Analysis of Immunoglobulin A Antibodies to *Helicobacter pylori* in Serum and Gastric Juice in Relation to Mucosal Inflammation

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*Helicobacter pylori* is a major etiologic agent in gastroduodenal disorders. In this study, immunoglobulin A (IgA) antibodies to *H. pylori* in serum and gastric juice specimens obtained from patients with gastritis or peptic ulcers by utilizing antibody capture enzyme-linked immunosorbent assays (ACELISAs). Urease α subunit (UA), urease β subunit (UB), the 66-kDa heat shock protein (HSP), and the 25-kDa protein (25K) were used as antigens for the ACELISAs. The antibody titers were expressed as the ratio of *H. pylori*-specific IgA to total IgA. The ratio is stable, although the antibody concentration fluctuates in gastric juice. By using ACELISAs it was possible to evaluate quantitatively not only serum IgA antibodies but also gastric juice secretory IgA (S-IgA) antibodies. In both serum IgA and gastric juice S-IgA ACELISAs, the titers of antibody to HSP and 25K were remarkably correlated with the histologic grade of gastritis, whereas those to UA and UB were not strongly correlated with histologic grade. Thus, it is useful for estimating the histologic grade of gastritis to quantify serum IgA and gastric juice S-IgA antibodies to HSP and 25K.

**MATERIALS AND METHODS**

**Sera and gastric juice.** Serum and gastric juice samples were obtained from 19 *H. pylori*-positive patients for this study. The ages of the patients ranged between 20 and 80 years, and there were 16 males and 3 females. Eleven patients had chronic gastritis, and eight had peptic ulcers. All of the patients were diagnosed through endoscopic examinations. Serum and gastric juice samples were also collected from six *H. pylori*-negative healthy male volunteers. The age of the volunteers ranged between 19 and 29 years. The volunteers were used as a negative-control group. Serum samples were stored at −80°C until tested. Gastric juice samples were acquired by washing gastric mucosa with phosphate-buffered saline (PBS) (pH 7.4). The washings were neutralized and dialyzed with distilled water (32). After dialysis, the samples were lyophilized and stored at −20°C until tested.

**Gastric biopsy specimens.** Biopsies were performed by utilizing endoscopy from both the disease and the control groups. Three biopsy specimens were taken from the gastric antrum of each subject. One antral biopsy specimen was used for culture, and another was used for a rapid urease test (Minitek Disk; BBL, Cockeysville, Md.) (14). The third specimen was fixed with formalin for histopathology, and immunostaining was performed by using anti-*H. pylori* monoclonal antibody (24). *H. pylori* infection was judged positive when the result of either the culture test, the rapid urease test, or the immunostaining was positive.

The biopsy tissue sections were also stained with hematoxylin and cosin, and the numbers of infiltrated cells were counted per 0.015 mm². Three sections from antral biopsy tissue were examined for each *H. pylori*-positive patient, and the median value of the three examinations was considered the histologic grade of gastritis (1).

**H. pylori antigens.** *H. pylori* (ATCC 43504) was inoculated onto brain heart infusion agar (Difco, Detroit, Mich.) containing 8% horse blood and incubated microaerobically (GasPak System without catalyst; BBL) at 37°C for 5 days (12). The organisms were harvested, washed three times with PBS, and resuspended in electrophoresis sample buffer (10 mM Tris-HCl [pH 6.8] containing 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 10% glycerol, and 1 mM phe- nylmethylsulfonyl fluoride). The resulting suspension was sonicated and heated at 100°C for 5 min. Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 12.5% separating gel and a 5% stacking gel. After electrophoresis, the 66-kDa, 60-kDa, 30-kDa, and 25-kDa proteins were cut out and electrodialyzed from the separating gel. The solution containing each antigen was dialyzed against 0.1 M NaHCO₃, (pH 8.4). After dialysis, the protein concentrations of these antigen solutions were assayed by using the Bio-Rad protein assay kit (Bio-Rad, Richmond, Calif.) and adjusted to 100 μg/ml each. Subsequently, 2 μl of each antigen solution was mixed with 120 μl of N-hydroxysuccinimidyl biotin (1 mg/ml) in dimethyl sulfoxide, kept at room temperature for 4 h, and dialyzed against PBS (17). After dialysis, the protein concentrations of
The plates were then washed, and 100 μl containing 1% bovine serum albumin (PBS-BSA) and incubating for 1 h at 37°C. 0.1% Tween 20. The free binding sites were blocked by adding 150 μl of PBS-BSA, added to each well, and incubated for 1 h at 37°C. After a wash, 100 μl of test gastric juice was dissolved in 100 μl of PBS-BSA, added to each well, and incubated for 2 h at 37°C, after which a fixed amount of S-IgA was captured per well. Under this condition, 39 ng of S-IgA was defined as the antibody titer.

RESULTS

IgA and S-IgA ACELISAs. The results from the IgA and S-IgA ACELISAs are shown in Fig. 1. With all antigens, the H. pylori-positive patients had significantly higher serum IgA antibody titers than the H. pylori-negative controls. The titers of gastric juice S-IgA antibody to any H. pylori antigens were also significantly higher in the patients than in the control group.

Correlation between serum IgA and histologic grade. The histologic grade of gastritis was determined according to the number of infiltrated cells per 0.015 mm² obtained from the gastric biopsy. The results are summarized in Fig. 2. With all antigens, IgA antibody titers were positively correlated with histologic grade. The correlation between anti-25K and anti-HSP IgA antibody titer and histologic grade was especially...
significant. On the other hand, the correlation between anti-UA and anti-UB IgA antibody titer and histologic grade was statistically significant but not remarkable.

**Correlation between gastric juice S-IgA and histologic grade.** The anti-UA and UB S-IgA antibody titers were negatively correlated with the histologic grade of gastritis (Fig. 3); however, the correlation was not remarkable. On the other hand, S-IgA antibody titers to 25K and HSP were positively correlated with histologic grade (Fig. 3).

**DISCUSSION**

In general, the antibody titer indicates the concentration of antibody. This is strongly influenced by the concentration of total immunoglobulin. However, the antibody titer in the ACELISA does not reflect the concentration of antibody but, rather, the ratio of *H. pylori*-specific IgA to total IgA (4, 10). The ratio is negligibly influenced by the concentration of total IgA. Thus, the S-IgA ACELISA made it possible to evaluate quantitatively S-IgA antibodies to *H. pylori* antigens in gastric juice, even though the concentration of total S-IgA in gastric juice fluctuates greatly. The S-IgA ACELISA does not measure serum IgA which leaks into gastric juice, because it uses anti-secretory component. The S-IgA ACELISA may measure not only S-IgA but S-IgM associated with secretory component. However, the concentration of S-IgM in secretions is far less than that of S-IgA (2, 3, 21). We tried to measure the concentration of S-IgM in gastric juice but did not detect it (unpublished data). Thus, S-IgM would have little influence on the results of S-IgA ACELISA. On the other hand, the IgA ACELISA was well suited for detecting *H. pylori*-specific IgA in serum. In both ACELISAs, the antibody titers of the patients were significantly higher than those of the controls. Both are useful in the diagnosis of *H. pylori*-associated diseases. Furthermore, in this type of ELISA, false-positive results are rarely obtained, though highly positive sera occasionally give relatively low values (11).

In *H. pylori*-positive patients, the serum IgA antibody titers to each *H. pylori* antigen were positively correlated with the histologic grade of gastritis. The correlation was especially significant between anti-25K and anti-HSP serum IgA antibody titers and histologic grade. The titers of gastric juice S-IgA antibody to 25K and HSP were also positively correlated with histologic grade. These results suggest that IgA and S-IgA antibodies to 25K and HSP are closely associated with the histologic grade of gastritis. Thus, it is beneficial for evaluating the histologic grade of gastritis to quantify serum IgA and gastric juice S-IgA antibodies to 25K and HSP. On the other hand, titers of gastric juice S-IgA antibody to UA and UB were negatively correlated with histologic grade, though the correlation was not remarkable. The pathophysiological states of anti-UA and -UB S-IgA may be different from those of anti-25K and -HSP S-IgA.

Urease is an essential enzyme for *H. pylori* colonization (18). The gastric juice S-IgA antibodies to urease, induced by oral immunization, can prevent *Helicobacter* infections in animal models (6, 13, 15, 19). However, serum IgG antibodies, induced by intravenous or subcutaneous immunization, cannot (13, 16). Thus, gastric juice S-IgA antibodies would play an important role in *H. pylori* infection. Our studies demonstrated
that human serum IgA antibodies to urease were positively correlated with the histologic grade of gastritis. On the contrary, gastric juice S-IgA antibodies to urease were negatively correlated with histologic grade, though the correlation was not remarkable. These results suggest that the S-IgA antibodies to urease may bind to H. pylori in gastric mucosa and inhibit H. pylori colonization in humans as well as animal models.

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