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CDR3 Sequences of MALT Lymphoma Show Homology with Those of Autoreactive B-Cell Lines

Norio Yumoto,[1, 3] Katsushi Kurosu,[1, 2] Masakazu Furukawa[1] and Atsuo Mikata[1]

[1]First Department of Pathology and [2]Department of Chest Medicine, School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba City, Chiba 260-8670

We have examined the CDR3 sequence and adjacent regions of immunoglobulin genes from B-cell lymphoma of mucosa-associated lymphoid tissue (MALT). Twenty-nine sequences (15 sequences from 13 low-grade MALT lymphomas, marginal zone B-cell lymphomas; 7 sequences from 6 high-grade MALT lymphomas; 7 sequences from 7 diffuse large cell lymphomas) were obtained after cloning of the polymerase chain reaction-amplified segments. In the low-grade MALT, high-grade MALT and diffuse large cell lymphomas, the mean length of the CDR3 region was 47.6±10.31 (range 21 to 60), 38.71±10.37 (range 27 to 57) and 40.86±3.34 (range 39 to 48) nucleotides, respectively. The length of the CDR3 region was significantly greater in the low-grade MALT lymphoma group than in the other two groups. CDR3 sequences in lymphoma cell clones of 14 cases showed 60 to 81% homology with autoantibody-associated lymphocyte clones including rheumatoid factor. The incidences of these autoantibody-associated lymphocyte clones were higher in the high-grade MALT (4/6) and diffuse large lymphomas (5/7) than in the low-grade MALT lymphoma (5/13). Cases with more than 70% homology at the nucleotide level were found to have 71 to 82% homology with autoantibodies at the protein level in the low-grade MALT lymphomas (2/13), and 67% homology in the high-grade MALT lymphomas (2/7). These results indicate that MALT lymphomas may be derived from the malignant transformation of autoreactive B-cells.

Key words: MALT lymphoma — Immunoglobulin heavy chain gene — CDR3 — Autoreactive B-cell

Mucosa-associated lymphoid tissue (MALT) lymphomas occur in extranodal organs such as the stomach, salivary gland, thyroid and lung.[1, 2] The organization of MALT is acquired as a result of chronic inflammation caused by factors such as *Helicobacter pylori* (*H. pylori*) infection, chronic sialadenitis of Sjögren syndrome, and Hashimoto's thyroiditis. MALT lymphomas are considered to arise from these backgrounds.[3–5] In comparison with equivalent nodal B-cell lymphomas, the clinical behavior of MALT lymphoma is one of slow dissemination and the prognosis is more favorable. The participation of autoantigen in MALT lymphomagenesis has been suggested. The immunoglobulin derived from gastric MALT lymphoma cells was shown to be specifically responsive to autotissues.[6, 7] Moreover, it was demonstrated that proliferation of low-grade gastric MALT lymphoma cells is indirectly stimulated by *H. pylori* via specific tumor-infiltrating T cells.[8, 9] Low-grade gastric MALT lymphoma regresses after the eradication of *H. pylori*.[10] Thus antigen stimulation may play a role in the pathogenesis and development of MALT lymphomas.[11] B-Cell lymphomas occurring in certain organs can be classified into low-grade and high-grade MALT lymphomas, and non-MALT high-grade lymphomas. However, it frequently becomes problematic whether a high-grade lymphoma is of MALT type or not, because the characteristic features of low-grade MALT lymphoma, such as the presence of reactive follicles and lymphoepithelial lesions, are readily lost with high-grade transformation. Therefore, analysis of the antigen binding sites of these lymphomas may give clues to the nature of the immune response.

Immunoglobulin heavy chain (*IgH*) gene has three hypervariable regions, so-called complementarity determining regions (CDR1, 2 and 3). CDR3 directly contacts the antigen and is the most variable region of the Ig molecule. Antigen specificity is highly dependent on the composition of CDR3. CDR3 changes its structure in accordance with the antigen. The nucleotide sequence of CDR3 is developmentally regulated, with its length showing a tendency to increase as the antigenic stimuli increase with the maturation of the individuals.[12, 13] The increase of diversity of *IgH* gene in response to various antigens is generated through the recombination of multiple VH, D and JH segments, increased use of the longer *DH* and *JH* gene segments, increased number of D-D fusions, and additions to the N region. Owing to this diversity, the CDR3 region is unique in each rearrangement. Analysis of the CDR3 sequence, therefore, is a useful procedure for studying the clonality of malignant cells and obtaining information about the binding antigen.

The aim of the present study was to determine the CDR3 nucleotide sequence of MALT lymphoma cell clones and to evaluate the properties of their antigen bind-

[3] To whom correspondence should be addressed.
E-mail: yumoto@med.m.chiba-u.ac.jp

ing sites, and, moreover, to understand the origin and the role of antigen in the clonal selection of the lymphoma cells on the basis of its primary structure.

MATERIALS AND METHODS

Pathologic samples Specimens were obtained from patients who were clinically diagnosed as having primary B-cell lymphoma and who underwent tumor resection. Transbronchial lung biopsy (TBLB) specimens from two patients with chronic bronchitis served as reactive controls, because bronchial mucosa is exposed to various antigens. A portion of the specimen was snap-frozen in liquid nitrogen for immunohistochemical analysis.

Histopathologic studies and immunohistochemical analysis Routinely processed formalin-fixed, paraffin-embedded samples were sectioned at 3 μm and stained with hematoxylin-eosin. Classification of non-Hodgkin's lymphomas was based on the MALToma[14, 15] and REAL classifications.[16] High-grade lymphomas were classified according to the criteria of Hsi *et al*.[17]

The immunological phenotype of each lymphoma was determined by immunohistochemical methods using the standard avidin-biotin complex technique, and the results were considered in conjunction with those of the histopathologic studies. The following antibodies were used: CD19 (B4, Coulter Immunology, Hialeah, FL), CD20 (B1, Coulter Immunology; L26, Dakopatts, Glostrup, Denmark), CD21 (B2, Coulter Immunology), CDw75 (LN-1, Nichirei, Tokyo), CD3 (Leu4, Becton Dickinson, Mountain View, CA), CD4 (Leu3a, Becton Dickinson), CD5 (Leu1, Becton Dickinson), CD8 (Leu2a, Becton Dickinson), CD43 (MT-1, Bio-Science, Emmenbrücke, Switzerland) and CD45RO (UCHL-1, Dakopatts).

DNA preparation DNA extraction and microdissection from surgically resected tissues were performed according to the previously described methods.[18] A part of the frozen specimen was also homogenized mechanically and centrifuged at 35,000 rpm for 18 h at 20°C by the guanidinium/cesium chloride method. Isolated genomic DNA was incubated for 2 h at 50°C with 200 μg/ml proteinase K and 0.5% sodium dodecyl sulfate, extracted with phenol/chloroform, and subsequently dialyzed.

Polymerase chain reaction (PCR) for *IgH* genes *IgH* gene from paraffin-embedded or frozen samples was amplified according to the two-step PCR method of Wan *et al*.[19] The primers used were: 5′ ACACGGC[C/T][G/C]TGTATTACTGT 3′ (Fr3A), 5′ TGAGGAGACGGTGACC 3′ (LJH), and 5′ GTGACCAGGGT[A/G/C/T]CCTTGGCCCCAG 3′ (VLJH). Fr3A is the oligomer for the third framework portion of the V region. LJH and VLJH are based on the consensus sequence from the J region. For the first step of amplification, 1 μl of extracted DNA was subjected to PCR amplification with 2.5 units of *Taq* polymerase and with the primers for Fr3A and LJH at a final concentration of 0.25 μM in 100 μl of standard buffer. Each PCR experiment contained a sample without the DNA template as a negative control, and a sample with DNA extracted from Burkitt lymphoma (lymph node) as a positive control in which *IgH* gene rearrangement had been detected by Southern blotting. Denaturing was carried out for 2 min at 94°C, annealing for 2 min at 60°C, and extension for 2 min at 72°C for 30 cycles. The second step of 20 cycles with Fr3A and VLJH was performed with 10 μl of a 1/1000 dilution of the first step PCR product as template. The PCR product (40 μl) was extracted with phenol/chloroform and precipitated with ethanol. The precipitate was then dissolved in 5 μl of Tris-EDTA (TE) buffer and electrophoresed on 2% agarose gel, and the gel was stained with ethidium bromide to visualize the DNA under a short-wavelength UV light. The expected size of the amplified products was about 100 bp.

Sequence analysis The PCR product was ligated to the PCR™ vector and the ligation mixture was transformed into One Shot™ competent cells by using a TA Cloning Kit (Invitrogen Corp., San Diego, CA). The subcloned DNAs were picked up at random and DNA was purified. Sequencing was performed using a Taq Dye Primer Cycle Sequencing Core Kit (Applied Biosystems, Foster City, CA).

The character of the CDR3 region was identified by comparison of the sequences with those in the GenBank and EMBL databases using the FASTA program. For *D* genes, minimal homology consisted of six matches in a row or seven matches interrupted by one mismatch.[20] Homology search to published B-cell clones was done both at nucleotide and protein levels.

Statistical analysis Statistical analysis of differences between low-grade MALT and high-grade MALT or diffuse large lymphoma groups was performed using the one-tailed Student's *t* test.

RESULTS

Histopathology of primary B-cell lymphoma in extranodal organs All 26 cases were diagnosed histopathologically as marginal zone B-cell lymphoma or low-grade MALT lymphoma (13 cases; three from the stomach, two from the thyroid, eight from the lung), diffuse large B-cell lymphoma with evidence of high-grade MALT lymphoma, that is, large cell lymphoma with lymphoepithelial lesions but without a low-grade component (6 cases; three from the stomach, three from the thyroid), and diffuse large B-cell lymphoma without evidence of MALT lymphoma (7 cases; three from the stomach, three from the thyroid, one from the lung) (Table I). The findings were confirmed by the examination of 3 to 5 blocks in each case.

Detection of *IgH* gene rearrangement and sequence analysis of CDR3 region Clonal *IgH* gene rearrangements in paraffin or frozen-embedded specimens evaluated

able I. Dominant Clone Sequence of CDR3 Region in B-Cell Lymphoma Derived from MALT-related Organs

Case	V | ←	(N) CDR3	D	(N)	J → |		Ratio	CDR3 length (N-D-N length)
Low-grade MALT: marginal zone B-cell lymphoma								
2	TGT GCGAGACA	cacggtcc	(D1-26) ATgGTGGGA	aatccgtcgcaaacttagtt	GACTAC	TGG JH4	5/10	51 (37)
4	TGT GCGAGAG	ctgtccgtcc	(DXP'1) GTTCGGGGAGTTAaTATA	tacccacttt	GACTCC	TGG JH5	5/9	51 (38)
9	TGT GCGAGAGA	cggcaca	(DXP'1) TATGGTTCaGGGAGTTATTATAAC	cggctg	TACGGTATGGACGTC	TGG JH6	6/10	60 (37)
17	TGT GCGAG		(DXP'1) GGGGAG	g	TTCGACCCC	TGG JH5	2/9	21 (7)
19-1	TGT GCGAGAGA		(D2) GATATTGTAGTGGTGGTAGCTGCTA	aaactag	TGGTTCGACCCC	TGG JH5	2/7	52 (32)
-2	TGT GCGAGAGA	tgaga	(D3) AGCAcATcGcGGTGC (D1)CTGGTG	acttcggat	ACTACTTTaAtTAC	TGG JH4	2/7	57 (35)
21	TGT GCGAG	gggccaactcg	(DK1) GTGGCTACGATT (D1or2or3)TGGTGG	gc	TGGTTCGACcCC	TGG JH5	5/10	48 (31)
22	TGT GCGAGAG	tggccaggcctgac	(D21/9) ATAGTAGTaGTTgT	gtat	TTCGACCCC	TGG JH5	9/10	48 (32)
23	TGT GCGG	gagccc	(DK1) TcGTGGCTA	tatc	TACTACTACGGTATGGACGTC	TGG JH6	6/8	44 (19)
24-1	TGT GCGAGAG	ctcgccggcccgacttg	(DM1) ATAACTGGAAC (DXP'1)TTCGGG	ctgc	TTCgAGCAC	TGG JH1	4/9	54 (37)
-2	TGT GCGAG	tata	(DXP'1:inv) AATCTTAGTGGGGGGCT	c	TACTACTACGGTATGGACGTC	TGG JH6	4/9	48 (22)
25	TGT GCAAG	cg	(D4-14) ACGGTGACTAC (DK1)GTGGATtTtGaG		TAC	TGG JH4	7/9	33 (25)
26	TGT GCGA	taggggg	(DA1or4)TGACTACAGTAACTAC	aagagggtc	TAC	TGG JH4	7/9	39 (32)
27	TGT GCGAAAGA	taggtatgaggg	(DN1) TAGCAGtgGCTGG (D1)TACTGGaGGgG	gtac	TACTTTGACTAC	TGG JH4	7/8	60 (40)
28	TGT GCGAAAG	tccgacc	(DXP'1) CGGGGAcTTc (D3)GgTTGCTATTCC		GGTATGGACGTC	TGG JH6	7/10	48 (29)
High-grade MALT								
3	TGT GCGAGAG		(DXP'1) TgCGtGGGAGTT	cggga	TAC	TGG JH4	10/10	27 (17)
6-1	TGT GCAAAAGATA		(DXP'1) TgTGGTTCaGGGAGTTATTAT	gggtc	TACTACTACGGTATGGACGTC	TGG JH6	6/10	57 (26)
-2	TGT GCGA	caagttcaacga	(D4-23) TACGGTGGcA	ctcattgc	TTTGACAAC	TGG JH4	3/10	43 (30)
7	TGT GCGAGAGG	ttcaacga	(D4-17) ACGGTGACTAC	gttgcc	TTTGACTAC	TGG JH4	5/9	42 (25)
11	TGT GCGAGAGA		(D21-10) TGGGGG	g	TGGTTCGACCCC	TGG JH5	4/5	27 (7)
12	TGT GCGAGAGA	c	(DHQ52) AtATGGGGAT (DA1or4)CgACAGT	c	TTTGACTAC	TGG JH4	3/5	36 (19)
18	TGT GCGAGAGA	tgctctg	(DK1) GGATATAGTaGC	agtggcggg	AAC	TGG JH4	8/10	39 (28)
Diffuse large cell								
1	TGT GCGAGAGG	gga	(D6-19) AGCAGTGGCTGGTA	gc	TACTTTGACTAC	TGG JH4	10/10	39 (19)
5	TGT GCGAGAA		(D3-16) GGGGAaTgtTtG (DK5)AaAGTcTCTAC	ctg	GACGTC	TGG JH6	10/10	39 (26)
8	TGT GCGAG	catagaca	(DK4)GTGGATACAGCTATGGTTA	aaggc	TAC	TGG JH4	7/8	40 (32)
13	TGT GCGAGAG	cggcgag	(DXP'1) TTACTATGGTTCGGGG	gca	AACTGGTTCGACCCC	TGG JH5	4/7	48 (26)
14	TGT GCGAGAGA		(DXP1) TGaCGATAT (D1C)AGctCAATcTCC	c	TTTGACTAC	TGG JH4	9/9	39 (22)
16	TGT GCGAGAG	ctca	(DN1) GTATAGCAGCAGCTGGTAC		TACTTTGACTAC	TGG JH4	10/10	42 (23)
29	TGT GCGAGAA		(DHQ52) GGGGA (DXP4)ATgTTTGaA	agtctctacctg	GACGTC	TGG JH6	3/6	39 (26)

Parentheses indicate homology with D segments. Nucleotides of the N region and mutation in gene segments are indicated in lower-case characters. Case numbers 1–9 show gastric lymphoma, case numbers 11–19 thyroid lymphoma, and case numbers 21–29 pulmonary lymphoma.

Table II. CDR3 Region Homology Sequence of Tumor Clone Rearrangements Compared to Reported Rearrangements

Case	CDR3 Region	Source	Homology(%)	
			Nucleotide	Amino acid
Low-grade MALT: marginal zone B-cell lymphoma				
2	GCGAGACACACGGTCCATGGTGGGAAATCCGTCGCAAACTTAGTTGACTAC			
	GCGAGACATAAGGGGAATAGTGG---CTACG----AACCGT--TTGACTAC	Clone L12198[e]	63%	
		(CD5+ EBV-transformed B cell)	(67%)*	
4	GCGAGAGCTGTCCGTCCGTTCGGGGAGTTAATAT-ATACCCA----CTTTGACTCC			
	GCGAGAG-TG-CGGGGGGTTCGGGGAGTTATTATAAGACAGAGGGCTTTTGATATC	Clone U00493[b]	66%	
		(natural autoantibody)	(90%)*	
	A R D G T Y G S G S Y Y N R L Y G M D V			
9	GCGAGAGAC---GGCACATATGGTTCAGGGAGTTATTATAAC--------CGGCTG-TACGGTATGGACGTC			71%
	GCGAGAGATTCAGGGTACTATGATTCGGGGAGTTATTATAACGTTATCCACTACTACTACGATATGGACGTC	Clone X75023[c]	70%	
	A R D S G T Y D S G S Y Y N V I H Y Y Y D M D V	(autoantibody of Hashimoto's thyroiditis)	(88%)*	
	A R G R F D P			
17	GCGAGGGGGAGGTTCGACCCC			71%
	GCAAGGGAGTGGTTCGACCCC	Clone Z14208[d]	86%	
	A R E W F D P	(fetal liver)	(67%)*	
	E R D I V V V V A A K T S G S T P			
19-1	GAGAGAGATATTGTAGTGGTGGTAGCTGCTAAAACTAGTGGTTCGACCCC			82%
	GAGAGGGATATTGTAGTAGTACCAGCTGCTCGAACT---GGTTCGACCCC	Clone M65093[e]	80%	
	E R D I V V V P A A R T G S T P	(G6-reactive tonsillar lymphocyte)	(80%)*	
19-2	GCGAGA--GATGAGAAGCACATCGCGGTGCCTGGTGACT----TC--GGATACTACTTTAATTAC			
	GCGAGAGTGACGGGCTCCACATTTTGGAG--TGGTTATTATACTCGGGGTTACTACTTTGACTAC	Clone X54445[f]	64%	
		(rheumatoid factor mAb)	(57%)*	
	A G A L V A I S T T T V W T S			
23	GCGGGAGCCCTCGTGGCTATATC---TACTACTACGGTATGGACGTC			60%
	CAAGATTCC---GTGGCTACGACTACTACTACTACGGTATGGACGTC	Clone N1[g]	71%	
	Q D S V A T T T T T V W T S	(Cord blood)	(44%)*	
	A S I N L S G G L Y Y Y G M D V			
24	GCGAGTATAAATCTTAG-TGGGGGGCTCTACTACTACGGTATGGACGTC			56%
	GCAAG-ATGGTCAGCAGCTGGTCCGGGTTACTACTACGGTATGGACGTC	Clone 6.7[d]	67%	
	A R W S A A G P G Y Y Y G M D V	(fetal liver)	(35%)*	
25	GC--AAGCGA---CGGTGACTACGTG-GATTTTGAGTAC			
	GCGAGAGGGAGTACGGTGACTAAGGGAGATTTTGACTAC	Clone L17[e]	74%	
		(G6-reactive lymphocyte)	(85%)*	
26	GCGATAGGGGGTGACTACAGTAACTACAAGAGGGTCT----AC			
	GCGAGAGGGAGT----ACGGTGACTACGGGAGATTTTGACTAC	Clone L37[e]	60%	
		(G6-reactive lymphocyte)	(63%)*	
27	GCGAAAGATAGGTATGAGGGTAGCAGTGGCTGGTACTGGAGGGGGTACTACTTTGACTAC			
	GCGAGAGGTA-GTA-GCGTATTACTATGGTTCG-GGGAGTTATTGTACTACTTTGACTAC	Clone 4.58[d]	61%	
		(fetal liver)	(33%)*	
High-grade MALT				
	A R V R G S S G T			
3	GCGAGAGTGCGTGGGAGTTCGGGATAC			67%
	GCGAGAGTGCG-GGGGGTTCGGGG-AG	Clone U00493[b]	81%	
	A R V R G V R G V	(natural autoantibody)	(83%)*	
6-1	GCAAAAGATATG--TGGTTCAGGGAGTTATTA--TGGGTCTACTACTACGGTATGGACGTC			
	GCGAGAGAGGTGGTTGGTTGTAGTAG-TACCAGCTGCTACTACTACTACGGTATGGACGTC	Clone M65094[e]	70%	
		(G6-reactive lymphocyte)	(56%)*	
-2	GCGACAAGTTCAACGATA-CGGTGGCACTCATTGCTTTGACAAC			
	GCGAAAGGCTCTATGATAGTAGTGGCA--AGATACTTTGACTAC	Clone Z18830[h]	66%	
		(anti-self antibody)	(73%)*	
7	GCGAGAGGTTCAACGAAC-GGTGACTAC-GT--TGCCTTTGACTAC			
	GCGAGA-GAGGGACGATCTTCTGACTACAGTAATCCCTTTGACTAC	Clone L14455[i]	72%	
		(rheumatoid B-1a cell high affinity RF)	(67%)*	

Table II. (Continued)

Case	CDR3 Region	Source	Homology(%) Nucleotide	Homology(%) Amino acid
	A R D G G W F D P			
11	GCGAGAGATGGGGGGTGGTTCGACCCC			
	::::::: : :::::::::::::			67%
	GCGAGAGGGGACAACTGGTTCGACCCC	Clone M65096[e]	74%	
	A R G D N W F D P	(G6-reactive tonsillar lymphocyte)	(17%)*	
	A R D I W G S T V F D Y			
12	GCGAGAGACATCTG-GGGATCGACAG--TCTTTGACTAC			
	:::::::::::: : :::: : : : :::::::::::			62%
	GCGAGAGACCTCCGAGGGAGCAAGGGCTACTTTGACTAC	Clone X81724[j]	74%	
	A R D L R G S K G Y F D Y	(pre B cell from adult bone marrow)	(55%)*	
	A R D A L G Y S S S G G D			
18	GCGAGAGATGCTCTGGGATATAGTAGCAGTGGCGGGAAC			
	:::::: :: :::::::::::: ::::: : :: :			67%
	GCGAGACAT---CTGGGATATAGCAGCAG---CTGGTA-	Clone X84339[k]	72%	
	A R H L G Y S S S W	(IgG+ CD5+ chronic lymphocytic leukemia B cell)	(92%)*	
Diffuse large				
1	GCGAGAGGGGAAGCAGTGGCTGGTAGCTACTTTGACTAC			
	:::: :: : ::::: : :: ::::::::::::			
	GCGAAAGATCATATAGTGGGAGCTACTTACTTTGACTAC	Clone Z14179[d]	69%	
		(fetal liver)	(57%)*	
5	GCGAGAAGGGGAATGTTTGAAAGT--CTCTACCTGGACGTC			
	::::::::: ::::: : ::: :: ::: : :			
	GCGAGAAGGATAATGTATTGTAGTGGTGGTAGCTG--CTAC	Clone Z49135[l]	61%	
		(lupus, anti-DNA antibody)	(52%)*	
8	GCGAGCATAGACAGT--GGATACAGCTATGGTTAA------AGGCTAC			
	::::: :: : :::::::::::::::::: : ::::			
	GCGAGAGAAGGGTATCCGGATACAGCTATGGTTAACCCCTTTGACTAC	Clone L19288[m]	65%	
		(rheumatoid factor)	(86%)*	
	A R A A S Y Y G S G A N W F D P			
13	GCGAGAGCGGCGAGT----TACTATGGTTCGGGG-GCAA--ACTGGTTCGACCCC			
	:::: :: ::: ::: ::::::::::::::: : : ::::::::::::::			56%
	GCGAAAG-GGCAAGTGTTGTACTATGGTTCGGGGAGTTATCACTGGTTCGACCCC	Clone M17746[e]	78%	
	A K G Q V L Y Y G S G S Y H W F D P	(anti-DNA immunoglobulin)	(80%)*	
14	GCGAGAGA-TGACGATAT----AGCTCAAT-CTCCCTTTGACTAC			
	:::::::: :::::: : : :: : ::::::::::::::			
	GCGAGAGAGGGACGATCTTCTGACTACAGTAATCCCTTTGACTAC	Clone L14455[j]	71%	
		(rheumatoid B-1a CD5+ B cell)	(54%)*	
	A R A Q Y S S S W Y Y F D Y			
16	GCGAGAGCTCAGTATAGCAGCAGCTGGTACTACTTTGACTAC			
	:::::: ::: ::::::::: ::::::: ::::::: ::			57%
	GCGAGACCTCGAGATAGCAGCACCTGGTACCCCTTTGACGAC	Clone U00528[b]	81%	
	A R P R D S S T W Y P F D D	(natural autoantibody)	(84%)*	

a) From Van der Heijden *et al*., 1993. Unpublished. *b*) From Huang and Stollar,[21] 1993. *c*) From McIntosh *et al*.,[22] 1994. *d*) From Cuisinier *et al*.,[23] 1993. *e*) From Kipps and Duffy,[24] 1991. *f*) From Harindranath *et al*.,[25] 1991. *g*) From Van Es *et al*.,[26] 1992. *h*) From Griffiths *et al*.,[27] 1993. *i*) From Mantovani *et al*.,[28] 1993. *j*) From Milili *et al*.,[29] 1996. *k*) From Hashimoto *et al*.,[30] 1995. *l*) From Spellerberg *et al*.,[31] 1995. *m*) From Youngblood *et al*.,[32] 1994. *n*) From Dersimonian *et al*.,[33] 1987.
∗ shows the degree of homology within the D segment. Underlines show the D segment. Deduced amino acid sequence is given in cases with more than 55% homology.

by PCR showed a sharp band in all cases (data not shown). The sequencing of the CDR3 regions revealed a single dominant clone in all cases except cases 6, 19 and 24, in each of which two major sequences were identified (Table I). The D region of the sequences in all cases had significant homology to one of the presently known germline *D* genes. Cases 3, 5, 6 (clone 1), 11, 14, 16, 17, 19 (clone 1), 25, 28 and 29 showed the absence of the N segment at either the VH-DH or DH-JH junction. DH-DH fusions were observed in cases 5, 12, 14, 19 (clone 2), 21, 24 (clone 1), 25, 27, 28 and 29, and were especially frequent in low-grade pulmonary MALT lymphomas. In 8 (cases 3, 4, 6, 9, 13, 17, 24 and 28) of the 26 cases the DH segment sequence shared a portion of the germline DXP′1 segment and, interestingly, 7 of these 8 cases were low- or high-grade MALT lymphomas. The most frequently utilized JH segments in tumor clones were JH4 (52%), JH6 (26%) and JH5 (22%).

Length of the CDR3 region In low-grade MALT, high-grade MALT and diffuse large cell lymphomas, the mean length of the CDR3 region was 47.6±10.31 (range 21 to 60), 38.71±10.37 (range 27 to 57) and 40.86±3.34 (range

39 to 48) nucleotides, respectively. The mean length of the CDR3 in 40 clones of the reactive control was 50.72±8.98 (range 36 to 75). The length of the CDR3 region was significantly greater in the low-grade MALT lymphoma group than in the other two groups ($P<0.05$), and was significantly greater in the reactive control than in the other two groups ($P<0.01$). The mean length of N-D-N was 30.2±8.82 (range 7 to 40), 21.71±7.99 (range 7 to 30) and 24.86±4.10 (range 19 to 32) nucleotides, respectively. That in the reactive control was 35.7±6.63 (range 22 to 50). The length of N-D-N was significantly greater in the low-grade MALT lymphoma group than in the high-grade MALT group ($P<0.05$), but was significantly smaller than in the reactive controls from two patients with chronic bronchitis ($P<0.01$).

Homology search of CDR3 region compared to published rearrangements Homology search indicated that the lymphoma cell clones of 14 cases (5 of 13 low-grade MALT lymphomas; cases 4, 9, 19-1, 19-2, 25 and 26; 4 of 6 high-grade MALT lymphomas; cases 3, 6-1, 6-2, 7 and 11; 5 of 7 diffuse large cell lymphomas; cases 5, 8, 13, 14 and 16) exhibited 60 to 81% homology with autoantibody-associated lymphocyte clones (Table II).[21, 22, 24, 25, 27, 28, 31–33] The incidence of these autoantibody-associated lymphocyte clones was higher in the high-grade MALT and diffuse large lymphomas than in low-grade MALT lymphoma. Eight of these 14 cases were rheumatoid factor-associated lymphocyte clones including G6. In the lymphoma cell clones of cases 1, 17, 23, 24 and 27, there was 61 to 86% homology with previously reported clones derived from fetal liver or cord blood.[23, 26] Case 12 showed 74% homology with a pre B-cell clone.[29] Case 18 had 72% homology with an IgG^+, $CD5^+$, CLL B-cell clone.[30] At the protein level, 2 cases of low-grade MALT lymphomas showed 71 and 82% homology with CDR3 of autoreactive B-cell clones, and 2 high-grade MALT lymphomas showed 67% homology. However in diffuse large cell lymphomas, homology at the protein level was 56 and 57% (Table II).

DISCUSSION

MALT lymphomas often occur in association with autoimmune disorders, and are believed to produce autoreactive immunoglobulin, as shown in gastric MALT lymphoma. This specific nature of MALT lymphoma cells must be reflected in the properties of CDR3 in *IgH* gene. So far, VH analyses suggest that low-grade MALT lymphomas are derived from post-germinal center B cells,[34] as well as a large proportion of diffuse large cell lymphomas. Therefore, precise analysis of the CDR3 sequence derived from each lymphoma clone may shed some light on the relation of MALT lymphomas and "*de novo*" large B-cell lymphomas occurring in mucosal organs.

As the same nucleotide sequence of the IgH CDR3 region is shared by only one in 20,000 circulating B cells,[35] it is a useful marker for determining the clonality of the malignant clone in lymphomas. The sequencing of the CDR3 regions revealed a single dominant clone in 23 cases, and two major sequences in the other 3 cases (6, 19 and 24) (Table I). In cases 6, 19 and 24, either these two major sequences were derived from different cells, or the *IgH* gene of both alleles from the same cell was expressed. Concerning the latter possibility, there is recent evidence that IgH allelic exclusion is not absolute and that a subset of B-CLL expresses more than one functional Ig heavy chain.[36]

The D region of the sequences in all cases had significant homology with one of the presently known germline *D* genes. Among them, eight cases used the DXP′1 gene. The DXP group gene is reported to be the most frequently used D segment both in fetal liver lymphocytes[13] and in adult peripheral B cells.[35] DHQ52, which is preferentially used at the fetal stage,[37] was observed in one case each of the high-grade MALT and diffuse large cell lymphoma groups, but in none of the low-grade MALT group. Ten of the 26 cases used two germline *D* genes, possibly by D-D fusion. This D-D fusion was frequently observed in low-grade MALT, especially in pulmonary MALT lymphoma (5 of the 8 cases). Immunoglobulin D-D fusion seemed to be central to the generation of antibody diversity. Although lymphocytes in both organs are likewise exposed to various antigens, the present study indicates that the diversity of *IgH* gene is higher in the lung than in the stomach.

The nucleotide sequence of CDR3 is developmentally regulated. The N sequence is found in 68% of fetal B cells, 86% of neonatal B cells, and 91 to 100% of mature adult B cells.[12] The average length of the N-D-N region in the fetal liver lymphocytes, neonatal and adult peripheral B cells is 22 (range 12 to 45), 24 (range 9 to 56) and 31 (range 13 to 54) nucleotides, respectively, showing a tendency to increase with age.[13] Therefore, the presence of the N sequence and the length of CDR3 can act as indicators of immunological selections on B-cell maturation. As the present study shows, the length of the CDR3 region was significantly greater in the low-grade MALT lymphoma group than in the other two groups. In the low-grade MALT, high-grade MALT and diffuse large cell lymphomas, the mean length of N-D-N was 30.2±8.84 (range 7 to 40), 21.71±7.99 (range 7 to 30) and 24.86±4.10 (range 19 to 32) nucleotides, respectively. Together with the usage of DHQ52, these findings indicate that the properties of CDR3 in high-grade MALT lymphoma resemble those in diffuse large cell lymphoma rather than in low-grade MALT lymphoma. The maturation stage of low-grade MALT lymphoma is similar to ontogenetic mature B cells. Diffuse large cell lymphoma

does not show a distinct difference from high-grade MALT lymphoma and therefore is at an ontogenetic early maturation stage.

The differentiating B cell undergoes positive selection directed by the presence of surface IgH with low affinity for self-antigen. Thus, B cells that express self-reactive receptors mature into functional B cells before entering the germinal center. In the germinal center of the lymphoid follicle, *IgH* gene causes somatic mutation for an adaptive response to foreign antigen. The B cells whose antigen receptors are bound to this antigen selectively proliferate under the influence of helper T cells.[12, 38] Positive ligand selection by low-affinity interaction with self antigen is also considered to shape the primary repertoire, which includes natural autoantibodies. Thus, the formation of some repertoires is performed by antigen-driven selection. Accumulating evidence has indicated that usage of *V* gene segment is preferential in some repertoires.[39–43] Although VH segment usage may be influenced by the ability of VH segments to facilitate certain ligand interactions, CDR3 is likely to play a major role in specific ligand interaction and repertoire development.[44] The specificity of antibody to antigen is highly dependent on the composition of CDR3. Therefore, similarities in the nucleotide sequence of the CDR3 region may be useful for detecting the origin of lymphoma cells and for determining whether a specific antigen participates in the clonal proliferation of lymphoma cells. Accordingly, we investigated the particular properties of the CDR3 region derived from tumor cell clones. The lymphoma cell clones of 14 cases showed 60 to 81% homology with autoantibody-associated lymphocyte clones, which include natural autoantibodies, anti-DNA antibodies, Hashimoto thyroiditis-related antibody and rheumatoid factor.[21, 22, 24, 25, 27, 28, 31–33] The incidences of these autoantibody-associated lymphocyte clones were higher in the high-grade MALT (4/6, 67%) and diffuse large cell lymphomas (5/7, 71%) than in low-grade MALT lymphoma (5/13, 39%). We also investigated the sequences of VH segment in 9 gastric lymphomas. Seven cases (2 from 3 low-grade MALT lymphomas; 2 from 3 high-grade MALT lymphomas; 3 from 3 diffuse large lymphomas) showed 85 to 98.3% homology with those of autoantibodies (data not shown). There are 4 cases in which the deduced amino acid sequences of CDR3 in low- and high-grade MALT lymphomas show a high degree (67 to 82%) of homology with natural autoantibody and rheumatoid factor. Some authors have suggested that the specificity of autoantibodies can be influenced not only by the structure of heavy chain CDR3, but also by mutation of that of CDR2 and light chain CDR1, and furthermore, reactivity of autoantibodies can be changed by several amino acid substitutions in IgH CDR3.[45,46] It seems highly significant that our lymphoma cells showed homology with CDR3 of a known autoantibody clone. Therefore, our findings suggest that the cells of MALT lymphoma are autoantigen-related, and that some diffuse large cell lymphomas may also be derived from selected autoreactive B-cell clones, even in the absence of histological evidence of MALT lymphoma.

Concerning the histogenesis, low-grade MALT lymphomas are considered to arise from acquired lymphoid tissues formed in response to insults such as *H. pylori* infection. Moreover, *H. pylori*-associated MALT lymphomas have been shown to result from both polyclonal and monoclonal B-cell proliferations.[47] High-grade MALT lymphoma has been considered to arise from transformed low-grade MALT lymphoma cells. Indeed, the findings that the range of length of CDR3 showed a narrowing tendency with high-grade malignant transformation and that the incidence of autoantibody-associated lymphocyte clones was higher in high-grade lymphomas than in low-grade MALT lymphoma support the hypothesis that high-grade lymphoma B cells are derived from cells highly selected by autoantigen, leading to a monoclonal proliferation of B cells, within the milieu of polyclonal MALT lymphoma cells. Thus, high-grade lymphoma cells may have acquired immaturity of differentiation and the ability to destroy auto-tissues.

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