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ORIGINAL ARTICLE

**Toru Hiyama · Ken Haruma · Yasuhiko Kitadai
Masaki Miyamoto · Sinji Tanaka
Masaharu Yoshihara · Koji Sumii
Fumio Shimamoto · Goro Kajiyama**

B-Cell monoclonality in *Helicobacter pylori*-associated chronic atrophic gastritis

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Abstract B-cell monoclonality has been reported not only in gastric lymphoma, but also in 1.3–21% of *Helicobacter pylori*-associated chronic gastritis (*Hp*-CG) cases. The aim of this study was to determine the significance of B-cell monoclonality in *Hp*-CG. We examined 134 gastric biopsy specimens from 99 patients with *Hp*-CG. The density of *Hp*, polymorphonuclear neutrophil activity, chronic inflammation, glandular atrophy, and intestinal metaplasia (IM) were scored according to the updated Sydney System. B-cell monoclonality was analyzed for immunoglobulin heavy chain gene rearrangement using polymerase chain reaction amplification. B-cell monoclonality was detected in 6% of informative samples. B-cell monoclonality was found in 18% of the samples from *Hp*-CG patients with marked glandular atrophy but in none of the samples from *Hp*-CG patients with none to moderate glandular atrophy. Monoclonality was also detected in 20% of the samples from *Hp*-CG patients with marked IM, in 11% of the samples from *Hp*-CG patients with moderate IM, and in none of the samples from *Hp*-CG patients without IM. Therefore, B-cell monoclonality was significantly more frequent in *Hp*-CG patients with marked glandular atrophy than in *Hp*-CG patients with none to moderate atrophy. It was also more significantly frequent in *Hp*-CG patients with moderate or marked IM than in *Hp*-CG patients without IM ($P<0.05$). Of 35 *Hp*-CG patients, 26 (74%) had identical B-cell populations in the antrum and the corpus, and all were polyclonal. The remaining nine (26%) *Hp*-CG patients had B-cell populations that differed in the antrum and the corpus. Four of the nine (44%) showed monoclonal B-cell populations in at least one gastric biopsy specimen. There were no patients with monoclonal B-cell populations in both the antrum and the corpus. These data suggest that glandular atrophy and IM in gastric biopsy specimens may be markers for gastric mucosa-associated lymphoid tissue (MALT) lymphomagenesis and that multiple gastric biopsy specimens from both the antrum and the corpus may be needed to assess the risk of gastric MALT lymphoma.

Keywords Chronic gastritis · B-cell monoclonality · Immunoglobulin heavy chain · *Helicobacter pylori*

T. Hiyama (✉) · K. Haruma · M. Miyamoto · K. Sumii
G. Kajiyama
First Department of Internal Medicine,
Hiroshima University School of Medicine,
1-2-3, Kasusmi, Minami-ku, Hiroshima, 734-8551, Japan
e-mail: t-hiyama@mcai.med.hiroshima-u.ac.jp
Tel.: +81-82-2575193, Fax: +81-82-2575194

Y. Kitadai · S. Tanaka
Department of Endoscopy, Hiroshima University Hospital,
Hiroshima, Japan

M. Yoshihara
Health Service Center, Hiroshima University,
Higashihiroshima, Japan

F. Shimamoto
Department of Anatomical Pathology,
Hiroshima University Hospital, Hiroshima, Japan

Introduction

Helicobacter pylori (*Hp*) infects nearly one-half of the world's population [15]. *Hp* likely plays a causative role not only in chronic gastritis, peptic ulcer disease, and gastric carcinoma but also in gastric lymphoma, especially of the mucosa-associated lymphoid tissue (MALT) type [2, 8, 13, 14, 18, 19, 24, 28]. Gastric MALT lymphomas comprise a few percent of all gastric malignancies [12, 17, 30]. Interestingly, 70–80% of gastric low-grade MALT lymphomas regress in response to eradication of *Hp* [1, 10, 27], and treatment of these lymphomas with antibiotics directed against *Hp* is advocated.

During B-cell differentiation, the genes coding for immunoglobulin heavy chain (IgH) and light chains rearrange to determine the structure of the Ig produced by the cell. This occurs through processes of deletion and splicing of DNA segments from the variable, diversity,

joining, and constant regions of the gene to form unique coding sequences [4, 7]. The rearranged Ig gene has been shown to contain three hypervariable regions surrounded by framework regions, which are well conserved [25]. These regions are called complementarity determining regions (CDR) I, II, and III [29]. The DNA sequence of CDR III is unique to each IgH and useful for identifying the clonality of B-cell lineage. Detection of IgH gene rearrangement is thought to provide information for distinguishing gastric lymphomas from reactive diseases [20]. However, monoclonal B-cell populations have been detected by several investigators not only in gastric lymphomas but also in *Hp*-associated chronic gastritis (*Hp*-CG) [3, 5, 6, 11, 18, 22]. The reported frequencies of clonal B-cell populations in *Hp*-CG range from 1.3% to 21%. In addition, Nakamura et al. reported that B-cell monoclonality precedes the development of gastric MALT lymphoma in *Hp*-CG patients [18]. To clarify the significance of B-cell monoclonality in *Hp*-CG, we assessed monoclonality for IgH gene rearrangement using polymerase chain reaction (PCR) amplification and compared the results with histological assessment of *Hp*-CG based on the updated Sydney System [6].

Materials and methods

Gastric biopsy specimens (77 antrum and 57 corpus) were obtained from 99 patients who tested positive for serum *Hp*-IgG antibody at the Hiroshima University Hospital or its affiliated hospitals in 1994 and 1995. *Hp* infection was also confirmed by means of histological examination.

Sections (4-µm thick) were prepared from formalin-fixed and paraffin-embedded specimens. The sections were stained with hematoxylin and eosin (HE) for histological examination and with Giemsa stain for *Hp* identification. Density of *Hp*, polymorphonuclear neutrophil (PMN) activity, chronic inflammation, glandular atrophy, and intestinal metaplasia (IM) were scored according to the updated Sydney System as follows: 0, normal; 1, mild; 2, moderate; and 3, marked [6]. All HE- and Giemsa-stained sections were reviewed by two investigators (T. H. and F. S.). Disagreements were resolved by joint discussion to reach consensus. The Wotherspoon system of histological scoring was used for diagnosis of MALT lymphoma [27].

Tissue from each specimen was placed in a tube containing 20 µl extraction buffer (100 mM Tris-HCl, 2 mM ethylene diamine tetraacetic acid, and 400 µg/ml proteinase K, pH 8.0) and incubated overnight at 55°C. The tubes were boiled for 7 min to inactivate proteinase K, and 2 µl DNA extract was used for each PCR amplification.

Analysis of IgH gene rearrangement was performed using semi-nested PCR amplification according to the method of Ono et al. but with some modification [20]. Briefly, each 25-µl reaction mixture contained 1×PCR buffer II (8.0 mM Tris-HCl, 40 mM KCl, pH 8.3; Perkin-Elmer, Branchburg, N.J.), 4 mM $MgCl_2$, 0.3 mM each deoxynucleotide triphosphate, 100 pmol each primer, 2 µl DNA extract, and 1.25 U *Taq* Gold DNA polymerase (Perkin-Elmer). Each PCR cycle consisted of denaturation at 95°C for 2 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. The primer sequences were as follows: V region, 5'-ACA'CGG'C(C/T)(G/C)'TGT'ATT'ACT'GT-3' (termed FR3A); J region, 5'-TGA'GGA'GAC'GGT'GAC'C-3' (termed LJH); or 5'-GTG'ACC'AGG'GTN'CCT'TGG'CCC'CAG-3' (termed VLJH). The first round of PCR amplification consisted of 30 cycles with primers LJH and FR3A. The first PCR amplification product (2 µl) used as the template for the second round of PCR amplification, and 30 cycles were carried out with primers VLJH and FR3A. The products of the second round of PCR amplification were electrophoresed on a 12% polyacrylamide gel and stained with ethidium bromide. The gel was observed with Eagle Eye II (Stratagene, Calif.). In this assay, a single DNA band 100–200 bp in length, corresponding to rearrangement of one IgH gene, indicates a monoclonal B-cell population. Within the same size range, several distinct bands indicate an oligoclonal B-cell population, whereas a broad smear indicates a polyclonal B-cell population. The DNA extraction and analysis of IgH gene rearrangement were repeated at least twice to confirm the results.

Patients with B-cell monoclonality were observed for more than 4 years by means of a physical examination, chest X-ray, abdominal ultrasound examination, and endoscopy at least every 12 months. B-cell monoclonality in follow-up gastric biopsy specimens was also examined. Fisher's exact probability test was used for statistical analysis. Statistical significance was defined at $P<0.05$.

Results

Microscopic observation of *Hp*-CG biopsies showed mild density of *Hp* in 86 (64%) specimens, moderate density in 34 (26%) specimens, and marked density in 13 (10%) specimens. PMN activity was normal in 19 (14%) specimens, mild in 70 (53%) specimens, moderate in 37 (28%) specimens, and marked in 6 (5%) specimens. Chronic inflammation was normal in 18 (13%) *Hp*-CG specimens, mild in 50 (37%), moderate in 37 (28%), and marked in 29 (22%). Lymphoepithelial lesion (LEL) was absent in all samples, and these *Hp*-CG specimens scored 0–2 according to the Wotherspoon system. Glandular atrophy was absent in 26 (19%) *Hp*-CG specimens, mild in 37 (28%), moderate in 27 (20%), and marked in 44 (33%). Of 64 (48%) *Hp*-CG specimens having detectable IM, 16 (25%) specimens showed mild IM, 31 (48%) showed moderate IM, and 17 (27%) showed marked IM. None of the specimens examined showed histological features of MALT lymphoma.

IgH gene rearrangement analysis showed 116 of the 134 (87%) *Hp*-CG specimens to be informative. B-cell monoclonality was detected in 7 of 116 (6%) informative samples. Representative examples are shown in Fig. 1. B-cell monoclonality was detected in 7 of 72 (10%) *Hp*-CG specimens with mild *Hp* density and in none of the specimens with moderate or marked *Hp* density. B-cell monoclonality frequency in *Hp*-CG specimens having mild *Hp* density did not differ significantly from the frequency in specimens with moderate or marked *Hp* density.

Specimens were graded for PMN activity and examined for B-cell monoclonality. B-cell monoclonality was found in 1 of 16 (6%) *Hp*-CG specimens without PMN activity, in 6 of 62 (10%) specimens with mild PMN activity, and in none of the specimens with moderate or marked PMN activity. No significant differences in B-cell monoclonality frequency were detected between specimens of the four PMN activity grades.

B-cell monoclonality was also assessed among specimens graded for chronic inflammation. Monoclonality was detected in 1 of 15 (7%) *Hp*-CG specimens without chronic inflammation, in 4 of 45 (9%) specimens with

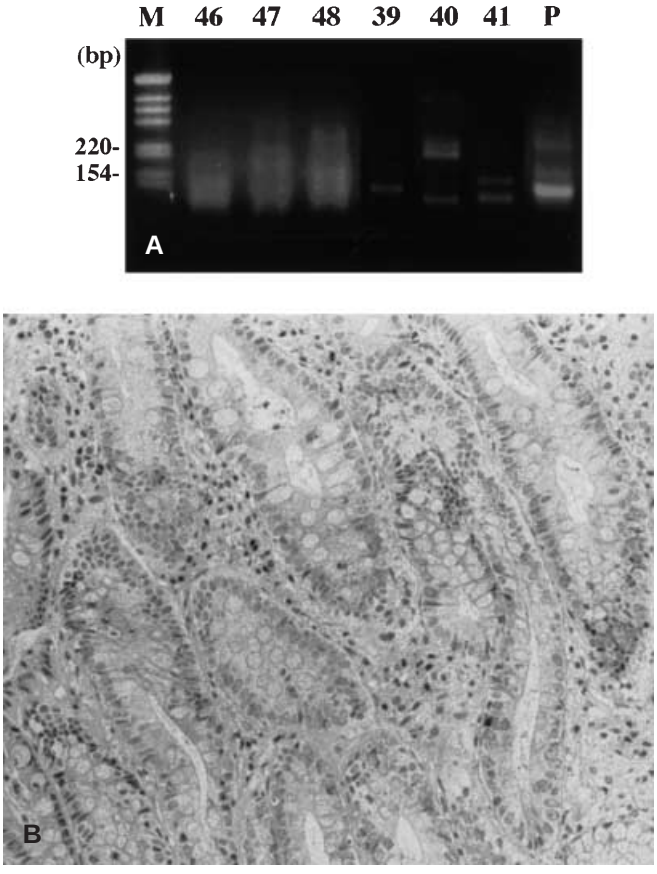

Fig. 1 **A** Analysis of the immunoglobulin heavy chain (IgH) gene rearrangement in samples from *Helicobacter pylori*-associated chronic gastritis patient. Patient numbers are shown above the lane. *P* and *M* are lanes containing a known gastric lymphoma sample and size marker, respectively. DNA from patients 46, 47, and 48 display a polyclonal B-cell pattern. Patient 39 displays a monoclonal pattern, and patients 40 and 41 display oligoclonal patterns. **B** Histological findings of the specimen from patient 39. (Hematoxylin and eosin staining; original magnification ×40). In this specimen, glandular atrophy and intestinal metaplasia are marked. However, the density of *H. pylori*, polymorphonuclear neutrophil activity, and chronic inflammation are mild

mild inflammation, in 1 of 30 (3%) specimens with moderate inflammation, and in none of the 26 specimens with marked inflammation. All specimens with B-cell monoclonality received scores of 0–1 according to the Wotherspoon system.

Table 1 summarizes the correlation between glandular atrophy and B-cell monoclonality. Of 40 *Hp*-CG specimens with marked glandular atrophy, 7 (18%) displayed B-cell monoclonality. Conversely, none of the *Hp*-CG specimens with none to moderate glandular atrophy showed B-cell monoclonality. The frequency of B-cell monoclonality in *Hp*-CG specimens with marked glandular atrophy was significantly different from that of monoclonality in specimens with none to moderate glandular atrophy ($P<0.05$). In specimens having the same grade of glandular atrophy, B-cell monoclonality did not differ significantly in frequency between the antrum and the corpus (Table 2 and Table 3).

Table 1 Correlation between grades of glandular atrophy and immunoglobulin heavy chain (IgH) gene rearrangement in *Heliobacter pylori*-associated chronic gastritis (total). Glandular atrophy in *Heliobacter pylor*-associated chronic gastritis was graded according to the updated Sidney System. *M* monoclonal; *O oligoclonal; P* polyclonal; *N.I.* not informative

Grade of glandular atrophy	IgH gene rearrangement				
	M	O	P	N.I.	Frequency of M
0	0	2	24	0	0/26 (0%)
1	0	3	25	9	0/28 (0%)
2	0	6	17	4	0/23 (0%)
3	7	0	33	4	7/40 (18%)***

***$P<0.05$ compared with frequency of monoclonal B-cell populations in *Helicobacter pylori*-associated chronic gastritis with grade 0, 1 and 2 of glandular atrophy, respectively, by using Fisher's exact probability test

Table 2 Correlation between grades of glandular atrophy and immunoglobulin heavy chain (IgH) gene rearrangement in *Heliobacter pylori*-associated chronic gastritis (antrum). Glandular atrophy in *Heliobacter pylor*-associated chronic gastritis was graded according to the updated Sidney System. *M* monoclonal; *O oligoclonal; P* polyclonal; *N.I.* not informative

Grade of glandular atrophy	IgH gene rearrangement				
	M	O	P	N.I.	Frequency of M
0	0	1	8	0	0/9 (0%)
1	0	2	12	4	0/14 (0%)
2	0	3	10	3	0/13 (0%)
3	4	0	20	3	4/24 (17%)

Table 3 Correlation between grades of glandular atrophy and immunoglobulin heavy chain (IgH) gene rearrangement in *Heliobacter pylori*-associated chronic gastritis (corpus). Glandular atrophy in *Heliobacter pylor*-associated chronic gastritis was graded according to the updated Sidney System. *M* monoclonal; *O oligoclonal; P* polyclonal; *N.I.* not informative

Grade of glandular atrophy	IgH gene rearrangement				
	M	O	P	N.I.	Frequency of M
0	0	1	16	0	0/17 (0%)
1	0	1	13	5	0/14 (0%)
2	0	3	7	1	0/10 (0%)
3	3	0	13	1	3/16 (19%)

B-cell monoclonality was also compared among specimens graded for IM (Table 4). B-cell monoclonality was observed in none of the *Hp*-CG specimens without IM, in 1 of 11 (9%) specimens with mild IM, in 3 of 27 (11%) specimens with moderate IM, and in 3 of 15 (20%) specimens with marked IM. The B-cell monoclonality in *Hp*-CG specimens with moderate or marked IM was significantly more frequent than that in *Hp*-CG specimens without IM ($P<0.05$). In specimens having the same grade of IM, B-cell monoclonality did not differ significantly in frequency between the antrum and the corpus (Table 5 and Table 6).

Table 4 Correlation between grades of intestinal metaplasia and immunoglobulin heavy chain (IgH) gene rearrangement in *Heliobacter pylori*-associated chronic gastritis (total). Intestinal metaplasia in *Heliobacter pylor*-associated chronic gastritis was graded according to the updated Sidney System. *M* monoclonal; *O oligoclonal; P* polyclonal; *N.I.* not informative

Grade of intestinal metaplasia	IgH gene rearrangement				
	M	O	P	N.I.	Frequency of M
0	0	5	57	7	0/62 (0%)
1	1	2	8	5	1/11 (9%)
2	3	6	18	4	3/27 (11%)***
3	3	0	12	2	3/15 (20%)****

***$P<0.05$, ****$P<0.01$ compared with frequency of monoclonal B-cell populations in *Helicobacter pylori*-associated chronic gastritis with grade of 0 of intestinal metaplasia by using Fisher's exact probability test

Table 5 Correlation between grades of intestinal metaplasia and immunoglobulin heavy chain (IgH) gene rearrangement in *Heliobacter pylori*-associated chronic gastritis (antrum). Intestinal metaplasia in *Heliobacter pylor*-associated chronic gastritis was graded according to the updated Sidney System. *M* monoclonal; *O* oligoclonal; *P* polyclonal; *N.I.* not informative

Grade of intestinal metaplasia	IgH gene rearrangement				
	M	O	P	N.I.	Frequency of M
0	0	3	26	4	0/29 (0%)
1	0	0	4	2	0/4 (0%)
2	2	4	13	2	2/19 (11%)
3	2	0	8	0	2/10 (20%)

Table 6 Correlation between grades of intestinal metaplasia and immunoglobulin heavy chain (IgH) gene rearrangement in *Heliobacter pylori*-associated chronic gastritis (corpus). Intestinal metaplasia in *Heliobacter pylor*-associated chronic gastritis was graded according to the updated Sidney System. *M* monoclonal; *O oligoclonal; P* polyclonal; *N.I.* not informative

Grade of intestinal metaplasia	IgH gene rearrangement				
	M	O	P	N.I.	Frequency of M
0	0	2	31	3	0/31 (0%)
1	1	2	4	3	1/7 (14%)
2	1	2	5	2	1/8 (13%)
3	1	0	4	2	1/5 (20%)

Table 7 Immunoglobulin heavy chain (IgH) gene rearrangement in specimens obtained from the antrum and the corpus of *Heliobacter pylori*-associated chronic gastritis. *M* monoclonal; *O* oligoclonal; *P* polyclonal; *DP* different polyclonal; *DO* different oligoclonal

Antrum	Corpus	Number of patients (percentage)
Identical pattern		26/35 (74%)
P	P	26/26 (100%)
Different pattern		9/35 (26%)
P	DP	3/9 (33%)
P	O	1/9 (11%)
O	DO	1/9 (11%)
O	M	2/9 (22%)
M	P	1/9 (11%)
M	O	1/9 (11%)

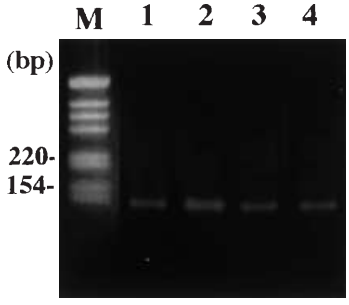

Fig. 2 Analysis of the immunoglobulin heavy chain (IgH) gene rearrangement in follow-up gastric samples (patient 39). Identical monoclonal B-cell populations were observed in a gastric biopsy specimen obtained at initial endoscopic examination (*lane 1*), 1 year later (*lane 2*), 2 years later (*lane 3*), and 4 years later (*lane 4*)

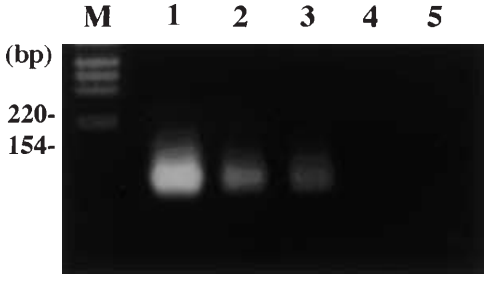

Fig. 3 Analysis of the immunoglobulin heavy chain (IgH) gene rearrangement in dilutions of a polyclonal sample. The amount of DNA template used in the polymerase chain reaction amplification were 10 ng (*lane 1*), 2 ng (*lane 2*), 0.4 ng (*lane 3*), 80 pg (*lane 4*), and 16 pg (*lane 5*)

In 45 *Hp*-CG patients, gastric biopsy specimens were obtained from both the antrum and the corpus. Thirty-five patients were informative for analysis of the IgH gene rearrangement. Of the 35 (74%) patients, 26 showed identical B-cell populations in the antrum and the corpus, and both populations were always polyclonal (Table 7). The remaining 9 (26%) patients showed differing B-cell populations between the antrum and the corpus. Four of the nine (44%) showed monoclonal B-cell populations in at least one gastric biopsy specimen. There were no patients with monoclonal B-cell populations in both the antrum and the corpus.

Seven *Hp*-CG patients had B-cell monoclonality. No evidence of gastric lymphoma was detected in patients with B-cell monoclonality during the follow-up period. However, follow-up gastric biopsy specimens showed identical monoclonal B-cell populations in all of the seven patients (Fig. 2).

The sensitivity of the semi-nested PCR amplification protocol for analysis of IgH gene rearrangement was confirmed by making fivefold limiting dilutions of DNA templates (Fig. 3). Known polyclonal *Hp*-CG samples were used. Polyclonal bands were obtained when the amount of DNA template added to the PCR was 0.4 ng or greater. When the DNA template was less than 0.4 ng, no bands were detected.

Discussion

An etiological association between *Hp* infection and development of gastric MALT lymphoma has been suggested [13, 18]. However, detection of B-cell monoclonality using PCR methodology remains controversial because monoclonality is detected not only in neoplastic lesions but also in histologically reactive lesions [3, 5, 6, 11, 18, 22]. Savio et al. reported that 4% of active-stage *Hp*-CG specimens showed a PCR pattern indicating B-cell monoclonality [22]. Hsi et al. also reported that, of all active-stage *Hp*-CG specimens examined, 15% of the specimens displayed B-cell monoclonality, and PCR patterns indicating monoclonality correlated with the presence of LELs [11]. However, few studies have reported a correlation in *Hp*-CG between B-cell monoclonality and histological parameters as defined by the updated Sydney System [18]. In this study, B-cell monoclonality was detected in 6% of *Hp*-CG specimens. Both marked glandular atrophy and IM were detected in all specimens having B-cell monoclonality. These results seem to conflict with those of previous reports in which monoclonality was observed frequently in massive chronic inflammation [11, 18]. There are controversies regarding whether high activity of gastritis increases the risk of developing MALT lymphoma. Herrera-Goepfert et al. examined the morphologic changes in gastric mucosa adjacent to MALT lymphomas and reported that IM and atrophy in an endoscopic biopsy may be markers of gastric lymphoma [9]. In addition, *H. heilmannii* gastritis, which is known to have a low activity, is overrepresented in cases with MALT lymphoma [16, 23]. Our data also suggest that glandular atrophy and IM in gastric biopsy specimens may be markers for gastric MALT lymphomagenesis.

Nakamura et al. reported that 79% of *Hp*-CG patients who developed gastric MALT lymphomas had monoclonal B-cell populations, whereas, 21% of *Hp*-CG patients who did not develop MALT lymphoma had monoclonal B-cell populations [18]. Thus, analysis of B-cell clonality could be used to assess the risk of gastric MALT lymphoma. However, the relationship between the site from which the biopsy specimen was taken and monoclonal B-cell populations was not addressed. There are no reports of whether B-cell populations in *Hp*-CG patients are identical throughout the stomach, or whether they differ area by area. This question is of great clinical interest because it could clarify how many and from which area(s) gastric biopsy specimens should be taken to assess the risk of gastric MALT lymphoma. Our results suggest that, initially in *Hp*-CG patients, a polyclonal B-cell population may spread to the entire stomach in response to *Hp* infection. In some cases, the stimuli in the antral mucosa and the corpus mucosa may differ, so that different polyclonal B-cell populations are induced 21. A monoclonal B-cell population may then develop, which is a prelymphomatous change, and may spread within the gastric mucosa but not the entire stomach. Therefore, to assess the risk of gastric MALT lymphoma, multiple gastric biopsy specimens from both the antrum and the corpus may be needed.

Since relatively few lymphoid cells were analyzed in our study, some may argue that B-cell monoclonality detected in our specimens may represent false positives. Wan et al. reported that false-positive monoclonal bands were detected when the number of PCR “targets” (cell equivalents) was reduced to an average of six per sample [26]. However, we can rule out this possibility since, in our hands, the DNA pattern did not change from a polyclonal-type to a monoclonal-type when relatively few lymphoid cells were analyzed (Fig. 3).

In conclusion, our data suggest that glandular atrophy and IM in gastric biopsy specimens may be markers for gastric MALT lymphomagenesis and that multiple gastric biopsy specimens from both the antrum and the corpus may be needed to assess the risk of gastric MALT lymphoma.

References

1. Bayerdörffer E, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S, Stolte M (1995) Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. Lancet 345:1591–1594
2. Blaser MJ (1990) Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. J Infect Dis 161:626–633
3. Calvert RJ, Evans PAS, Randerson JA, Jack AS, Morgan GJ, Dixon MF (1996) The significance of B-cell clonality in gastric lymphoid infiltrates. J Pathol 180:26–32
4. Cossman J, Uppenkamp M, Sundeen J, Coupland R, Raffeld M (1988) Molecular genetics and the diagnosis of lymphoma. Ann Pathol Lab Med 112:117–127
5. de Mascarel A, Dubus P, Belleannee G, Megraud F, Merlio JP (1998) Low prevalence of monoclonal B cells in Helicobacter pylori gastritis patients with duodenal ulcer. Hum Pathol 29:784–790
6. Dixon MF, Genta RM, Yardley JH, Correa P (1996) Classification and grading of gastritis: the updated Sydney System. Am J Surg Pathol 20:1161–1181
7. Gulley ML, Ross DW (1991) Progress in molecular diagnosis of lymphoma and leukemia. Clin Biotechnol 3:95–99
8. Hansson LE, Engstrand L, Nyren O, Evans DJ Jr, Lindgren A, Bergstrom R, Andersson B, Athlin L, Bendtsen O, Tracz P (1993) Helicobacter pylori infection: independent risk indicator of gastric carcinoma. Gastroenterology 105:1098–1103
9. Herrera-Goepfert R, Arista-Nasr J, Alba-Campomanes A (1999) Pathologic features of the gastric mucosa adjacent to primary MALT-lymphomas. J Clin Gastroenterol 29:266–269
10. Hiyama T, Haruma K, Kitadai Y, Tanaka S, Yoshihara M, Sumii K, Shimamoto F, Kajiyama G (2000) Eradication therapy of Helicobacter pylori for low-grade mucosa-associated lymphoid tissue lymphomas of the stomach. Exp Oncol 22: 78–81

11. Hsi ED, Greenson JK, Singleton TP, Siddiqui J, Schnitzer B, Ross CW (1996) Detection of immunoglobulin heavy chain gene rearrangement by polymerase chain reaction in chronic active gastritis associated with Helicobacter pylori. Hum Pathol 27:290–296
12. Hsi ED, Eisbruch A, Greenson JK, Singleton TP, Ross CW, Schnitzer B (1998) Classification of primary gastric lymphomas according to histologic features. Am J Surg Pathol 22:17–27
13. Isaacson PG (1995) The MALT lymphoma concept updated. Ann Oncol 6:319–320
14. Kawaguchi H, Haruma K, Komoto K, Yoshihara M, Sumii K, Kajiyama G (1996) Helicobacter pylori infection is the major risk factor for atrophic gastritis. Am J Gastroenterol 91:959–962
15. Marshall BJ (1993) Helicobacter pylori: a primer for 1994. Gastroenterology 105:241–247
16. Morgner A, Lehn N, Andersen LP, Thiede C, Bennedsen M, Trebesius K, Neubauer B, Neubauer A, Stolte M, Bayerdörffer E (2000) Helicobacter heilmannii-associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. Gastroenterology 118:821–828
17. Muller AF, Maloney A, Jenkins D, Dowling F, Smith P, Bessell EM, Toghill PJ (1995) Primary gastric lymphoma in clinical practice 1973–1992. Gut 36:679–683
18. Nakamura S, Aoyagi K, Furuse M, Suekane H, Matsumoto T, Yao T, Sakai Y, Fuchigami T, Yamamoto I, Tsuneyoshi M, Fujishima M (1998) B-cell monoclonality precedes the development of gastric MALT lymphoma in Helicobacter pylori-associated chronic gastritis. Am J Pathol 152:1271–1279
19. Nakamura S, Yao T, Aoyagi K, Iida M, Fujisawa M, Tsuneyoshi M (1997) Helicobacter pylori and primary gastric lymphoma: a histopathologic and immunohistochemical analysis of 237 patients. Cancer 79:3–11
20. Ono H, Kondo H, Saito D, Yoshida S, Shirao K, Yamaguchi H, Yokota T, Hosokawa K, Fukuda H, Hayashi S, Ochiai A, Oguro Y (1993) Rapid diagnosis of gastric malignant lymphoma from biopsy specimens: detection of immunoglobulin heavy chain rearrangement by polymerase chain reaction. Jpn J Cancer Res 84:813–817
21. Ruiz B, Correa P, Fontham ETH, Ramakrishnan T (1996) Antral atrophy, Helicobacter pylori colonization and gastric pH. Am J Clin Pathol 105:96–101
22. Savio A, Franzin G, Wotherspoon AC, Zamboni G, Negrini R, Buffoli F, Diss TC, Pan L, Isaacson PG (1996) Diagnosis and posttreatment follow-up of Helicobacter pylori-positive gastric lymphoma of mucosa-associated lymphoid tissue: histology, polymerase chain reaction, or both? Blood 87:1255–1260
23. Stolte M, Kroher G, Meining A, Morgner A, Bayerdörffer E, Bethke B (1997) A comparison of Helicobacter pylori and H. heilmannii gastritis. A matched control study involving 404 patients. Scand J Gastroenterol 32:28–33
24. The Eurogast Study Group (1993) An international association between Helicobacter pylori and gastric cancer. Lancet 34:1359–1362
25. Tonegawa S (1983) Somatic generation of antibody diversity. Nature 302:575–581
26. Wan JH, Sykes PJ, Orell SR, Morley AA (1992) Rapid method for detecting monoclonality in B cell lymphoma in lymph node aspirates using the polymerase chain reaction. J Clin Pathol 45:420–423
27. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG (1993) Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 342:575–577
28. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG (1991) Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 338:1175–1176
29. Yamada M, Hudson S, Tounay O, Bittenbender S, Shane SS, Lange B, Tsujimoto YJ, Caton AJ, Rovera G (1989) Detection of minimal disease in hematopoietic malignancies in the B-cell lineage by third complementarity determining region (CDR-3) specific probes. Proc Natl Acad Sci USA 86:5123–5127
30. Yoshino T, Akagi T (1998) Gastric low-grade mucosa-associated lymphoid tissue lymphomas: their histogenesis and high-grade transformation. Pathol Int 48:323–331