Med. J. Kagoshima Univ., Vol. 51. Suppl. 48-50, July, 1999

Somatic mutation of immunoglobulin heavy chain variable region genes in gastric low-grade MALT type lymphoma

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Malignant lymphoma arising from mucosa-associated lymphoid tissue (MALT) occurs in various extranodal organs including the gastrointestinal (GI) tract, lungs, salivary gland and thyroid. Since Isaacson and Wright first described a new concept of MALT lymphoma in 1983, its essential and distinctive difference from nodal lymphoma has been characterized and generally accepted. Low-grade MALT type (L-MALT) lymphoma is morphologically characterized by a proliferation of centrocyte-like cells, lymphoepithelial lesions, plasmocytic differentiation and follicular colonization. The immunophenotype was considered to show a similar phenotype to nodal marginal zone B-cell lymphoma or splenic marginal cell lymphoma: sIgM+, sIgD-, cIg-/+, pan B+, CD5-, CD10-, CD23-, and CD43-/+.

In response to antigen stimulation, somatic hypermutation of V_H (immunoglobulin heavy chain variable region) genes is generally believed to occur in germinal center B-cells, which results in the generation of memory B-cells, producing antibodies with a high specificity and affinity. These mutations are distributed in both complementary determining regions (CDR) and the framework regions (FR) with a high ratio of replacement (R) to silent (S) mutations in the CDR indicating antigen selection. Ongoing mutation indicates the intraclonal variation of V_H genes and suggests the genetic evidence that antigen stimulation plays an important role in clonal expansion in follicular lymphoma. Sequence analysis of rearranged V_H genes has been used to determine where B-cell lymphoma derives from pre-germinal center or post-germinal center B-cells. In order to clarify the cell characteristics and origin of L-MALT lymphoma, and to asses the difference of somatic mutation between L-MALT lymphoma and gastric diffuse large B-cell lymphoma (DLBL), we analyzed the V_H genes from eight cases of L-MALT lymphomas and two cases of gastric DLBL. To further elucidate the role of antigen selection in L-MALT lymphoma, we analyzed intraclonal variations of VH genes.

Materials and Methods

Fifty-seven gastric lymphomas obtained from the files of the First Department of Pathology, Fukushima Medical University, School of Medicine, collected between 1983 and 1998, were reviewed. Fifty-seven lymphomas were subclassified as L-MALT lymphoma (24 cases), mantle cell lymphoma (MCL) (1), L-MALT lymphoma with diffuse large B cell lymphoma (DLBL) (9), and DLBL (23). Clinical and follow-up data were also obtained from referring clinicians. Paraffin and frozen immunohistochemistry were performed using the following antibodies: CD3, CD5, CD10, CD20 (FB1), CD21, CD23, CD30, CD43, CD45RO, CD74, CDw75, immunoglobulins, bcl2, bcl6, Pax5, cyclinD1, p53, p16, p27, MDM2, MIB1, LMP1, EBNA2 and keratin. Immunophenotypic study using the ABC method was helpful for confirming the diagnosis reached by morphologic examination of the H.E. stained sections.

Table 1. Distribution of Mutations of VH genes in L-MALT lymphoma

Case No.	Germline	Region	Replacement	Silent	Total
1	V3-23	CDRII	7 (13.7)	3 (3.9)	10 (19.6)
		FRIII	5 (5.2)	5 (5.2)	10 (10.4)
2	DP-51	CDRII	7 (13.7)	1 (2.0)	8 (15.7)
		FRIII	2 (2.1)	3 (3.1)	5 (4.2)
3	B42	CDRII	2 (3.9)	3 (5.9)	5 (9.8)
		FRIII	2 (2.1)	2 (2.1)	4 (4.2)
4	VH311	CDRII	6 (11.8)	0	6 (11.8)
		FRIII	6 (6.3)	2 (2.1)	8 (8.3)
5	VH311	CDRII	1 (2.0)	2 (3.9)	3 (5.9)
		FRIII	7 (7.3)	7 (7.3)	14 (14.6)
6	DP-49	CDRII	4 (7.8)	1 (2.0)	5 (9.8)
		FRIII	6 (6.3)	1 (1.0)	7 (7.3)
7	VH311	CDRII	4 (7.8)	0	4 (7.8)
		FRIII	8 (8.3)	4 (4.2)	12 (12.5)
8	DP53	CDRII	7 (13.7)	3 (3.9)	10 (19.6)
		FRIII	9 (9.4)	8 (8.3)	17 (17.7)

Using BLATIN and the GenBank Database. Parentheses indicate percentage.
CDR: Complementary determining region. FR: Framework lesion.

Sequences of the CDRII and FRIII region of V_H genes were analyzed in twelve cases of L-MALT lymphoma and two cases of DLBL using semi-nested PCR. DNA preparation was performed from a whole section of either frozen or paraffin-embedded tissue. The two-step semi-nested PCR reaction was performed in either a GeneAmp 9700 System (Perkin Elmer Applied Systems, Chiba, Japan) or a DNA Thermal Cycler (Perkin Elmer), as described previously. In brief, in the first round PCR amplification was carried out using consensus primers of the second framework portion of the V_H region (FR2A) and the JH region (LJH) from genomic DNA. In the second round of PCR, the PCR products of the first round were amplified using primer FR2A and another JH primer (VLJH). The primer sequences were follows: 5'TGG (A/G)TC CG(CA) CAG (G/C)C(T/C) (T/C)C(A/G/T/C) GG3' (termed FR2A), 5'TGA GGA GAC GGT GAC C3' (termed LJH), 5'GTG ACC AGG GT(A/G/C/T) CCT TGG CCC CAG3'5 (termed VLJH). PCR products were electrophoresed on 2% agarose gel and visualized by ethidium bromide staining. Appropriately sized PCR products were isolated. PCR products were cloned in the pCR II vector using the TA cloning system according to the manufacturer's protocol (Invitrogen, San Diego, CA, USA). Sequencing was performed with an SQ5500 automated sequencer (Hitachi, Tokyo, Japan) by the dideoxy chain termination method with fluorescent dyes using a Thermo Sequenase Core Sequencing Kit (Amersham International plc., Little Chalfont, UK). DNA sequences were analyzed using BLATIN and the GenBank Database. Mutations in the V_H were identified by comparing the consensus sequence of each case with the corresponding germline to estimate somatic mutations.

Results

Twenty-four gastric L-MALT lymphomas were examined in the present study. The mean age of the patients in the 24 L-MALT lymphomas was 56 years (20 to 80 years) with a M/F ratio of 1:2. Twenty-two cases (92%) of L-MALT lymphoma were restricted to the submucosa, and two cases showed an infiltration of propria muscle. The morphologic characteristics of L-MALT lymphomas were commonly found in all our cases. L-MALT lymphoma showed a preferential involvement of epithelial structures. Follicular colonization was found in 18 cases of L-MALT lymphoma (75%) with an irregularly expanded and segmented FDC network pattern. The neoplastic cells usually were centrocyte-like cells and/or monocytoid B-cells, and a small number of transformed blasts and plasmocytic differentiation was also found. The neoplastic cells expressed CD20 but lacked T-cell-associated antigens. Immunohistochemistry revealed that L-MALT lymphoma was negative for CD5, CD34, CD43, and sIgD. The bcl2 protein was identified in 18 cases of MALT lymphoma (75%). p53 protein reacted with L-MALT lymphomas showing intranuclear staining, and positive cells were less than 10% of the neoplastic cells except one case.

All twelve cases of L-MALT lymphoma examined in the present study showed IgH gene rearrangement and eight cases of them showed frequent somatic mutation of the rearranged V_H genes, with a much higher R/S mutation ratio in the CDRII than FRIII. (Tables 1, 2). The average mutation frequency was 10.9% (range: 6.1-18.4%), and the averages of R/S ratio of the CDR II and FRIII were 2.9 and 1.8, respectively. The V_H family used was found to be V_H3 in all eight cases. The mutation frequency of two cases in DLBL was 6.25% and 13.61%, respectively. The ongoing mutation (intraclonal variation) was observed in all three cases of L-MALT lymphomas (Table 3).

Table 2. Somatic Mutation Analysis of DNA in L-MALT Lymphoma

Average mutation frequency	10.9 %
Range	6.1 - 18.4
Replacement/silent	
CDRII	2.9
FRIII	1.4
VH family usage	
VH3	8/8 cases

Average mutation frequency: total number of somatic mutations / total number of bases.

Discussion

Sequence analysis of rearranged V_H genes has been used to determine whether B-cell lymphomas derive from pre-germinal center B-cells, germinal center B-cells or post-germinal center B-cells, and to asses the role of antigen stimulation in lymphomagenesis. Pre-germinal center B-cells usually exhibit unmutated V_H genes with a germline sequence. Antigen-activated proliferating germinal center B-cells show mutated V_H genes and intraclonal variations by ongoing mutations. Post-germinal center memory B-cells show mutated V_H genes with a higher ratio of R/S mutation in the CDRs in comparison with FRs, but lack ongoing mutations. Ongoing mutation occurs in germinal centers but not in marginal zones. From the date of somatic mutation analysis of B-cell lymphomas, L-MALT lymphoma is considered to originate from post-germinal center B-cells (memory B-cells) that have undergone antigen selections. However, recent several reports have showed that ongoing mutations as indicated by intraclonal variations of V_H gene sequences are found in L-MALT lymphoma. Ongoing mutations are known to exist in follicular lymphoma and provide the genetic evidence that antigen stimulation plays an important role in clonal expansion of this lymphoma. In the present study, L-MALT lymphomas showed mutated V_H gene with a high ratio of R/S mutations and ongoing mutations. These data including ours suggest that antigen-driven-high-affinity somatic mutations may play an important role in the clonal expansion of L-MALT lymphomas. The ongoing mutation of L-MALT lymphoma may reflect reentry in to a germinal center pathway, and may occur in follicular colonization in which lymphoma cells infiltrate to reactive follicles.

Table 3. VH Sequences of L-MALT Lymphoma Clones

Case No.	Germline Clone	VH Sequence																
									CDR II									
	V3-23	GCT	ATT	AGT	GGT	AGT	GGT	GGT	AGC	ACA	TAC	TAC	GCA	GAC	TTC	GTG	AAG	GGC
1	1-1*	T--	---	-C-	A--	-C-	--a	-CA	---	-Tt	--t	---	---	---	---	---	---	---
	1-2	T-c	---	-C-	A--	---	--a	--A	---	--t	--t	---	---	---	---	---	---	---
	1-3	T-c	---	-C-	A--	---	--a	--A	---	--t	--t	---	---	---	---	---	---	---
	1-4	T--	---	-C-	A--	-C-	--a	CCA	---	-Tt	--t	---	---	---	---	---	---	---
	1-5	T--	---	-C-	A--	-C-	--a	-CA	---	-Tt	--t	---	---	---	---	---	---	---
									FR III									
	V3-23	CGG	TTC	ACC	ATC	TCC	AGA	GAC	AAT	TCC	AAG	AAC	ACG	CTG	TAT	CTG	CAA	
	1-1*	---	---	---	---	---	---	---	--A	G--	---	--t	--a	---	---	G--	G--	
	1-2	---	---	---	---	---	---	---	G-A	G--	---	--A	--a	--a	---	G--	G--	
	1-3	---	---	---	---	---	---	---	G-A	G--	-C-	--A	--a	--a	---	G--	G--	
	1-4	---	---	---	---	---	---	---	--A	G--	---	--t	--a	---	---	G--	G--	
	1-5	---	---	---	---	---	---	---	--A	G--	---	---t	--a	---	---	G--	G--	
	V3-23	ATG	AAC	AGC	CTG	AGA	GCC	GAG	GAC	ACG	GCC	GTA	TAT	TAC	TGT	GCG	AAA	
	1-1*	---	---	---	--c	---	t--	---	---	---	---	--c	---	--t	---	---	---	
	1-2	---	---	---	--c	---	t--	---	---	---	---	--c	---	--t	---	---	-C-	
	1-3	---	---	---	--c	---	t--	---	---	---	---	--c	---	--t	---	---	-C-	
	1-4	---	---	---	--c	---	t--	---	---	---	---	--c	---	--t	---	---	---	
	1-5	---	-G-	---	--c	---	t--	---	---	---	---	--c	---	--t	---	---	---	
									CDR II									
4	VH3-11	AAC	ATA	AAG	CAA	GAT	GGA	AGT	GAG	AAA	TAC	TAT	GTG	GAC	TCT	GTG	AAG	GGC
	4-1*	--G	---	--C	-G-	---	---	C--	---	---	G--	---	A--	---	---	---	---	---
	4-2	--G	---	--C	-G-	---	---	C--	---	---	G--	---	A--	---	---	---	---	---
	4-3	--G	---	--C	-G-	---	---	C--	---	---	G--	---	A--	---	---	---	---	---
	4-4	--G	---	--C	-G-	---	---	C--	---	---	G--	---	A--	---	---	---	---	---
	4-5	--G	---	--C	-G-	---	---	C--	---	---	G--	---	A--	---	---	---	---	---
	4-6	--G	---	--C	-G-	---	---	C--	---	---	G--	---	A--	---	---	---	---	---
									FR III									
	VH3-11	CGA	TTC	ACC	ATC	TCC	AGA	GAC	AAC	GCC	AAG	AAC	TCA	CTG	TAT	CTG	CAA	
	4-1*	---	---	---	---	---	---	---	---	---	---	---	---	---	-T-	--c	---	
	4-2	---	---	---	---	---	---	---	---	---	---	---	---	---	-T-	--c	---	
	4-3	---	---	---	---	---	---	---	---	---	---	---	---	---	-T-	--c	---	
	4-4	---	---	---	---	---	---	---	---	---	---	---	---	--a	-T-	--c	---	
	4-5	---	---	---	---	---	---	---	---	---	---	---	---	---	-T-	--c	---	
	4-6	---	---	---	---	---	---	---	---	---	---	---	---	---	-T-	--c	---	
	VH3-11	ATG	AAC	AGC	CTG	AGA	GCC	GAG	GAC	ACG	GCT	GTG	TAT	TAC	TGT	GCG	AGA	
	4-1*	---	---	---	---	-A-	A--	---	---	G--	T--	--c	---	-T-	---	---	---	
	4-2	---	---	---	---	-A-	A--	---	---	G--	T--	--c	---	CT-	---	---	---	
	4-3	---	---	---	---	-A-	A--	---	---	G--	T-c	--c	---	-T-	---	---	---	
	4-4	---	---	---	---	-A-	A--	---	---	G--	T--	--c	---	-T-	---	---	---	
	4-5	---	---	---	---	-A-	A--	-G-	---	G--	T--	--c	---	-T-	---	---	---	
	4-6	---	---	---	--c	-A-	A--	---	---	G--	T--	--c	---	-T-	---	---	---	
									CDR II									
7	VH3-11	AAC	ATA	AAG	CAA	GAT	GGA	AGT	GAG	AAA	TAC	TAT	GTG	GAC	TCT	GTG	AAG	GGC
	7-1*	---	---	---	---	---	---	---	---	C--	A-t	---	---	---	---	---	-G-	---
	7-2	---	---	---	G--	---	---	---	---	C--	---	-T-	---	---	---	---	-G-	---
	7-3	---	---	---	G--	---	---	---	---	C--	---	-T-	---	---	---	---	-G-	---
	7-4	---	---	---	---	---	---	---	---	C--	A-t	---	---	---	---	---	-G-	---
	7-5	---	---	---	G--	---	---	---	---	C--	-G-	-T-	---	---	---	---	-G-	---
	7-6	---	---	---	G--	---	---	---	-C-	C--	---	-T-	---	---	---	---	-G-	---
	7-7	---	---	---	G--	---	---	---	---	C--	---	-T-	---	---	---	---	-G-	---
	7-8	---	---	---	G--	---	---	---	---	C--	---	-T-	---	---	---	---	-G-	---
									FR III									
	VH3-11	CGA	TTC	ACC	ATC	TCC	AGA	GAC	AAC	GCC	AAG	AAC	TCA	CTG	TAT	CTG	CAA	
	7-1*	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	7-2	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	7-3	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	7-4	---	---	---	---	---	---	--t	---	---	---	---	---	--a	---	---	---	
	7-5	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	7-6	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	7-7	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	7-8	---	---	---	---	---	---	---	---	---	---	---	---	--a	---	---	---	
	H3-11	ATG	AAC	AGC	CTG	AGA	GCC	GAG	GAC	ACG	GCT	GTG	TAT	TAC	TGT	GCG	AGA	
	7-1*	T--	---	---	---	---	---	---	---	---	--c	A-a	---	-T-	---	TTc	GCT	
	7-2	T--	---	---	---	---	---	---	---	---	--c	A-a	---	-TG	---	-Tt	GCT	
	7-3	T--	---	---	---	---	---	---	---	---	--c	A-a	---	-T-	---	TTc	GCT	
	7-4	T--	---	---	---	---	---	---	---	---	--c	A-a	---	-T-	---	TTc	GCT	
	7-5	T--	---	---	---	---	---	---	---	---	--c	A-a	---	-T-	---	TTc	GCT	
	7-6	T--	---	---	---	---	---	---	---	---	--c	A-a	---	-TG	---	-Tt	GCT	
	7-7	T--	---	-C-	---	---	---	---	---	---	--c	A-a	---	-T-	---	TTc	GCT	
	7-8	T-A	---	---	---	---	---	---	---	---	--c	A-a	---	-TG	---	-Tt	GCT	

*: Major monoclonal population in lymphoma clones