Original

**Anti-CagA Antibodies in Peptic Ulcer Patients
Infected with *Helicobacter pylori***

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Abstract: We investigated the clinical significance of serum anti-CagA antibody in peptic ulcer patients infected with *Helicobacter pylori* and assessed its association with histologic changes in gastric mucosa. The subjects were 62 Japanese patients with *H. pylori* infection and endoscopically diagnosed peptic ulcers (24 men and 19 women with gastric ulcers and 14 men and 5 women with duodenal ulcers). Serum samples were assayed for the presence of antibodies to CagA with an enzyme-linked immunosorbent assay procedure. The *cagA* gene was assayed in the gastric juice of 42 of the 62 patients. Histologic examinations were performed according to the histologic classification of the Updated Sydney System. Serum anti-CagA antibody was detected in 76.7% of patients with gastric ulcer and in 73.7% of those with duodenal ulcer. Among the patients with duodenal ulcers, the scores of chronic inflammatory cell infiltration were significantly higher in seropositive patients than in seronegative patients ($P=.020$). Serum titers of anti-CagA antibody correlated with scores of chronic inflammatory cell infiltration ($r=0.525$, $P=0.026$). The *cagA* gene of *H. pylori* was detected in the gastric juice of 28 of 29 patients with gastric ulcers and all in 13 patients with duodenal ulcers. The absence of anti-CagA antibody in the serum does not necessarily reflect the absence of the *cagA* gene of *H. pylori*. However, serum titers of anti-CagA antibodies correlated with chronic inflammatory cell infiltration into the gastric mucosa of the duodenal ulcer patients. This may indicate that infection with *cagA*-positive strains of *H. pylori* is associated with chronic inflammation in the gastric mucosa of Japanese patients with duodenal ulcers.

Key words: serum anti-CagA antibody, chronic inflammatory cell infiltration, peptic ulcer, *Helicobacter pylori*

Introduction

Helicobacter pylori (*H. pylori*) is now considered a central factor in the pathogenesis of peptic ulcers. A vacuolating cytotoxin and a *H. pylori* protein with a molecular weight of approximately 128 kd were discovered in the supernatant of cultured *H. pylori*¹⁾. The gene encoding the protein was found to be closely associated with the vacuolating cytotoxin and was named the cytotoxin-associated gene A (*cagA*)²⁾. The CagA protein stimulates production of interleukin-8 (IL-8) by cultured human gastric cancer cells³⁾. Moreover, *H. pylori*

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expressing CagA stimulates IL-8 production via gastric epithelial cells *in vitro*, and neutrophil cells induced by *H. pylori* injure the gastric mucosa⁴). On the other hand, serum titers of an antibody against the 128 kd protein are significantly higher in patients with gastric cancer⁵). In a series of Western blotting tests conducted in Japan, high percentages of patients with *H. pylori* were seropositive for anti-CagA antibody regardless of whether they had peptic ulcers⁶). In a series of patients from a North American population, neither peptic ulcers nor asymptomatic *H. pylori* gastritis were correlated with serum anti-CagA antibodies⁷). In Japan, patients with gastric ulcers outnumber those with duodenal ulcers. Mucosal atrophy of the gastric corpus may be more severe in Japanese patients than in Western patients because of the high prevalence of *H. pylori* infection. In the present study, we investigated the clinical significance of anti-CagA antibodies in peptic ulcer patients infected with *H. pylori* and assessed the association of anti-CagA antibody with histologic changes in gastric mucosa.

Materials and Methods

Subjects

The subjects were 62 patients with *H. pylori* infection and endoscopically diagnosed gastric ulcers (24 men and 19 women, mean age 55.1 ± 2.1 years) and duodenal ulcers (14 men and 5 women, mean age 42.4 ± 3.7 years) treated at Showa University Hospital from January 1996 to January 2000. These were patients who had given consent for detailed analysis of any *H. pylori* infection. The subjects were treated with H₂-receptor antagonists or proton pump inhibitors. The examinations were performed within a week of start of treatment. None of the subjects had undergone eradication therapy for *H. pylori* or long-term treatments with antibiotics or nonsteroidal anti-inflammatory drugs (Table 1). The protocol was approved by the ethics committee of Showa University Hospital. Informed consent was obtained from all subjects.

Endoscopies and Histologic examinations

Gastroduodenal examinations were performed with a gastroduodenoscope (Q20, Q30, Q200, or Q240, Olympus; Tokyo, Japan) that had been washed in an automatic endoscope washer. Three samples of mucosa were obtained with disposable biopsy forceps (Scientific Corporation, Boston, MA, USA) from both the gastric antrum and corpus on the greater curvature. The biopsy specimens were taken from areas of mucosa distant from any focal lesions. One specimen was cultured on blood agar medium in a microaerophilic environment, the second was used for polymerase chain reaction (PCR) assay, and the third examined microscopically (hematoxylin-eosin stain and Giemsa stain). The PCR assays

Table 1. Demographics and clinical characteristics of patients

Anti-CagA antibody	Gastric Ulcer (N=43)			Duodenal Ulcer (N=19)		
	positive	negative	p values	positive	negative	p values
Sex (male/female)	16/17	8/2	0.88	10/4	4/1	>0.99
Age	53 $\pm$ 2	62 $\pm$ 4	0.08	45 $\pm$ 5	36 $\pm$ 4	0.14
CagA Titers	69.6 $\pm$ 12.0			82.0 $\pm$ 19.9		
Hp-IgA Titers	389.2 $\pm$ 41.4	369.4 $\pm$ 73.0	0.82	304.2 $\pm$ 66.3	174.4 $\pm$ 63.9	0.18

were performed as per the method of Valentine et al⁸⁾ using a pair of primers that amplified a 1.9-kb fragment of *H. pylori* genomic DNA. Histologic examinations were performed according to the histologic classification of the Updated Sydney System⁹⁾.

Anti-H. pylori antibody and H. pylori PCR assay

Serum samples of patients were examined for anti-*H. pylori* immunoglobulin G (IgG) with an *H. pylori* enzyme immunoassay kit (AMRAD, Kew, Victoria, Australia)¹⁰⁾. The results are expressed quantitatively, and a titer of less than 30 U/ml was considered negative. To confirm *H. pylori* infection, positive results were required from at least two of the following tests: culture, microscopic examination, PCR assay and *H. pylori* serum enzyme immunoassay.

Anti-CagA antibodies

Serum samples were stored at -80°C and assayed for the presence of antibodies to CagA with an enzyme-linked immunosorbent assay procedure (Radim, Pomezia, Italy)¹¹⁾. Serum was considered IgG-positive if the concentration was 15 Radim units (UR) per ml or more.

cagA gene in gastric juice

The *cagA* gene in gastric juice was assayed in 42 of the 62 patients. Gastric juice samples were not taken from the remaining 20 patients. Gastric juices were pulled through a suction tube at endoscopic examinations and stored at -80°C . A new suction tube was used for each sample. Approximately 1 ml of gastric juice was neutralized with 1 N NaOH and centrifuged at 15000 rpm for 10 minutes. The pellet was resuspended in 0.5 ml of tissue extraction buffer, and the DNA was extracted. The *H. pylori* DNA was amplified with a primer pair for *cagA* as described by Tummuru et al²⁾. Nested PCR was used to detect *cagA*: first primer pair, 5'-(GATAACAGGCAAGCTTTGAGG)-3' (sense) and 5'-(CTGCAA AAGATTGTTTGGCAGA)-3' (antisense); second primer pair, 5'-(AGGGAAGAATACTC CAATAAAGCGA)-3' (sense) and 5'-(CTGCAAAAGATTGTTTGGCAGA)-3' (antisense). The PCR conditions were denaturation at 94°C for 30 seconds, annealing at 55°C for 1.0 minute, and extension at 72°C for 1.0 minute (40 cycles). The amplified products were electrophoresed on a 3.0% agarose gel and stained with ethidium bromide. The primary PCR resulted in a 349 bp fragment and the nested PCR in a 313 bp fragment.

Statistical analysis

Data were analyzed with the Student's *t*-test, the Mann-Whitney U test, and the Fisher exact test. Correlations between titers of serum anti-CagA antibody and scores of chronic inflammation were examined with the Spearman rank correlation test. Differences with a *P* value less than .05 were considered statistically significant. All *P* values are two-tailed.

Results

Serum anti-CagA antibody was detected in 76.7% of patients with gastric ulcers and in 73.7% of those with duodenal ulcers. Among patients with gastric ulcers, the histologic features did not differ significantly between anti-CagA seropositive and seronegative patients (Fig. 1). Among patients with duodenal ulcers, there were no significant differences between the anti-CagA seropositive and seronegative patients in terms of *H. pylori* density,

Fig. 1. Histologic comparison of gastric ulcer patients infected with *H. pylori* who were anti-CagA seropositive or seronegative. Hp, *H. pylori* density, $P=.674$; N, polymorphonuclear neutrophil activity, $P=.204$; M, chronic inflammation, $P=.495$; A-at, atrophy of gastric antrum, $P=.893$; C-at, atrophy of gastric corpus, $P=.671$; Meta, intestinal metaplasia, $P=.431$; T, Total scores, $P=.838$.

Fig. 2. Histologic comparison of duodenal ulcer patients infected with *H. pylori* who were anti-CagA seropositive or seronegative. Hp, $P=.884$; N, $P=.165$; M, $P=.020$ *; A-at, $P=.434$; C-at, $P=.056$; Meta, $P=.896$; Total, $P=.815$.

polymorphonuclear neutrophil activity, glandular atrophy of the antrum or corpus, or intestinal metaplasia. However, scores of chronic inflammatory cell infiltration were significantly higher in seropositive patients than in seronegative patients ($P=.020$) (Fig. 2). Serum titers of anti-CagA antibody correlated with scores of chronic inflammatory cell

Fig. 3. Correlation between serum titers of anti-CagA antibody and scores of chronic inflammatory cell infiltration.

Table 2. The results of gastric juice *cagA* PCR assay and serum anti-CagA antibody status

Gastric juice		Gastric Ulcer (N=29)		Duodenal Ulcer (N=13)	
		<i>cagA</i> +	<i>cagA</i> -	<i>cagA</i> +	<i>cagA</i> -
Anti-CagA antibody	seropositive	21	1	10	0
	seronegative	7	0	3	0

infiltration (titers in patients seronegative for anti-CagA antibodies were regarded as zero) ($r=.525$, $P=.026$) (Fig. 3). The *cagA* gene of *H. pylori* was detected in the gastric juice of 28 of 29 patients with gastric ulcers and in all 13 patients with duodenal ulcers. Moreover, the *cagA* gene of *H. pylori* was found in the gastric juice of all patients seronegative for anti-CagA antibodies (7 with gastric ulcers and 3 with duodenal ulcers) (Table 2).

Discussion

Several factors, such as cytotoxins induced by *H. pylori*, cytokines induced by acute or chronic inflammatory cells, and the immune response of infected subjects, contribute to the gastric mucosal damage^{3, 12, 13}. The CagA protein induced by *H. pylori* is closely associated with gastroduodenal diseases^{5, 14, 15}. Nevertheless, the significance of the serum anti-CagA antibody is poorly understood. The rate of seropositivity for anti-CagA antibody is higher in patients with peptic ulcers than in patients with non-ulcer dyspepsia^{16, 17}. Also, the CagA-positive strain of *H. pylori* is associated with IL-8 induction in gastric epithelium^{4, 18}. Moreover, the *picB* gene of *H. pylori* plays a role in the induction of IL-8 in gastric epithelial cells¹⁹.

In the present study, immunoenzymatic assay showed that the seropositivity for anti-CagA antibody in patients with duodenal ulcers was associated with chronic inflammatory cell infiltration, but was not associated with the scores of *H. pylori* density,

polymorphoneutrophilic cell infiltration, glandular atrophy of antrum or corpus, or intestinal metaplasia. Furthermore, no relationship was found between seropositivity for anti-CagA antibody and histologic scores in patients with gastric ulcers. The previous studies have failed to find any correlation of histologic findings and IgG antibody for CagA protein in patients with peptic ulcers or asymptomatic *H. pylori* gastritis. In fact, the only histologic difference that has been found on the basis of anti-CagA antibody has been mononuclear cell infiltration of the gastric antrum⁷⁾. Moreover, serologic tests were less accurate than PCR for determining the CagA status of *H. pylori* strains²⁰⁾. In the present study, 28 of the 29 patients with gastric ulcers were positive for the *cagA* gene. The 1 patient negative for the *cagA* gene was seropositive for anti-CagA antibody. The *cagA* gene was found in the gastric juice of all 13 patients with duodenal ulcers. Together, these results suggested that the absence in serum of anti-CagA antibody did not necessarily reflect the absence of the *cagA* gene of *H. pylori*.

Inflammatory responses have been reported to be associated with the *cagA* gene^{21,22)}. Therefore, we investigated the correlation between serum titers of anti-CagA antibody and histologic scores in peptic ulcer patients infected with *H. pylori*. In patients with duodenal ulcers, serum titers of anti-CagA antibody correlated positively with scores of chronic inflammatory cell infiltration. However in patients with gastric ulcers, the scores of histologic features did not correlate with serum titers of anti-CagA antibody. Western blotting has shown that the rate of seropositivity for CagA antibody is high in Japanese patients with *H. pylori* infection, irrespective of the presence of a peptic ulcer⁶⁾. Our results of seropositivity for CagA antibody were less than the previously reported results, which may be due to the decreased sensitivity of assaying CagA protein in gastric juice samples by ELISA as compared to Western blotting analysis¹⁷⁾. Furthermore, most Japanese *H. pylori* strains have an intact *cag* pathogenicity island²³⁾. The present histologic examination showed a positive correlation between serum titers of anti-CagA antibody and the severity of chronic inflammatory cell infiltration into the gastric epithelium in patients with duodenal ulcers, but showed no correlation in patients with gastric ulcers. The differing results between patients with gastric ulcers and those with duodenal ulcers may be due to atrophy and intestinal metaplasia of the gastric mucosa, because the mucosal atrophy in the gastric antrum or corpus in patients with gastric ulcers is much more severe than in patients with duodenal ulcers. We found that the scores of intestinal metaplasia and glandular atrophy in the gastric antrum were significantly higher in patients with gastric ulcers than in patients with duodenal ulcers. Scores of glandular atrophy were also slightly but not significantly higher in the gastric corpus. The number of inflammatory cells and lymphoid follicles is lower in intestinalized mucosa than in non-intestinalized gastric mucosa²⁴⁾. In patients with gastric ulcers, the grade of atrophy in gastric mucosa may not correlate with chronic inflammatory cell infiltration. Therefore, the presence in serum of anti-CagA antibodies was not associated with chronic inflammatory cell infiltration in patients with gastric ulcers.

In conclusion, the absence of serum anti-CagA antibodies does not necessarily reflect the absence of the *cagA* gene of *H. pylori*. However, serum titers of anti-CagA antibodies correlated with chronic inflammatory cell infiltration into the gastric mucosa of Japanese duodenal ulcer patients. This may indicate that infection with *cagA*-positive strains of *H. pylori* is associated with chronic inflammation in the gastric mucosa of Japanese patients

with duodenal ulcers.

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References

- 1) Cover TL and Blaser MJ: Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. *J Biol Chem* **267** : 10570-10575 (1992)
- 2) Tummuru MKR, Cover TL and Blaser MJ: Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. *Infect Immun* **61** : 1799-1809 (1993)
- 3) Crabtree JE, Wyatt JI, Trejdosiewicz LK, Peichl P, Nichols PH, Ramsay N, Primrose JN and Lindley IJD: Interleukin-8 expression in *Helicobacter pylori* infected, normal, and neoplastic gastroduodenal mucosa. *J Clin Pathol* **47** : 61-66 (1994)
- 4) Crabtree JE, Covacci A, Farmery SM, Xiang Z, Tompkins DS, Perry S, Lindley IJD and Rappuoli R: *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial cells is associated with CagA positive phenotype. *J Clin Pathol* **48** : 41-45 (1995)
- 5) Crabtree JE, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN and Morgan AG: Systemic and mucosal humoral responses to *Helicobacter pylori* in gastric cancer. *Gut* **34** : 1339-1343 (1993)
- 6) Maeda S, Kanai F, Ogura K, Yoshida H, Ikenoue T, Takahashi M, Kawabe T, Shiratori Y and Omata M: High seropositivity of anti-CagA antibody in *Helicobacter pylori*-infected patients irrelevant to peptic ulcers and normal mucosa in Japan. *Dig Dis Sci* **42** : 1841-1847 (1997)
- 7) Graham DY, Genta RM, Graham DP and Crabtree JE: Serum CagA antibodies in asymptomatic subjects and patients with peptic ulcer: lack of correlation of IgG antibody in patients with peptic ulcer or asymptomatic *Helicobacter pylori* gastritis. *J Clin Pathol* **49** : 829-832 (1996)
- 8) Valentine JL, Arthur RR, Mobley HLT and Dick JD: Detection of *Helicobacter pylori* by using the polymerase chain reaction. *J Clin Microbiol* **29** : 689-695 (1991)
- 9) Dixon MF, Genta RM, Yardley JH and Correa P: Classification and grading of gastritis: The Updated Sydney System. *Am J Surg Pathol* **20** : 1161-1181 (1996)
- 10) Schembri MA, Lin SK and Lambert JR: Comparison of commercial diagnostic tests for *Helicobacter pylori* antibodies. *J Clin Microbiol* **31** : 2621-2624 (1993)
- 11) Navaglia F, Basso D, Piva MG, Brigato L, Stefani A, Dal Bo N, Mario FD, Rugge M and Plebani M: *Helicobacter pylori* cytotoxic genotype is associated with peptic ulcer and influences serology. *Am J Gastroenterol* **93** : 227-230 (1998)
- 12) Correa P: Human gastric carcinogenesis: A multistep and multifactorial process—first American Cancer Society award lecture on cancer epidemiology and prevention. *Cancer Res* **52** : 6735-6740 (1992)
- 13) Negrini R, Lisato L, Zanella I, Cavazzini L, Gullini S, Villanacci V, Poiesi C, Albertini A and Ghielmi S: *Helicobacter pylori* infection induces antibodies cross-reacting with human gastric mucosa. *Gastroenterology* **101** : 437-445 (1991)
- 14) Covacci A, Censini S, Bugnoli M, Petracca R, Burrone D, Macchia G, Massone A, Papini E, Xiang Z, Figura N and Rappuoli R: Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* **90** : 5791-5795 (1993)
- 15) Shimoyama T, Fukuda S, Tanaka M, Mikami T, Munakata A and Crabtree JE: CagA seropositivity associated with development of gastric cancer in Japanese population. *J Clin Pathol* **51** : 225-228 (1998)
- 16) Ching CK, Wong BCY, Kwok E, Ong L, Covacci A and Lam SK: Prevalence of CagA-bearing *Helicobacter pylori* strains detected by the anti-CagA assay with peptic ulcer disease and in controls. *Am J Gastroenterol* **91** : 949-953 (1996)
- 17) Cover TL, Glupczynski Y, Lage AP, Burette A, Tummuru MK, Perez PG, Perez-perez GI and Blaser MJ: Serologic detection of infection with *cagA*⁺ *Helicobacter pylori* strains. *J Clin Microbiol* **33** : 1496-1500 (1995)
- 18) Sharma SA, Tummuru MKR, Miller GG and Blaser MJ: Interleukin-8 response of gastric epithelial cell lines to *Helicobacter pylori* stimulation *in vitro*. *Infect Immun* **63** : 1681-1687 (1995)
- 19) Tummuru MKR, Sharma SA and Blaser MJ: *Helicobacter pylori* *picB*, a homologue of the *Bordetella pertussis* toxin secretion protein, is required for induction of IL-8 in gastric epithelial cells. *Mol Microbiol* **18** : 867-876 (1995)
- 20) Yamaoka Y, Kodama T, Graham DY and Kashima K: Comparison of serological tests to determine the CagA or VacA status of *Helicobacter pylori* strains. *J Clin Microbiol* **36** : 3433-3434 (1998)
- 21) Peek RM Jr, Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC and Blaser MJ: Heightened

- inflammatory response and cytokine expression *in vivo* to *cagA*⁺ *Helicobacter pylori* strains. *Lab Invest* **71** : 760-770 (1995)
- 22) Gunn MC, Stephens JC, Stewart JAD, Rathbone BJ and West KP: The significance of *cagA* and *vacA* subtypes of *Helicobacter pylori* in the pathogenesis of inflammation and peptic ulceration. *J Clin Pathol* **51** : 761-764 (1998)
- 23) Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F and Kato N: Structure of *cag* pathogenicity island in Japanese *Helicobacter pylori* isolates. *Gut* **44** : 336-341 (1999)
- 24) Tsutsumi Y, Nagura H and Watanabe K: Immune aspects of intestinal metaplasia of the stomach: an immunohistochemical study. *Virchows Arch A Pathol Anat* **403** : 345-359 (1984)

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