CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Sept. 1998, p. 617–621 Vol. 5, No. 5
1071-412X/98/$04.00+0

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

SHUNJI HAYASHI,[1]* TOSHIRO SUGIYAMA,[2] KENJI YOKOTA,[3] HIROSHI ISOGAI,[4] EMIKO ISOGAI,[5] KEIJI OGUMA,[3] MASAHIRO ASAKA,[2] NOBUHIRO FUJII,[6] AND YOSHIKAZU HIRAI[1]

Department of Microbiology, Jichi Medical School, Tochigi-ken 329-0498,[1] Third Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo 060-8638,[2] Department of Bacteriology, Okayama University Medical School, Okayama 700-8558,[3] Animal Experimentation Center[4] and Department of Microbiology,[6] Sapporo Medical University School of Medicine, Sapporo 060-8556, and Department of Hygiene, Health Sciences University of Hokkaido, Hokkaido 061-0293,[5] Japan

Received 29 December 1997/Returned for modification 24 March 1998/Accepted 12 June 1998

***Helicobacter pylori* is a major etiologic agent in gastroduodenal disorders. In this study, immunoglobulin A (IgA) antibodies to *H. pylori* antigens were evaluated 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 of the ACELISAs reflect 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.**

Helicobacter pylori is a causative agent in chronic gastritis (31), peptic ulcers (9), and gastric cancer (7). The detection of antibodies specific to *H. pylori* in serum is important in the diagnosis of these diseases (5, 24). However, *H. pylori* infections occur in the gastric mucosa (29). The mucosal immune response against *H. pylori* plays an important role in the development of gastric mucosal lesions (1, 20, 25, 26). Immunoglobulin A (IgA) is the main immunoglobulin in secretions (30). Thus, it is thought to be important to detect gastric juice secretory IgA (S-IgA) antibodies to *H. pylori*. It is, however, difficult to evaluate quantitatively S-IgA antibodies in gastric juice, because the concentration of S-IgA fluctuates greatly in gastric juice. To solve this problem, we have developed antibody capture enzyme-linked immunosorbent assays (ACELISAs) (10), which are used to assay serum IgA and gastric juice S-IgA antibodies specific to *H. pylori*. Urease α subunit (UA), urease β subunit (UB), the 66-kDa heat shock protein (HSP), and the 25-kDa protein (25K) were obtained from *H. pylori* and used as antigens for the ACELISAs (33). These are the major proteins which are recognized by the sera as well as the gastric juice of *H. pylori*-positive patients (24). In this report, we discuss the relationships between IgA antibodies to these *H. pylori* antigens and gastric mucosal inflammation.

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 eosin, and the numbers of infiltrated cells were counted per 0.015 mm^2. 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 phenylmethylsulfonyl 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 electroeluted from the separating gel. The solution containing each antigen was dialyzed against 0.1 M $NaHCO_3$ (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 ml of each antigen solution was mixed with 120 μl of *N*-hydroxysuccinimide 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

* Corresponding author. Mailing address: Department of Microbiology, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Tochigi-ken 329-0498, Japan. Phone: 81-285-58-7332. Fax: 81-285-44-1175. E-mail: shunhaya@jichi.ac.jp.

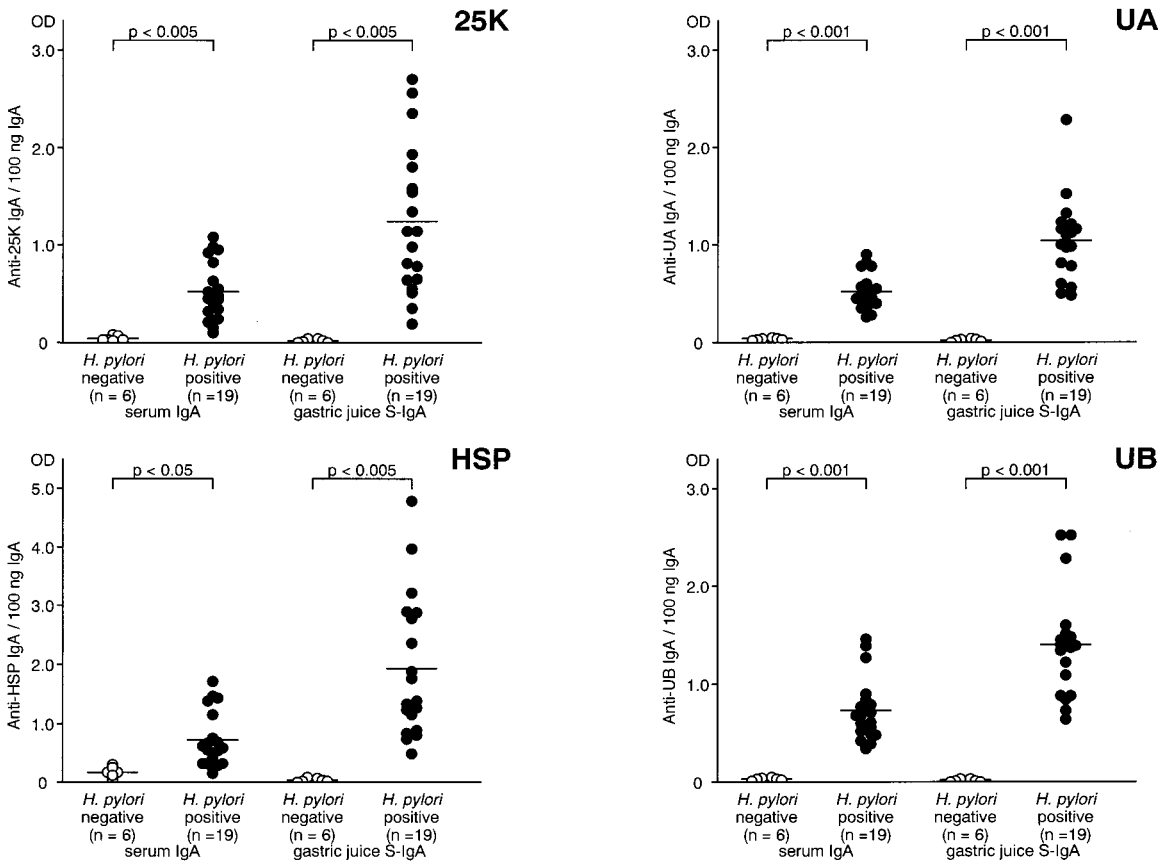

FIG. 1. Antibody titers to *H. pylori* antigens (25K, HSP, UA, and UB) measured by IgA and S-IgA ACELISAs. ●, *H. pylori*-positive patients; ○, *H. pylori*-negative healthy volunteers. Horizontal bars, medians.

these antigen solutions were assayed again and adjusted to 10 μg/ml each, and the solutions were stored at −80°C until tested.

The 30- and 60-kDa proteins were identified as UA and UB, respectively, by N-terminal amino acid analysis. The 66-kDa protein was identified as HSP in the same way (33). However, 25K could not be identified. The N-terminal 20 amino acids of 25K were similar (55% homology) to those of *H. pylori* ferritin with a molecular mass of 19.3 kDa (8, 27). On the other hand, *H. pylori* has a 25-kDa outer membrane protein which binds to laminin (28). Thus, either the ferritin or the laminin-binding protein may be 25K.

Serum IgA ACELISA. Flat-bottom 96-well microtiter plates (EIA Plate High Binding; Costar, Cambridge, Mass.) were coated with 100 μl of goat anti-human IgA (lot H075, monospecific for the α chain; BioMakor, Rehovot, Israel), diluted 1:100 in 50 mM carbonate-bicarbonate buffer (pH 9.6), per well. After an overnight incubation at 4°C, the plates were washed three times with PBS containing 0.1% Tween 20. The free binding sites were blocked by adding 150 μl of PBS containing 1% bovine serum albumin (PBS-BSA) and incubating for 1 h at 37°C. The plates were then washed, and 100 μl of test serum diluted 1:100 in PBS-BSA was added to each well and incubated for 2 h at 37°C, after which a fixed amount of IgA was captured per well. Under this condition, 126 ng of IgA (mean, 126.0 ± 1.1 ng; range, 123.8 to 128.1 ng) was consistently captured per well (10). The plates were then washed, and 100 μl of each biotinylated *H. pylori* antigen diluted 1:100 in PBS-BSA was added to each well and incubated for 2 h at 37°C. After a wash, 100 μl of alkaline phosphatase-conjugated streptavidin (Sumitomo Kinzoku Co., Tokyo, Japan) diluted 1:2,000 in PBS was added and incubated for 1 h at 37°C. After a wash, 50 μl of the substrate solution of an ELISA amplification system (Gibco BRL, Gaithersburg, Md.) (22, 23) was added and incubated at room temperature for 15 min, and 50 μl of an amplifier solution was added and incubated at room temperature for 15 min. The reaction was terminated by adding 50 μl of 0.3 M H_2SO_4. The optical density (OD) of the reaction was measured at 495 nm with a microplate reader (model 3550 EIA reader; Bio-Rad).

The OD represents the quantity of each *H. pylori* antigen-specific IgA per well. Thus, the OD corresponds to the quantity of *H. pylori* antigen-specific IgA contained in 126 ng of IgA. The amount of *H. pylori* antigen-specific IgA contained in 100 ng of IgA was considered the antibody titer.

Gastric juice S-IgA ACELISA. Microtiter plates were coated with 100 μl of goat anti-human secretory component (lot H211, monospecific for secretory component; BioMakor), diluted 1:1,000 in 50 mM carbonate-bicarbonate buffer (pH 9.6), per well and incubated overnight at 4°C. After the wells were washed, they were blocked with PBS-BSA. After a wash, 100 μg of lyophilized 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 (mean, 39.0 ± 0.6 ng; range, 37.5 to 40.2 ng) was consistently captured per well (10). The plates were consecutively treated with each biotinylated *H. pylori* antigen and alkaline phosphatase-conjugated streptavidin. Substrate, amplifier, and stop solutions were added consecutively, and the OD was then measured as mentioned above.

The OD corresponds to the quantity of each *H. pylori* antigen-specific S-IgA in 39 ng of S-IgA. The amount of *H. pylori* antigen-specific S-IgA contained in 100 ng of S-IgA was defined as the antibody titer.

Statistical analysis. The difference between patients and negative controls was evaluated by an unpaired Student's *t* test. The correlation between antibody titer and histologic grade was evaluated by Spearman's rank correlation. A two-tailed *P* value of <0.05 was considered statistically significant.

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 mm^2 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

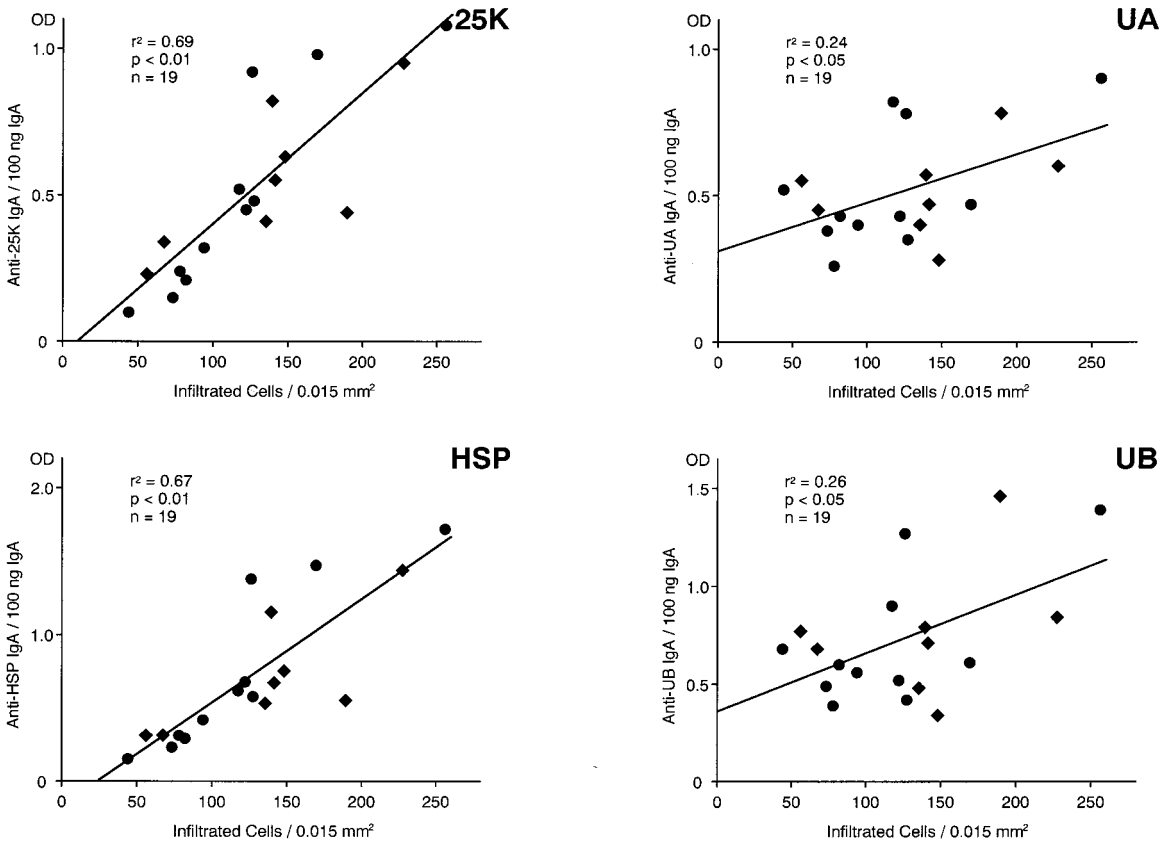

FIG. 2. Correlation between serum IgA antibody titers and histologic grade of gastritis in patients. ●, *H. pylori*-positive patients with chronic gastritis; ◆, *H. pylori*-positive patients with peptic ulcers.

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

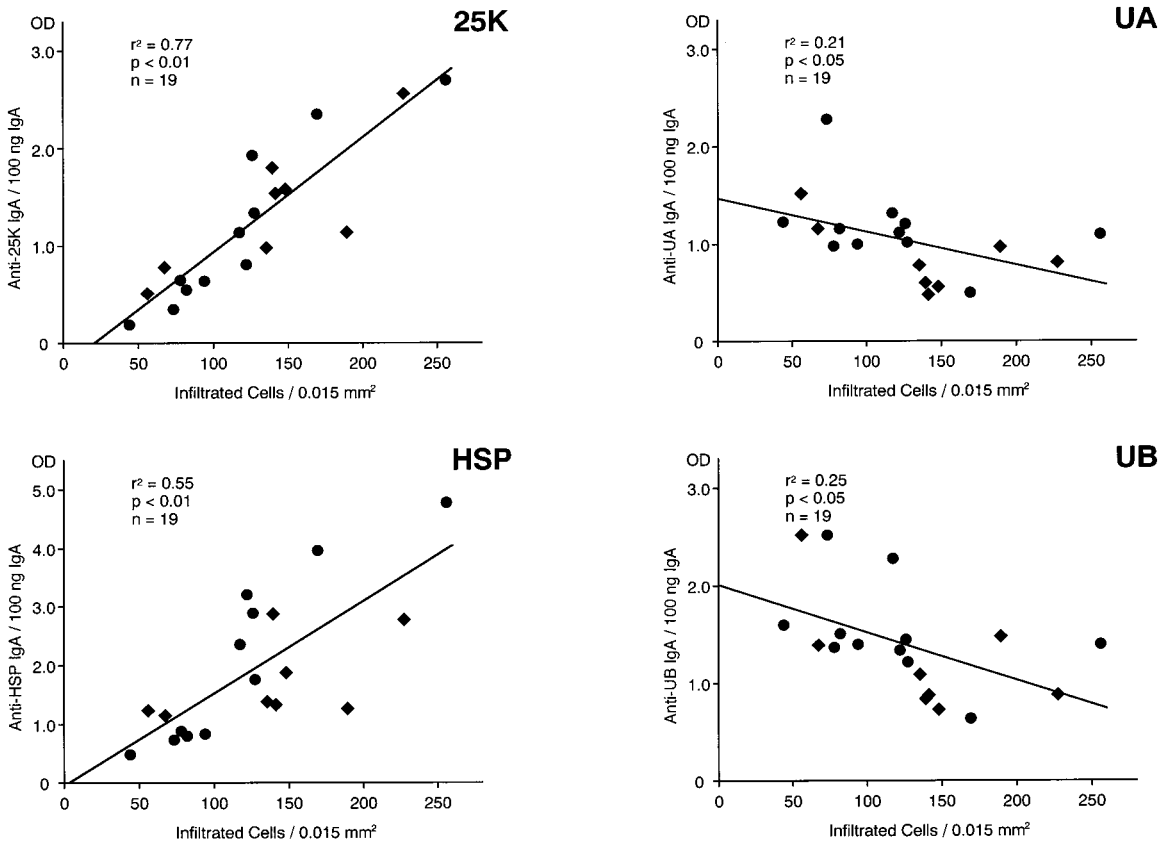

FIG. 3. Correlation between gastric juice S-IgA antibody titers and histologic grade of gastritis in patients. ●, *H. pylori*-positive patients with chronic gastritis; ◆, *H. pylori*-positive patients with peptic ulcers.

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.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research (no. 09770188) from the Japanese Ministry of Education, Science, Sports and Culture; a grant (no. 8-14) from the Japanese Ministry of Health and Welfare; and a grant from the Nakano Foundation.

REFERENCES

1. **Ando, T., K. Kusugami, M. Ohsuga, M. Shinoda, M. Sakakibara, H. Saito, A. Fukatsu, S. Ichiyama, and M. Ohta.** 1996. Interleukin-8 activity correlates with histological severity in *Helicobacter pylori*-associated antral gastritis. Am. J. Gastroenterol. **91:**1150–1156.
2. **Biewenga, J., A. E. Stoop, H. A. M. D. van der Heijden, S. van der Baan, and G. J. van Kamp.** 1995. Albumin and immunoglobulin levels in nasal secretions of patients with nasal polyps treated with endoscopic sinus surgery and topical corticosteroids. J. Allergy Clin. Immunol. **96:**334–340.
3. **Bresson-Hadni, S., M. Rossel, E. Seilles, D. A. Vuitton, K. Guennoune, B. Hory, J. P. Miguet, M. Gillet, C. Vincent, and J. P. Revillard.** 1991. Serum and bile secretory immunoglobulins and secretory component during the early postoperative course after liver transplantation. Hepatology **14:**1046–1053.
4. **Burke, D. S., A. Nisalak, and M. A. Ussery.** 1982. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J. Clin. Microbiol. **16:**1034–1042.
5. **Czinn, S. J., H. S. Carr, and W. T. Speck.** 1991. Diagnosis of gastritis caused by *Helicobacter pylori* in children by means of an ELISA. Rev. Infect. Dis. **13**(Suppl. 8)**:**S700–S703.
6. **Czinn, S. J., A. Cai, and J. G. Nedrud.** 1993. Protection of germ-free mice from infection by *Helicobacter felis* after active oral or passive IgA immunization. Vaccine **11:**637–642.
7. **Forman, D., and the Eurogast Study Group.** 1993. An international association between *Helicobacter pylori* infection and gastric cancer. Lancet **341:** 1359–1362.
8. **Frazier, B. A., J. D. Pfeifer, D. G. Russell, P. Falk, A. N. Olsén, M. Hammar, T. U. Westblom, and S. J. Normark.** 1993. Paracrystalline inclusions of a novel ferritin containing nonheme iron, produced by the human gastric pathogen *Helicobacter pylori*: evidence for a third class of ferritins. J. Bacteriol. **175:**966–972.
9. **Graham, D. Y.** 1991. *Helicobacter pylori*: its epidemiology and its role in duodenal ulcer disease. J. Gastroenterol. Hepatol. **6:**105–113.
10. **Hayashi, S., T. Sugiyama, K. Hisano, T. Awakawa, I. Kurokawa, A. Yachi, H. Isogai, E. Isogai, K. Yokota, Y. Hirai, K. Oguma, and N. Fujii.** 1996. Quantitative detection of secretory immunoglobulin A to *Helicobacter pylori* in gastric juice: antibody capture enzyme-linked immunosorbent assay. J. Clin. Lab. Anal. **10:**74–77.
11. **Hodgson, J., and P. Morgan-Capner.** 1984. Evaluation of a commercial antibody capture enzyme immunoassay for the detection of rubella specific IgM. J. Clin. Pathol. **37:**573–577.
12. **Itoh, T., Y. Yanagawa, M. Shingaki, M. Takahashi, A. Kai, M. Ohashi, and G. Hamana.** 1987. Isolation of *Campylobacter pyloridis* from human gastric mucosa and characterization of the isolates. Microbiol. Immunol. **31:**603–614.
13. **Lee, C. K., R. Weltzin, W. D. Thomas, Jr., H. Kleanthous, T. H. Ermak, G. Soman, J. E. Hill, S. K. Ackerman, and T. P. Monath.** 1995. Oral immunization with recombinant *Helicobacter pylori* urease induces secretory IgA antibodies and protects mice from challenge with *Helicobacter felis*. J. Infect. Dis. **172:**161–172.
14. **McNulty, C. A. M., and R. Wise.** 1985. Rapid diagnosis of *Campylobacter*-associated gastritis. Lancet **i:**1443–1444.
15. **Michetti, P., I. Corthesy-Theulaz, C. Davin, R. Haas, A. C. Vaney, M. Heitz, J. Bille, J. P. Kraehenbuhl, E. Saraga, and A. L. Blum.** 1994. Immunization of BALB/c mice against *Helicobacter felis* infection with *Helicobacter pylori* urease. Gastroenterology **107:**1002–1011.
16. **Negrini, R., L. Lisato, I. Zanella, L. Cavazzini, S. Gullini, V. Villanacci, C.**

Poiesi, A. Albertini, and S. Ghielmi. 1991. *Helicobacter pylori* infection induces antibodies cross-reacting with human gastric mucosa. Gastroenterology **101:**437–445.
17. **Nerurkar, L. S., M. Namba, G. Brashears, A. J. Jacob, Y. J. Lee, and J. L. Sever.** 1984. Rapid detection of herpes simplex virus in clinical specimens by use of a capture biotin-streptavidin enzyme-linked immunosorbent assay. J. Clin. Microbiol. **20:**109–114.
18. **Owen, R. J., S. R. Martin, and P. Borman.** 1985. Rapid urea hydrolysis by gastric *Campylobacters*. Lancet **i:**111.
19. **Pappo, J., W. D. Thomas, Jr., Z. Kabok, N. S. Taylor, J. C. Murphy, and J. G. Fox.** 1995. Effect of oral immunization with recombinant urease on murine *Helicobacter felis* gastritis. Infect. Immun. **63:**1246–1252.
20. **Pérez-Pérez, G. I., W. R. Brown, T. I. Cover, B. E. Dunn, P. Cao, and M. J. Blaser.** 1994. Correlation between serological and mucosal inflammatory responses to *Helicobacter pylori*. Clin. Diagn. Lab. Immunol. **1:**325–329.
21. **Prigent-Delecourt, L., B. Coffin, J. F. Colombel, J. P. Dehennin, J. P. Vaerman, and J. C. Rambaud.** 1995. Secretion of immunoglobulins and plasma proteins from the colonic mucosa: an *in vivo* study in man. Clin. Exp. Immunol. **99:**221–225.
22. **Self, C. H.** 1985. Enzyme amplification—a general method applied to provide an immunoassisted assay for placental alkaline phosphatase. J. Immunol. Methods **76:**389–393.
23. **Stanley, C. J., A. Johannsson, and C. H. Self.** 1985. Enzyme amplification can enhance both the speed and the sensitivity of immunoassays. J. Immunol. Methods **83:**89–95.
24. **Sugiyama, T., K. Imai, H. Yoshida, Y. Takayama, T. Yabana, K. Yokota, K. Oguma, and A. Yachi.** 1991. A novel enzyme immunoassay for serodiagnosis of *Helicobacter pylori* infection. Gastroenterology **101:**77–83.
25. **Sugiyama, T., T. Awakawa, S. Hayashi, K. Hisano, T. Yabana, I. Kurokawa, and A. Yachi.** 1994. The effect of the immune response to *Helicobacter pylori* in the development of intestinal metaplasia. Eur. J. Gastroenterol. Hepatol. **6**(Suppl. 1)**:**S89–S92.
26. **Sugiyama, T., T. Yabana, and A. Yachi.** 1995. Mucosal immune response to *Helicobacter pylori* and cytotoxic mechanism. Scand. J. Gastroenterol. **30**(Suppl. 208)**:**30–32.
27. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:** 539–547.
28. **Valkonen, K. H., T. Wadström, and A. P. Moran.** 1997. Identification of the *N*-acetylneuraminyllactose-specific laminin-binding protein of *Helicobacter pylori*. Infect. Immun. **65:**916–923.
29. **Warren, J. R., and B. Marshall.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet **i:**1273–1275.
30. **Williams, R. C., and R. J. Gibbons.** 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science **177:**697–699.
31. **Wyatt, J. I., and M. F. Dixon.** 1988. Chronic gastritis—a pathogenetic approach. J. Pathol. **154:**113–124.
32. **Yachi, A., Y. Akahonai, T. Suzuki, T. Abe, and S. Maeda.** 1984. The IgA type parietal cell antibody in gastric juice. Protides Biol. Fluids Proc. Colloq. **32:** 57–60.
33. **Yokota, K., Y. Hirai, M. Haque, S. Hayashi, H. Isogai, T. Sugiyama, E. Nagamachi, Y. Tsukada, N. Fujii, and K. Oguma.** 1994. Heat shock protein produced by *Helicobacter pylori*. Microbiol. Immunol. **38:**403–405.