.
10. Hummel M, Tamaru J, Kalvelage B, Stein H. Mantle cell (previously centrocytic) lymphomas express VH genes with no or very little somatic mutations like the physiologic cells of the follicle mantle. Blood. 1994;84:403-407.
11. Pittaluga S, Tierens A, Pinyol M, Campo E, Delabie J, De Wolf-Peeters C. Blastic variant of mantle cell lymphoma shows a heterogenous pattern of somatic mutations of the rearranged immunoglobulin heavy chain variable genes. Br J Haematol. 1998;102:1301-1306.
12. Thorselius M, Walsh S, Eriksson I, et al. Somatic hypermutation and V(H) gene usage in mantle cell lymphoma. Eur J Haematol. 2002;68:217-224.
13. Kuppers R, Zhao M, Hansmann ML, Rajewsky K. Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. EMBO J. 1993;12:4955-4967.
14. 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.
15. Rassenti LZ, Kipps TJ. Lack of allelic exclusion in B cell chronic lymphocytic leukemia. J Exp Med. 1997;185:1435-1445.
16. Bea S, Ribas M, Hernandez JM, et al. Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. Blood. 1999;93:4365-4374.
17. Cuneo A, Bigoni R, Rigolin GM, et al. Cytogenetic profile of lymphoma of follicle mantle lineage: correlation with clinicobiologic features. Blood. 1999; 93:1372-1380.
18. Ott G, Kalla J, Hanke A, et al. The cytomorphological spectrum of mantle cell lymphoma is reflected by distinct biological features. Leuk Lymphoma. 1998;32:55-63.
19. Durig J, Naschar M, Schmucker U, et al. CD38 expression is an important prognostic marker in chronic lymphocytic leukaemia. Leukemia. 2002; 16:30-35.
20. Ibrahim S, Keating M, Do KA, et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. Blood. 2001;98: 181-186.
21. Thunberg U, Johnson A, Roos G, et al. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. Blood. 2001;97:1892-1894.
22. Brezinschek HP, Foster SJ, Brezinschek RI, Dorner T, Domiati-Saad R, Lipsky PE. Analysis of the human VH gene repertoire: differential effects of selection and somatic hypermutation on human peripheral CD5(+)/IgM+ and CD5(−)/IgM+ B cells. J Clin Invest. 1997;99:2488-2501.
23. Guigou V, Cuisinier AM, Tonnelle C, Moinier D, Fougereau M, Fumoux F. Human immunoglobulin VH and VK repertoire revealed by in situ hybridization. Mol Immunol. 1990;27:935-940.