Antibodies to *Helicobacter pylori* and CagA protein are associated with the response to antibacterial therapy in patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma

Tomonori Sumida,[1] Yasuhiko Kitadai,[1,4] Toru Hiyama,[2] Kei Shinagawa,[1] Miwako Tanaka,[1] Michiyo Kodama,[1] Hiroshi Masuda,[1] Masanori Ito,[1] Shinji Tanaka,[3] Masaharu Yoshihara[2] and Kazuaki Chayama[1]

[1]Department of Medicine and Molecular Science, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima; [2]Health Service Center, Hiroshima University, Higashi Hiroshima; [3]Department of Endoscopy, Hiroshima University Hospital, Hiroshima, Japan

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The aim of this study was to clarify predictive factors for response to eradication therapy in cases of *Helicobacter pylori* (*H. pylori*)-positive *API2–MALT1*-negative gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Sixty-six patients who were examined for *H. pylori* infection and the presence of the *API2–MALT1* chimeric transcript and who underwent *H. pylori* eradication therapy as first-line therapy, were enrolled in this study. Immunohistochemical markers (p53, Ki-67, and BCL10), microsatellite instability, loss of heterozygosity, serum levels of antibodies (anti-*H. pylori* and anti-CagA), and markers for gastritis (gastrin and pepsinogens) were examined, and the results were compared between patients whose tumors regressed completely after eradication therapy (responders) and patients whose tumors did not regress (non-responders). Of the 66 patients with localized gastric MALT lymphoma, 47 (71.2%) showed complete remission after eradication therapy. None of the *H. pylori*-negative ($n = 9$) and/or *API2–MALT1*-positive ($n = 7$) patients responded to antibacterial treatment. Of 44 patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma, 38 (86.4%) showed complete remission after eradication therapy. Titers of antibodies against *H. pylori* and CagA protein were significantly higher in the responders than in the non-responders ($P = 0.0235$ and 0.0089, respectively). No significant difference between the groups was observed for the other factors. In conclusion, measurement of titers of serum antibodies to *H. pylori* and CagA protein may be useful for predicting the response to eradication therapy in patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma. (*Cancer Sci* 2009; 100: 1075–1081)

Malignant lymphoma of mucosa-associated lymphoid tissue (MALT) was first described by Isaacson and Wright.[1] Gastric MALT lymphoma arises from mucosal lymphoid tissue that is usually acquired as a reaction to *Helicobacter pylori* (*H. pylori*) infection.[2] Wotherspoon *et al*. first reported that eradication of *H. pylori* could induce regression of gastric MALT lymphoma.[3] Approximately 60–80% of patients show CR after eradication of *H. pylori*.[3–10]

The *API2* gene on chromosome 11 and the *MALT1* gene on chromosome 18 are fused as a result of a translocation, t(11;18)(q21;q21).[11,12] It has been reported that gastric MALT lymphoma expressing *API2–MALT1* does not respond to *H. pylori* eradication therapy[13,14] and these MALT lymphomas do not show transformation into high-grade diffuse large B-cell lymphoma (DLBCL).[15,16] On the other hand, *API2–MALT1*-negative MALT lymphomas have the potential to progress to DLBCL.[15,16] Expression of the *API2–MALT1* transcript is higher in *H. pylori*-negative gastric MALT lymphomas than in *H. pylori*-positive MALT lymphomas.[17,18] Gastric MALT lymphoma is genetically unstable and acquires other genetic abnormalities, such as trisomy 3, trisomy 18,[19] and p16 deletion.[20] t(1;14)(p22;q32) has also provided fresh insights into the pathogenesis of this disease like t(11;18)(q21;q21).[21] t(1;14)(p22;q32) occurs in approximately 5% of MALT lymphomas and causes deregulation of BCL10,[22,23] which specifically links antigen receptor signaling to the nuclear factor-κB pathway.[24]

Presently, *H. pylori* status and the presence of the *API2–MALT1* chimeric transcript are known as predictive factors for therapeutic response to *H. pylori* eradication therapy. In the present study, we selected patients with *H. pylori*-positive and/or *API2–MALT1*-negative gastric MALT lymphoma and investigated clinicopathological and genetic characteristics with reference to the response to *H. pylori* eradication therapy.

Materials and Methods

Patients. Between 1997 and 2007, 83 Japanese patients with low-grade gastric MALT lymphoma without a DLBCL component were treated at Hiroshima University Hospital (Hiroshima, Japan). Sixty-six patients who were examined for *H. pylori* infection status and the presence of the *API2–MALT1* chimeric transcript and who were treated with antibacterial therapy as a first line were enrolled in this study. All cases were classified as clinical stage I or II_1 according to the Lugano staging system for gastrointestinal lymphomas.[25] There were 29 men and 37 women with a mean age of 59.8 years (range, 20–89 years). The median follow-up period was 40 months (range, 13–135 months). At least five biopsy specimens were obtained from the tumor lesion in the stomach for histological diagnosis, and two biopsy specimens taken from the normal gastric mucosa and tumor lesion were frozen at –70°C immediately and stored for molecular analyses. MALT lymphoma was diagnosed according to the histological criteria of the World Health Organization classification system.[26] Informed consent for participation in the study was obtained from each patient after an explanation of the aims and protocol.

Assessment of *H. pylori* infection. One specimen each from the greater and lesser curvatures of the gastric antrum and corpus was obtained, and histological examination with Giemsa staining for *H. pylori* was carried out. Serum samples were tested independently for the presence of antibodies against *H. pylori* by ELISA

[4]To whom correspondence should be addressed. E-mail: kitadai@hiroshima-u.ac.jp
Abbreviations: CR, complete remission; CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; ELISA, enzyme-linked immunosorbent assay; EUS, endoscopic ultrasonography; LI, labeling index; LOH, loss of heterozygosity; MALT, mucosa-associated lymphoid tissue; MSI, microsatellite instability; NC, no change; PCR, polymerase chain reaction; PG, pepsinogen; PR, partial remission; UBT, ^{13}C-urea breath test.

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(Pylori Stat Kit; Whittaker Bioproducts, Walkersville, MD, USA). Results were considered positive when the serum anti-*H. pylori* IgG antibody titer was greater than or equal to 10.0 U/mL; a titer below 10.0 U/mL was considered negative. The standard UBT for *H. pylori* detection was carried out after an overnight fast. Infrared spectrometric analysis was carried out with a spectrometer (UbiT-100; Otsuka Electronics, Hiranaka, Japan). The breath samples were collected before and 20 min after drinking 100 mg ^{13}C-urea. The ratio of $^{13}CO_2$ to $^{12}CO_2$ ($\Delta^{13}C$) was calculated on the basis of absorption differences in the infrared regions for $^{13}CO_2$ and $^{12}CO_2$. The criterion for *H. pylori* positivity was a $\Delta^{13}C$ of more than 2.5. *H. pylori* infection was judged as positive when at least one of these tests for *H. pylori* showed a positive result and judged as negative only when the results of all the tests were negative.

Clinical staging of the disease. Staging involved a clinical physical examination, upper gastrointestinal endoscopy, abdominal ultrasonography, CT from the cervix to abdomen, colonoscopy, bone marrow examination, and whole-body gallium scintigraphy. Gastric EUS and positron emission tomography with CT were optional. Lymph nodes were considered to be infiltrated by lymphoma cells if they were spherical, hypoechogenic, and 1 cm or more in diameter. The degree of the disease was classified according to the Lugano staging system, a modification of the Ann-Arbor classification system.[25]

Treatment of patients with gastric MALT lymphoma with antibacterial therapy. Antibiotic therapy with lansoprazole (60 mg/day, twice daily) or omeprazole (40 mg/day, twice daily), clarithromycin (400 mg/day, twice daily), and amoxicillin (1500 mg/day, twice daily) was administered for 7 days for all patients as the first-line treatment regardless of *H. pylori* infection status. For patients in whom *H. pylori* infection persisted after the initial treatment, a combination of lansoprazole, amoxicillin, and metronidazole (500 mg/day, twice daily) was used as the second-line eradication therapy. The effect of *H. pylori* eradication was evaluated 6 weeks after treatment, and eradication therapy was considered successful when both Giemsa staining and UBT were negative. *H. pylori* was eradicated with these therapeutic protocols in all patients.

Follow-up study. Patients who underwent eradication therapy were followed up every 3 months for the first year, every 4 months for the second year, and every 6 months thereafter. *H. pylori*-negative patients were followed up every 3 months, and radiotherapy was started if there was no improvement in the histological grade of biopsy specimens upon two consecutive gastrointestinal endoscopic examinations after eradication therapy. CR of the lymphoma was considered as endoscopic improvement with a histological grade for MALT lymphoma of 2 or less, as described by Wotherspoon *et al.* on post-treatment biopsy specimens at any time during the follow-up period.[3] PR was considered as endoscopic improvement, but with a histological score of 4 or more on biopsy samples. No endoscopic and histological response for 8 months after successful eradication was considered to represent NC. CR was defined as characteristic of responders, and PR or NC was defined as characteristic of non-responders. Radiotherapy (1.5 Gy/day, total 30.0 Gy) was chosen as a second-line therapy for non-responders and all of the patients achieved CR.

Immunohistochemistry. Expression of p53, Ki-67, and BCL10 was examined by immunohistochemistry. Immunohistochemical staining was carried out by means of the labeled streptavidin–biotin method (LSAB Kit; Dako, Copenhagan, Denmark) on formalin-fixed, paraffin-embedded tissues. Tissue sections were treated for antigen retrieval in target retrieval solution (Dako) in a microwave oven for 15 min. To detect the p53 protein, a mouse monoclonal antibody (DO7; Novocastra, Newcastle, UK) was used. The level of p53 was calculated by expressing the number of p53-positive lymphoma cells as a percentage of the total number of lymphoma cells in a high-power field. Sporadic or no p53 staining was considered negative, whereas focal or diffuse immunoreactivity (more than 10% of cells) was considered positive. Ki-67 was stained with an anti-Ki-67 monoclonal mouse antibody (MIB-1; Dako). The Ki-67 LI was determined by counting the percentage of positive cells among 1000 tumor cells in 10 random regions in 400-fold fields. BCL10 was immunostained with a mouse monoclonal antibody (clone 151.1; Dako). Staining was considered positive for BCL10 when nuclear staining of the protein was present in more than 10% of tumor cells.

Extraction of DNA and RNA. Genomic DNA and total RNA were extracted from biopsy specimens with a High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) and an RNeasy Minikit (Qiagen, Valencia, CA, USA) according to the manufacturers' instructions.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis for the *API2–MALT1* chimeric transcript. Five micrograms of total RNA was converted to cDNA with reverse transcriptase (Superscript II; Invitrogen, Carlsbad, CA, USA) in a total volume of 40 μL, according to the manufacturer's protocol. We used primer pairs reported previously (sense primer API2/S 1203-1222, 5′-GTTCCTACCACTGTGCAATG-3′ and anti-sense primer MALT1/AS 1030–1049, 5′-CAAAGGCTGGTCAGTTGTTT-3′).[13] These primers and the cDNA solution produced as described above were used for PCR amplification. PCR was carried out in a total volume of 100 μL containing 2.5 U *Taq* (Takara, Otsu, Japan), 10 mM Tris (pH 8.3 at 25°C), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each dNTP, and 100 pmol of each primer. The PCR regimen was as follows: 10 min at 94°C for initial denaturation followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, and a final extension for 10 min at 72°C. The PCR products were separated by electrophoresis on a 1.2% agarose gel and stained with ethidium bromide.

Microsatellite assay. Nine microsatellite loci, including *TP53*, *D3S1229*, *D6S310*, *D6S447*, *D17S855*, *D18S35*, *D18S58*, *D18S61*, and *BAT26*, reported to be informative markers to examine for microsatellite instability (MSI) and loss of heterozygosity (LOH), were used (Table 1).[27,28] The microsatellite assay was carried out as described elsewhere.[29,30] Briefly, each 15-μL reaction mixture containing 20 ng genomic DNA, 6.7 mM Tris-HCl (pH 8.8), 6.7 mM ethylenediaminetetraacetic acid, 6.7 mM $MgCl_2$, 0.33 μM primer labeled with [γ-^{32}P] dATP, 0.175 μM unlabeled primer, 1.5 mM of each dNTP, and 0.75 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ, USA) was amplified with 50 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C followed by a final extension at 72°C for 10 min. PCR products were separated by electrophoresis on 6% polyacrylamide–8 M urea–32% formamide gels and exposed to Fuji RX Film (Fuji Photo Film, Minamiashigara, Japan) overnight for autoradiography. MSI in tumors was assessed as the presence of novel-sized alleles that were not present in normal tissues from the same individuals. LOH was also detected by microsatellite assay. Only patients heterozygous for a given locus were considered informative. Homozygosity and MSI rendered a particular locus unevaluable for LOH. LOH was confirmed as the presence of only one band for a microsatellite sequence tumor DNA, whereas two major bands were present in normal tissues from the same individual.

Measurement of serum levels of anti-CagA antibody, gastrin, and PG. Serum gastrin, PG-I, and PG-II levels were measured by radioimmunoassay as described previously.[31] ELISA was carried out with a CagA Kit (*Helicobacter* P120 [CAG A] ELISA; Ravo Diagnostika, Freiburg, Germany) according to the manufacturer's instructions.

Statistical analysis. Fisher's exact test was used to analyze the association between *H. pylori* infection and clinicopathological findings (sex, number of tumor lesions, *API2–MALT1* expression, and immunohistochemistry data). The level of gastrin, PG-I/PG-II ratio, and serum anti-*H. pylori* IgG and anti-CagA IgG antibody titers obtained before eradication therapy were compared by Mann–Whitney *U*-test. A *P*-value of less than 0.05 was considered statistically significant.

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Table 1. Microsatellite markers used in the analysis of loss of heterozygosity and microsatellite instability

Marker	Location	Primer sequence
TP53	17p13.1	5′-ACTGCCACTCCTTGCCCCATTC-3′
		5′-AGGGATACTATTCAGCCCGAGGTG-3′
D3S1229	3q26.2-27	5′-GCCTTTAAAAAATCTGAACAG-3′
		5′-ATTACAGTCCTTCACACATC-3′
D6S310	6q23.3-q25	5′-GATCCAGATTGCAGAAGG-3′
		5′-GAAACAGGACCAGTAGGATATG-3′
D6S447	6q21-q22.1	5′-CTTTCTCTCGCTCTCTCACA-3′
		5′-GGTTCTGTGCTTGCTAAATG-3′
D17S855	17q-17q	5′-GGATGGCCTTTTAGAAAGTGG-3′
		5′-ACACAGACTTGTCCTACTGCC-3′
D18S35	18q21	5′-AGCTAGATTTTTACTTCTCTG-3′
		5′-CTGGTTGTACATGCCTGAC-3′
D18S58	18q22.3-18q23	5′-GCTCCCGGCTGGTTTT-3′
		5′-GCAGGAAATCGCAGGAACTT-3′
D18S61	18q22.3-18q22.3	5′-ATTTCTAAGAGGACTCCCAAACT-3′
		5′-ATATTTTGAAACTCAGGAGCAT-3′
BAT26	2p16-2p16	5′-TGACTACTTTTGACTTCAGCC-3′
		5′-AACCATTCAACATTTTTAACCC-3′

Results

Comparison of *H. pylori*-positive and *H. pylori*-negative MALT lymphomas. We first examined the clinicopathological characteristics of patients with *H. pylori*-positive and *H. pylori*-negative MALT lymphomas (Table 2). Fifty-seven of the 66 patients (86.4%) were infected with *H. pylori*, nine (13.6%) were *H. pylori*-negative. There was no significant difference in sex, endoscopic appearance, dominant site, number of tumor lesions, or p53 staining status between the *H. pylori*-positive and *H. pylori*-negative groups. In most *H. pylori*-positive cases of MALT lymphoma (32/34; 94.1%), the infiltrated layers were identified as mucosa by EUS. In contrast, half of *H. pylori*-negative cases (3/6) showed submucosal infiltration. Positivity for *API2–MALT1* expression and the degree of BCL10 staining were significantly higher in the *H. pylori*-negative group than in the *H. pylori*-positive group ($P = 0.005$ and 0.032, respectively).

Clinicopathological characteristics of the responders and non-responders. The clinicopathological characteristics of the responder and non-responder groups are summarized in Table 3. There was no significant difference in sex, endoscopic appearance, dominant site, number of tumor lesions, p53 staining status, or Ki-67 LI between responders and non-responders. However, *API2–MALT1* expression and the degree of BCL10 staining were significantly higher in the non-responder group than in the responder group ($P = 0.00006$ and 0.0099, respectively). The proportion of tumors with submucosal infiltration was also significantly higher in the non-responder group than in the responder group. None of the patients without *H. pylori* infection or with *API2–MALT1*-positive tumors responded to antibacterial therapy.

Comparison of responder status of *H. pylori*-positive *API2–MALT1*-negative patients with gastric MALT lymphoma. Factors predictive of resistance to antibiotic therapy in *H. pylori*-positive *API2–MALT1*-negative patients were unknown. We next examined the clinicopathological and genetic features of patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma with reference to response to *H. pylori* eradication therapy.

Out of 54 patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma, serum levels of markers for gastritis and antibodies against *H. pylori* and CagA proteins were measured in 44 patients. These comprised 38 responders and 6 non-responders (Table 4). The differences in the gastrin level and PG-I/PG-II ratio between the responders and non-responders were not statistically significant ($P = 0.8734$ and 0.1508, respectively) (Fig. 1). In responders, the serum anti-*H. pylori* IgG antibody titer and the anti-CagA IgG antibody titer were significantly higher than those in the non-responders (24.3 ± 14.9 vs 105.2 ± 166.4 U/mL, $P = 0.0235$, and 16.5 ± 13.2 vs 43.7 ± 25.1 U, $P = 0.0089$, respectively) (Fig. 2). Most of the patients with *H. pylori*-positive MALT lymphoma (42/44, 95.5%) showed positive reaction for CagA antibody. The titers of both antibodies were significantly correlated ($r = 0.44$, $P = 0.0039$) (Fig. 3). We also compared the titer of UBT before eradication therapy between the responders and non-responders; however, no significant difference was observed

Table 2. Clinicopathological and molecular features of *H. pylori*-positive and *H. pylori*-negative patients

Clinicopathological feature	*H. pylori* positive ($n = 57$)	*H. pylori* negative ($n = 9$)	*P*-value
Sex ratio (male/female)	26/31	3/6	Not significant
Endoscopic appearance			
Superficial	41	4	Not significant
Ulcers	10	2	
IIc-like	0	1	
Protuberance	5	2	
Cobble-stone-like	1	0	
Depth of invasion by endoscopic ultrasonography			
Mucosa	32	3	$P = 0.018$
Submucosa	2	3	
Not examined	23	3	
Dominant site of lesion			
Proximal third	4	0	Not significant
Distal two thirds	53	9	
Number of lesions			
Single	19	5	Not significant
Multiple	38	4	
API2–MALT1 transcript positive	5.26% (3/57)	44.4% (4/9)	$P = 0.005$
p53 staining positive	15.8% (9/57)	0.00% (0/9)	Not significant
Ki-67 labeling index (mean ± SD)	10.9 ± 2.12	8.64 ± 3.64	Not significant
BCL10 staining positive	19.3% (11/57)	55.6% (5/9)	$P = 0.032$

Table 3. Clinicopathological and molecular features of responders and non-responders to *H. pylori* eradication

Clinicopathological feature	Responders ($n = 47$)	Non-responders ($n = 19$)	*P*-value
Sex ratio (male/female)	21/26	8/11	Not significant
H. pylori infection			
Positive	47	10	$P < 0.00001$
Negative	0	9	
Endoscopic appearance			
Superficial	35	10	Not significant
Ulcers	8	4	
IIc-like	0	1	
Protuberance	3	4	
Cobble-stone-like	1	0	
Depth of invasion by endoscopic ultrasonography			
Mucosa	26	4	$P = 0.00002$
Submucosa	1	9	
Not examined	20	6	
Dominant site of lesion			
Proximal third	4	0	Not significant
Distal two thirds	43	19	
Number of lesions			
Single	16	8	Not significant
Multiple	31	11	
API2–MALT1 transcript positive	0.00% (0/47)	36.8% (7/19)	$P = 0.00006$
p53 staining positive	14.9% (7/47)	11.1% (2/19)	Not significant
Ki-67 labeling index (mean ± SD)	10.6 ± 12.7	8.46 ± 13.9	Not significant
BCL10 staining positive	14.9% (7/47)	47.4% (9/19)	$P = 0.0099$

Table 4. Clinicopathological and genetic features of patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma

Clinicopathological feature	Responders ($n = 38$)	Non-responders ($n = 6$)	*P*-value
Sex ratio (male/female)	16/22	4/2	Not significant
Endoscopic appearance			
Superficial	28	3	Not significant
Ulcers	6	3	
IIc-like	0	0	
Protuberance	3	0	
Cobble-stone-like	1	0	
Depth of invasion by endoscopic ultrasonography			
Mucosa	19	5	Not significant
Submucosa	1	0	
Not examined	18	1	
Dominant site of lesion			
Proximal third	2	0	Not significant
Distal two thirds	36	6	
Number of lesions			
Single	14	1	Not significant
Multiple	24	5	
p53 staining positive	7.89% (3/38)	0.00% (0/6)	Not significant
Ki-67 labeling index (mean ± SD)	10.6 ± 12.7	14.0 ± 20.7	Not significant
BCL10 staining positive	15.8% (6/38)	50.0% (3/6)	Not significant

(15.0 ± 11.9 vs 2.95 ± 3.19, $P = 0.078$). There were no significant differences in p53 or BCL10 positivity and Ki-67 LI between the two groups (Table 4).

An association between MSI and response to eradication therapy has been reported previously.[2,27,28] In the present study, only 26 patients were informative for microsatellite analysis. Three patients had LOH at *D6S310*, two had LOH at *D18S35*, and one had LOH at *D3S1229*, *D17S855*, *D18S58*, and *D18S61*. MSI was detected in 3 of the 26 (11.5%) patients at *TP53* and in 1 of the 26 (3.85%) at *D18S61*. There were no significant differences in the frequencies of LOH and MSI between responders and non-responders (data not shown).

Discussion

H. pylori infection induces acquisition of MALT in the gastric mucosa and promotes malignant transformation of reactive B cells.[2] Eradication of *H. pylori* with antibiotics leads to regression of gastric MALT lymphoma in approximately 70% of cases and is now used as a first-line therapy for this disease.[3–10] However, some patients with gastric MALT lymphoma show no evidence of *H. pylori* infection, and the pathogenesis is still poorly understood.

In the present study, we compared the clinicopathological characteristics of patients with *H. pylori*-positive and *H. pylori*-negative gastric MALT lymphomas. Tumor invasion to the

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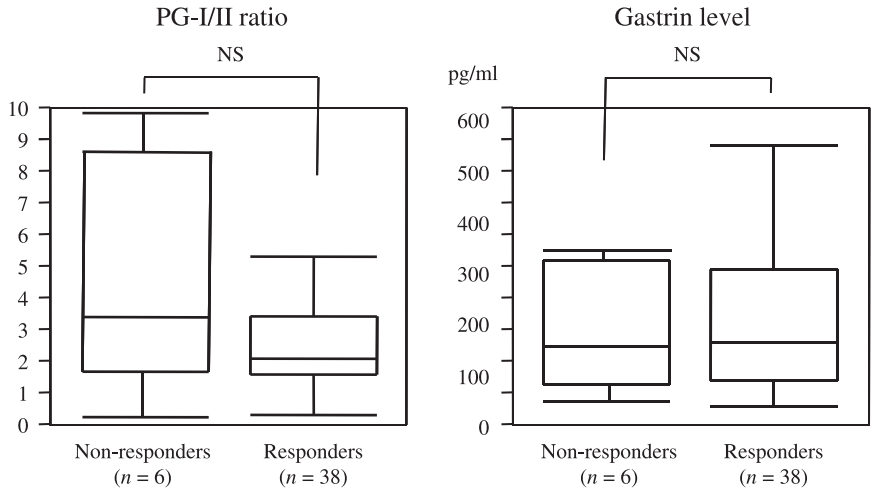

Fig. 1. Pre-treatment levels of serum markers in *H. pylori*-positive *API2-MALT1*-negative responders and non-responders. Differences in the gastrin level and the pepsinogen (PG)-I/PG-II ratio were not statistically significant (NS).

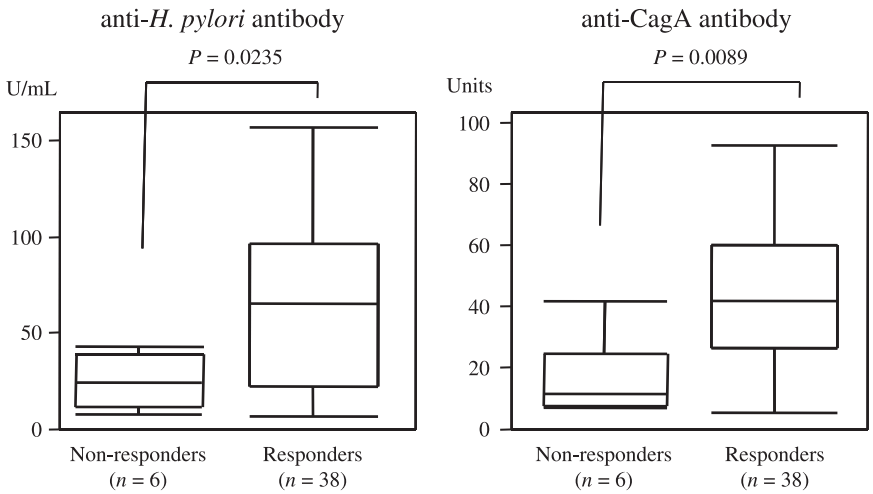

Fig. 2. Anti-*H. pylori* IgG and anti-CagA IgG antibody titers between responders and non-responders. Among patients who responded to eradication therapy, the serum anti-*H. pylori* IgG and anti-CagA IgG antibody titers were significantly higher than those in non-responders ($P = 0.0235$ and 0.0089, respectively).

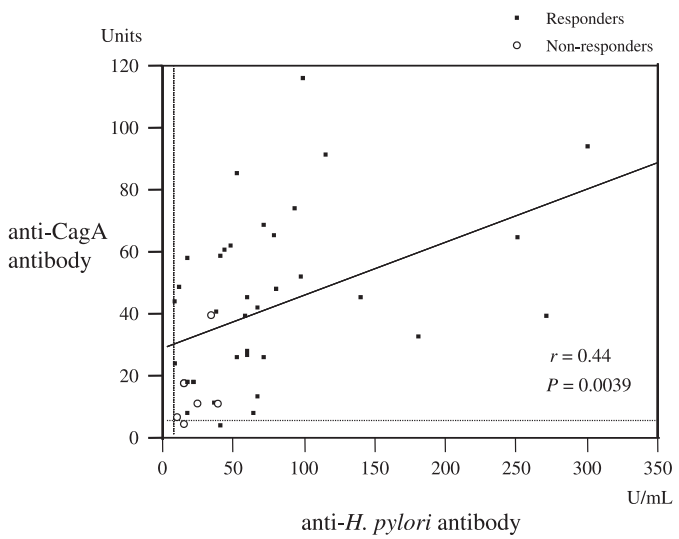

Fig. 3. Correlation of the serum anti-*H. pylori* IgG titer with the anti-CagA IgG antibody titer. The titers of both antibodies were significantly correlated ($r = 0.44$, $P = 0.0039$). The vertical and horizontal dotted lines indicate the lower detection limits of the anti-*H. pylori* IgG and anti-CagA IgG antibodies, respectively.

submucosa, expression of *API2–MALT1*, and staining of BCL10 were detected more frequently in *H. pylori*-negative patients than in *H. pylori*-positive patients, consistent with previous reports.[18,32] When we compared the clinicopathological characteristics of responders and non-responders to antibacterial treatment, *H. pylori*-negative or *API2–MALT1*-positive gastric MALT lymphomas did not regress in response to antibacterial treatment.

There are several reports concerning the efficacy of antibacterial treatment for gastric MALT lymphoma.[6,7,19,32–34] Akamatsu *et al*. recommended that antibacterial therapy should not be the first-line treatment for *H. pylori*-negative gastric MALT lymphoma on the basis of some considerations of the influences of delay in undergoing an effective treatment such as radiation therapy and the progression of the disease during antibacterial therapy and the subsequent follow up.[33] In contrast, Nakamura *et al*. summarized their cases and previously reported cases and found that 9 of 46 (19.6%) *H. pylori*-negative cases responded to antibacterial eradication therapy.[32] They proposed that all patients, regardless of *H. pylori* infection status, should be given antibacterial treatment initially. However, some of their patients with *H. pylori*-negative gastric MALT lymphoma had atrophic gastritis or intestinal metaplasia in the background gastric mucosa, suggesting the presence of infection with a very small quantity of *H. pylori* or *Helicobacter heilmannii* cells.[32,33] In the present study, atrophic change, intestinal metaplasia, and histological inflammation were not observed in the background gastric mucosa of the patients with *H. pylori*-negative gastric MALT lymphoma, suggesting that false-negative *H. pylori* infection may be excluded.

The association between *API2–MALT1* expression and *H. pylori* infection is also controversial. Nakamura *et al*. reported that expression of *API2–MALT1* is related to the absence of *H. pylori* infection.[17] Ye *et al*. reported that t(11;18)(q21;q21) occurs at high frequency in *H. pylori*-negative gastric MALT lymphoma.[18] These findings are consistent with our results. Liu *et al*. detected the *API2–MALT1* chimeric transcript only in *H. pylori*-positive MALT lymphoma,[35] whereas Baens *et al*. found no correlation between *API2–MALT1* expression and *H. pylori* status.[36] In general, gastric MALT lymphoma with *API2–MALT1* is thought to be resistant to eradication therapy regardless of *H. pylori* infection status.[13,14,35,37,38]

In patients with *H. pylori*-positive *API2–MALT1*-negative gastric MALT lymphoma, markers predictive for responsiveness to anti-*H. pylori* therapy have not been identified. In the present study, we showed that there were no differences in expression of p53 and BCL10 or Ki-67 LI between responders and non-responders. Genetic alterations, including MSI and LOH, were rare events in gastric MALT lymphoma and did not differentiate between the groups.

In the present study, we examined serum levels of gastritis markers in patients with gastric MALT lymphoma. The serum level of PG, the precursor of pepsin, is considered a reliable marker of atrophic gastritis. Reduction in the area of the fundic mucosa due to gastritis is associated with stepwise reduction in the PG-I/PG-II ratio.[39–41] Gastrin is another popular marker of gastritis. Hypergastrinemia is induced by increased intragastric pH due to atrophic change of the gastric corpus. Eck *et al*. reported that almost all patients with MALT lymphoma (95.6%, 65 of 68 patients) were infected with a $CagA^+$ *H. pylori* strain.[42] Umehara *et al*. reported that CagA may play a role in the development of MALT lymphoma by impairing p53-dependent apoptosis, although their data were obtained by transfection of the *CagA* gene into B lymphocytes.[43] Therefore, we also examined titers of antibody against CagA protein. We found no differences in serum gastritis markers between responders and non-responders; however, we found significant differences in titers of serum antibodies against *H. pylori* and CagA protein. Titers of both antibodies were significantly higher in responders than in non-responders. Delchier *et al*. reported that there was no relationship between the prevalence of anti-CagA antibody and the response to *H. pylori* eradication therapy in MALT lymphoma;[44] however, they did not measure the CagA antibody titer. Takenaka *et al*. investigated pre-treatment titers of serum antibodies against *H. pylori* and *H. pylori*-recombinant heat shock protein 60 in patients with *H. pylori*-positive gastric MALT lymphoma.[45] They found that titers of these antibodies were significantly higher in responders than in non-responders; however, they did not examine expression of the *API2–MALT1* chimeric transcript in their patients. The mechanism that underlies these findings has not yet been elucidated, but it is possible that the immune reaction of the host to *H. pylori* may be involved in progression of gastric MALT lymphoma via induction of various cytokines and growth factors. Although antibacterial therapy decreases cytokine levels in gastric mucosa, this effect may be insignificant in patients with weak immune responses to *H. pylori*. Because titers of both antibodies against *H. pylori* and CagA were related to response to the eradication therapy, generalized higher immune responses rather than specific immune response to CagA seem to be involved in the pathogenesis of gastric MALT lymphoma.

The mechanism for development of gastric MALT lymphoma in patients with weak immune response is also unknown. There is a possibility that other genomic alterations may have existed in these patients. Fukuhara *et al*. reported that numerous genomic alterations were seen by array-based comparative genomic hybridization in *API2–MALT1*-negative MALT lymphoma without any response to eradication therapy.[46] Many of these alterations were similar to those found in DLBCL.[46] These findings may in part explain why *API2–MALT1*-negative MALT lymphomas have the potential to transform into DLBCL.

In conclusion, our present data suggest that the differential response to antibiotic therapy of *H. pylori*-positive *API2–MALT*-negative gastric MALT lymphoma is associated with the host immune responses to *H. pylori* infection and CagA protein. Among patients who do not respond to eradication therapy, the anti-*H. pylori* IgG and anti-CagA IgG antibody titers are good predictive markers of responsiveness to eradication therapy.

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