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☐ Original Article ☐

Relationship of CagA to Serum Gastrin Concentrations and Antral G, D Cell Densities in *Helicobacter pylori* Infection

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Abstract

The purpose of this study was to investigate whether the densities of antral gastrin and somatostatin-immunoreactive cells in *Helicobacter pylori* (*H. pylori*) infection were related to the bacterial expression of cytotoxin-associated gene A (CagA). 32 patients who had underwent diagnostic esophagogastroduodenoscopy were studied. On the histologic examination all patients had antral gastritis. We divided the subjects into three groups. Group I consisted of 6 patients who had chronic superficial gastritis, group II, 9 patients who had *H. pylori*-associated gastritis but with no expression of CagA, and group III, 17 patients who had *H. pylori*-associated gastritis with the expression of CagA. In group I and II, serum gastrin levels, and antral G cell and D-cell were measured. In group III, serum gastrin levels, and antral G cell and D-cell were measured, before and after the eradication of *H. pylori*. The results were as follows. Firstly, serum gastrin concentrations were significantly higher in the patients with *H. pylori* infection than in the negative controls. Nextly, there was no correlation between the changes in antral G or D-cell density and *H. pylori* infection. Thirdly, group III had a significant increase in serum gastrin concentrations and a significant decrease in antral D-cell density than group I. Forthly, eradication of *H. pylori* in group III showed a significantly increased antral D-cell density. Our results suggest that hypergastrinemia in *H. pylori*-associated gastritis is relevant to the presence of CagA, and the possible mechanism of hypergastrinemia may be related to antral D-cell deficiency, which is caused by *H. pylori* infection with the expression of CagA.

Key Words: *Helicobacter pylori*, CagA, G cell, D cell, gastrin

INTRODUCTION

Gastrin and somatostatin are well known as regulators of gastric acid secretion. Gastrin is released from antral G-cells and stimulates gastric acid secretion from the parietal cells.[1] Somatostatin is released from antral D-cells[1] and suppresses gastric acid secretion by acting directly on the parietal cell and by suppressing gastrin release from G-cells.[2] *H. pylori* is known as an etiologic factor of chronic active gastritis and the major pathogenic factor in peptic ulcer disease.[3] Also, *H. pylori* infection is associated with increases in serum gastrin concentration in patients with gastritis. Circulating gastrin concentrations are higher in patients positive for *H. pylori* than in subjects without the bacterium.[4,5] Indeed, *H. pylori*-positive patients present increased levels of basal, postprandial, and meal-stimulated plasma gastrin concentrations.[6,7] Moreover, clearance of the microorganism with antimicrobial agents leads to a rapid decrease in serum gastrin concentration among patients with and without duodenal ulcer.[8] Previous studies show that *H. pylori*-positive patients present a significantly lower antral somatostatin concentration than *H. pylori*-negative individuals.[9,10] This somatostatin deficiency is thought to be a factor contributing to hypergastrinemia through decreased paracrine inhibition of the gastrin-producing G cells in the antrum.[11,12] Another study demonstrates the eradication of *H. pylori* is followed by increases in the antral D-cell density and somatostatin mRNA, which is accompanied by a decrease in neither G-cell nor gastrin mRNA.[13] *H. pylori* strains show phenotypic heterogeneity in their ability to express CagA, which is thought to be virulent factor. Strains expressing the CagA protein have been strongly associated with

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severe gastritis, duodenal ulceration, and gastric adenocarcinoma.[14] The relation of CagA on G cells and D cells has not been assessed previously in *H. pylori* positive subjects with gastritis.

To elucidate further mechanism of hypergastrinemia in *H. pylori*-associated gastritis, we measured serum gastrin concentrations and antral G-cell and D-cell densities in the antral gastric mucosa of patients with *H. pylori*-positive and *H. pylori*-negative gastritis. we then compared the results of these measurements to the serologic recognition of CagA. We also studied the effects of the eradication of *H. pylori* on the densities of antral G-cell and D-cell.

MATERIALS AND METHODS

We studied 32 patients (13 men, 19 women; mean age 43.3±12.9 years) who underwent diagnostic esophagogastroduodenoscopy (GIF-XQ 230, Olympus, Tokyo, Japan) for investigation of dyspepsia. 26 patients were positive for *H. pylori* and 6 were negative by H&E and Giemsa stain for antral biopsy. For the eradication of *H. pylori* infection, we treated the 26 patients with Omeprazole 40 mg, amoxicillin 1.5 g and metronidazole 750 mg for 10 days. A total of 7 patients were cured completely. Serum levels of gastrin were measured by radioimmunoassay using a double antibody (Gastrin Products Corporation, Los Angeles, USA). Bacterial expression of CagA was determined indirectly by Western Blot assay, using a Helicoblot 2.0, of serum IgG antibodies to these proteins (Genelabs Diagnostics, Singapore).

For the measurement of the densities of immunoreactive cells for somatostatin (D cells) and gastrin (G cells), 3 to 4 biopsy samples from the antrum were immediately fixed in Bouin's solution for 18 h, dehydrated, and embedded in paraffin. 2 μm thick sections were cut perpendicular to the surface of the mucosa and were deparaffinised. The deparaffinized sections were immersed in methanol containing 3 percent hydrogen peroxidase for 30 minutes at room temperature to eliminate endogenous peroxidase activity. The specimens were immunostained with polyclonal antibodies to gastrin (1 : 100, DAKO, Corporation, Carpinteria, CA, USA) and somatostatin (1 : 100, DAKO Corporation, Carpinteria, CA, USA) by the ABC method (Fig. 1 and 2). Finally, the sections were counterstained with haematoxylin. We examined only the sections showing the whole area between the surface and the muscularis mucosae with an intact mucosa. At least 10 adjacents areas encompassing the mid-zone of the mucosa from 3 or 4 different sections were used for counting G and D cells on the microscope (×200). Each cell was identified as G- or D-cell if a dark-brown granular reaction was produced by the ABC method. The cells were counted independently by two observers and the mean number of cells through the square field, mm^2 of mucosae, were calculated. To ensure manual analysis, we undertook quantitation using a computer-derived image analysis. Results were expressed as means±

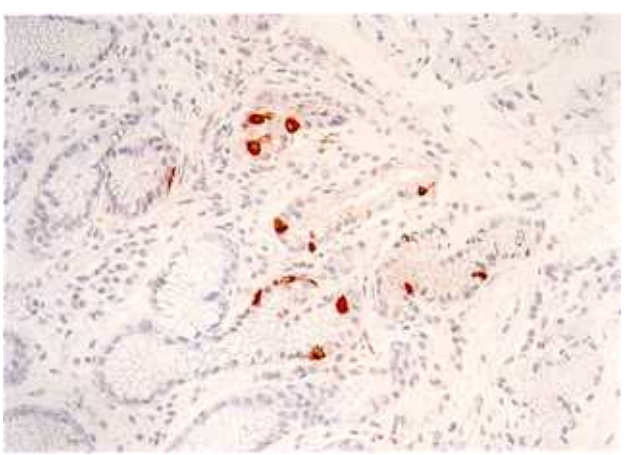

Fig. 1. Gastrin-immunoreactive cells in the antral mucosa was identified as a dark-brown granular reaction (avidin-biotin peroxidase complex method, ×200).

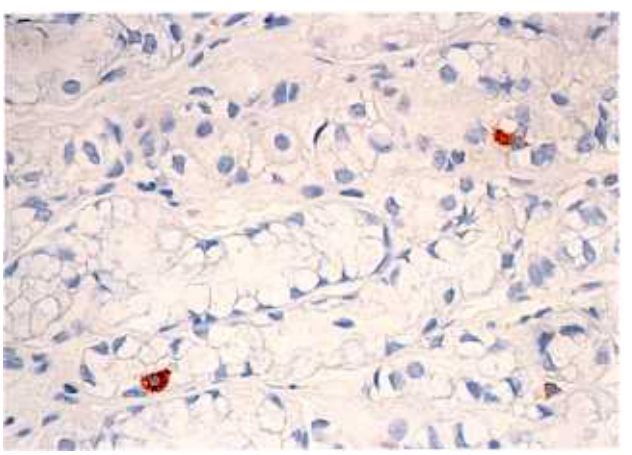

Fig. 2. Somatostatin-reactive cells in the antral mucosa was identified as a dark-brown granular reaction (avidin-biotin peroxidase complex method, ×400).

SEM. The Mann-Whitney *U* test for unpaired data was used in statistical analysis. The differences were taken as significant when $p<0.05$.

RESULTS

On histology, all patients had antral gastritis, which was active (polymorphonuclear neutrophils were present in the lamina propria or glandular and superficial epithelia, or both), but no atrophy or intestinal metaplasia was observed. In 7 of the 26 patients positive for *H. pylori* who received triple antimicrobial therapy, *H. pylori* was eradicated, as confirmed by a negative histology of the antrum. Antral G-cell and D-cell densities were measured before and after eradication.

On the basis of *H. pylori* infection and serologic recognition of CagA, patients were divided into three groups: group I=*H. pylori*-negative gastritis (n=6); group II=*H. pylori*-positive, CagA-negative gastritis (n=9); and group III=*H. pylori*-positive, CagA-positive gastritis (n=17). Serum gastrin concentrations were significantly higher in patients with *H. pylori* positive compared to *H. pylori* negative subjects (98.8 ±96.7 vs 33.5±8.6 pg/ml; $p<0.05$) (Fig. 3). In antral G-cell and D-cell densities, no significant difference was found between the positive and negative patients (Table 1). Significantly higher serum gastrin concentrations were found in group III than group I (111.7±108.3 vs 33.5±8.7 pg/ml; $p<0.05$) (Fig. 4). Antral D-cell density was significantly lower in group III compared with group I (12.9±10.1 vs 31.4 ±17.8/mm^2; $p<0.05$) (Fig. 5). There was no significant difference in antral G-cell density between the groups (Fig. 5). No significant difference in the gastrin level and antral D-cell density were found between groups I & II. Eradication of *H. pylori* in group III was accompanied by a significant increase in D-cell density (40.1±18.0 vs 17.8±12.0/mm^2; p

Table 1. Serum Gastrin Concentrations, Antral G- and D-cell Densities

Group	HP (−) N=6	HP (+) N=26
Gastrin (pg/ml)	33.5±8.6	98.8±96.7*
G-cell (No./mm^2)	120.0±42.2	123.7±61.5
D-cell (No./mm^2)	31.4±17.8	16.5±12.4

HP, *Helicobacter pylori*. *$p<0.05$.

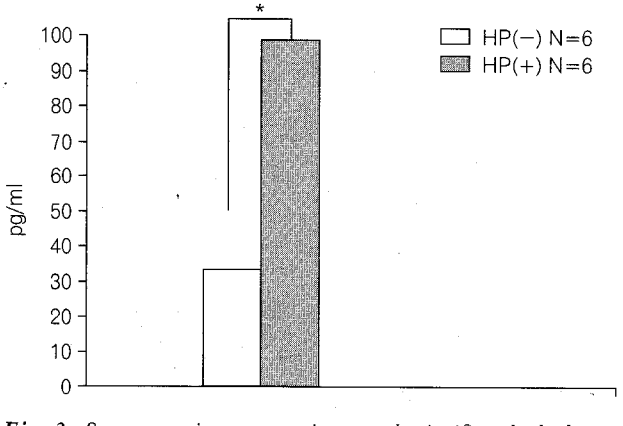

*Fig. 3. Serum gastrin concentration reveals significantly higher in the patients with H. pylori positive than in the negative subjects (*p <0.05).*

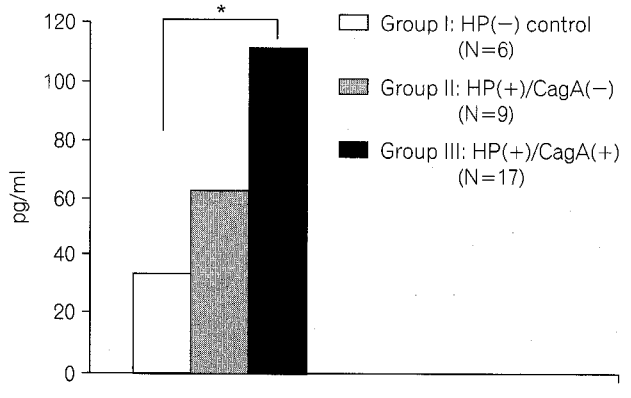

*Fig. 4. Serum gastrin concentrations are higher in group III than group I (*p <0.05).*

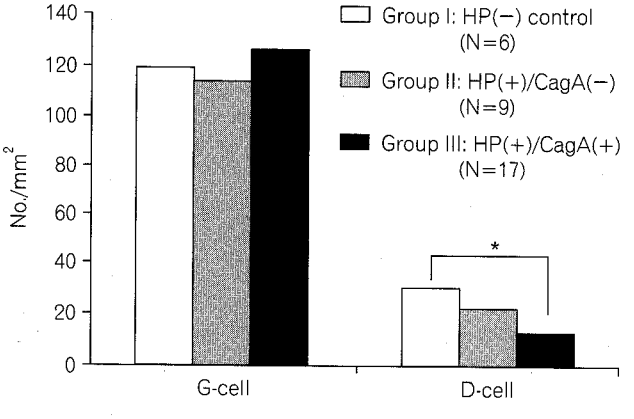

*Fig. 5. Antral G-cell and D-cell densities reveal that antral D cell density is significantly lower in group III than group I (*p <0.05).*

Table 2. Antral G- and D-cell Densities before and after Eradication of HP in Group III (N=7)

Group	before	after
G-cell (No./mm^2)	120.0±101.4	118.4±42.4
D-cell (No./mm^2)	17.8±12.0	40.1±18.0*

*$p<0.05$.

<0.05) (Table 2).

DISCUSSION

Earlier work showed that antibody response to a particular protein of *H. pylori* was associated with duodenal ulcer disease.[15] The protein was named cytotoxin-associated gene A (CagA). The gene encoding for CagA is not present in all strains of *H. pylori*.[16] *H. pylori* strains show a phenotypic heterogeneity in their ability to express CagA, which is thought to be a virulent factor.[17,18] It can be detected by hybridization or by a combination of polymerase chain reactions (PCRs) with different primers, since there is some variability within its structure. The protein has a Mwt. of 120 kDa and is highly immunogenic. Only the strains of *H. pylori* which are CagA (+) can induce the production of interleukin 8 by epithelial cells.[19] Therefore, CagA seems to be responsible for severe inflammation observed in the gastric mucosa.

H. pylori strains are subdivided into two different major types according to the expression of CagA. In several studies, about 60−70% of the *H. pylori* isolates are shown to be CagA positive.[20,21] Strains expressing the CagA protein are considered to produce more severe gastritis than CagA (−) strains and are linked with duodenal ulceration.[22] Although we still do not know the mechanism of CagA, the detection of the CagA protein or the *cag*A gene in a *H. pylori* strain is currently considered the best marker of pathogenicity of peptic disease.[23]

Patients positive for *H. pylori* have abnormalities in the regulation of gastrin release, which may be the link between *H. pylori* and duodenal ulcer, since gastrin could play an important role in acid hypersecretion. Gastrin-stimulated acid secretion increases in patients positive for *H. pylori* with or without duodenal ulcer, but more so in patients with duodenal ulcer. Eradication of *H. pylori* leads to a reduction in gastrin-mediated acid secretion.[24] Many studies have been performed to elucidate the mechanism that causes hypergastrinemia in *H. pylori* infection. The mechanism by which *H. pylori* changes gastrin metabolism, however, has been only partly elucidated. Also, the mechanism of gastrin release by *H. pylori* remains uncertain, but studies on the regulation of gastrin secretion have shown that gastrin (G)-cells are under constant restraint by somatostatin (D)-cells in the antral mucosa. Since a delicate balance of hormonal mechanisms regulate the secretion of gastrin, hypergastrinemia may be due to a decrease in somatostatin, a powerful and effective inhibitor of antral gastrin cells and gastric acid secretion.[25] The changes may also be caused by cytokines, interleukin-2 and interferon, released from the inflammatory cells of the antral mucosa, which can stimulate G cells.[26] Other suggested explanations for hypergastrinemia include alkalinization of the gastric mucous layer by bacterial urease.[27] However, other studies have failed to demonstrate a direct effect of elevated pH level on the antral surface in hypergastrinemia.[28]

This study confirms the existence of an antral D-cell density deficiency in CagA-positive *H. pylori*-associated gastritis (group III). The present results indicate that the eradication of *H. pylori* is accompanied by an increase in antral D-cell density. Although these changes do not directly reflect D-cell activity, indirectly they may imply that there is a decrease in synthesis and release of somatostatin. Similar observations have been found that D-cell density and somatostatin mRNA increase after *H. pylori* eradication.[13,29] The antral D-cell density deficiency does not differ significantly between groups I and II. Furthermore, our study shows decreased concentrations of serum gastrin in group I compared with group III. The finding that antral D-cell density deficiency depends on the presence of CagA in *H. pylori* infection points to an important role of the CagA in the mechanism of hypergastrinemia in *H. pylori*-associated gastritis. With regard to G-cells, our results are in agreement with another study[30] that the number of G-cells in the antral mucosa does not seem to be affected by the presence of *H. pylori*. Moreover, antral G-cell density was not affected by serologic recognition of CagA.

Although we observed an increase in the antral

D-cell density after *H. pylori* eradication in group III, no differences in the number of G cells have been found between patients positive and negative for *H. pylori* or after eradication of the microorganism.[12] Another study, however, describs an adult with *H. pylori*-associated duodenal ulcer and primary gastrin-cell hyperplasia in whom eradication of the microorganism was accompanied by a return to normal serum gastrin concentrations and a significant decrease in gastrin cell density.[31]

In conclusion, the present study shows that hypergastrinemia in CagA-positive *H. pylori*-associated gastritis is dependent on antral D-cell density deficiency, and is partially affected by the presence of CagA.

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