Volume 10 · Number 1 · 2005
HELICOBACTER

Effect of Dietary Anti-Urease Immunoglobulin Y on *Helicobacter pylori* Infection in Mongolian Gerbils

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ABSTRACT

Background and aim. *Helicobacter pylori* is known to be a major pathogenic factor in the development of gastritis, peptic ulcer disease and gastric cancer. Recently, chicken egg yolk immunoglobulin Y (IgY) has been recognized as an inexpensive antibody source for passive immunization against gastrointestinal infections. The present study was designed to investigate the effect of anti-urease IgY on *H. pylori* infection in Mongolian gerbils.

Methods. *H. pylori*-infected Mongolian gerbils were administered a diet containing anti-urease IgY, with or without famotidine (F). After 10 weeks, bacterial culture and measurement of the gastric mucosal myeloperoxidase (MPO) activity were performed. In a second experiment, another group of gerbils was started on a diet containing F + IgY a week prior to *H. pylori* inoculation. After 9 weeks, these animals were examined.

Results. In the *H. pylori*-infected gerbils, there were no significant differences in the level of *H. pylori* colonization among the different dietary and control groups. However, the MPO activity was significantly decreased in the *H. pylori* group administered the F + IgY diet compared with that in the *H. pylori* group administered the IgY, F, or control diet. Furthermore, in the gerbils administered the F + IgY diet prior to the bacterial inoculation, inhibition of *H. pylori* colonization and suppression of the elevated gastric mucosal MPO activity were observed.

Conclusions. Oral administration of urease-specific IgY not only inhibited *H. pylori* disease activity in *H. pylori*-infected gerbils, but also prevented *H. pylori* colonization in those not yet infected. These encouraging results may pave the way for a novel therapeutic and prophylactic approach in the management of *H. pylori*-associated gastroduodenal disease.

Keywords. *Helicobacter pylori*, egg yolk immunoglobulin, oral passive immunization, Mongolian gerbil, antacid.

Helicobacter pylori infection is the most common cause of gastritis and gastric ulcers and plays a pivotal role in the development of gastric carcinoma [1–3]. Successful treatment of the infection most often employs antibiotic therapy, consisting of some combination of metronidazole, amoxicillin, clarithromycin and either bismuth or a proton pump inhibitor [4]. However, antibiotic therapy fails in 10–15% of cases because of the development of antibiotic resistance [5,6]. Consequently, it is important to seek new approaches for the treatment of *H. pylori* infection that would not entail the risk of drug resistance.

Passive immunization with orally administered antibodies has been shown to be effective against a variety of intestinal pathogens in both prophylactic and therapeutic studies [7–9]. Although the role of passive immunization in the treatment of *H. pylori* infection remains largely unclarified, a few studies have suggested that specific antibodies might be protective. Bovine colostral antibodies against *H. pylori* were reported to be useful for the prevention of *Helicobacter* infection in a mouse model [10], and specific colostral antibodies have also been shown to eradicate or reduce the number of bacteria in infected mice [11].

Recently, chicken egg yolk has been recognized as an inexpensive, alternative source of

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antibodies, and the usefulness of oral administration of egg yolk immunoglobulin Y (IgY) for passive immunization against intestinal infections has been demonstrated [12–16]. Furthermore, administration of the egg yolk of chickens immunized with *H. pylori* was found to effectively reduce the serum titer of anti-*H. pylori* IgG [17] and attenuate the severity of gastritis [18] in *H. pylori*-colonized Mongolian gerbils. However, IgY produced by immunization of chickens with whole-cell lysates of *H. pylori* may cross-react with other bacteria, including the normal human intestinal flora [19], and this could decrease its efficiency and specificity. Therefore, immunization using a selective antigen is necessary.

Icatlo et al. previously reported on the bioadhesive function of the *H. pylori* urease [20]. They identified the adhesive specificity of urease for certain sugar-containing biomaterials, such as gastric mucin and sulfated cell membrane glycolipids [21]. Thus, it was expected that anti-*H. pylori*-urease IgY would capture bacterium-associated urease within the gastric mucus layer, causing bacterial aggregation and clearance via the continuous washing action of the gastric mucus. Further evidence was provided by the study which showed that urease-specific monoclonal antibodies prevented *Helicobacter* infection in mice [22]. More recently, we evaluated the effect of anti-urease IgY in humans [23], and showed that this IgY partially attenuated gastric urease activity, and when administered with antacids it ameliorated *H. pylori*-associated gastric inflammation.

For more detailed examination, the present study aimed to determine the effect of anti-*H. pylori*-urease IgY on *H. pylori* colonization and on *H. pylori*-induced mucosal inflammation in the Mongolian gerbil. Since the passage of chicken egg yolk IgY through the stomach is thought to be associated with considerable loss of antibodies because of the action of gastric acid [24], anti-*H. pylori*-urease IgY was administered with famotidine, an H_2-receptor antagonist.

Methods

Preparation of urease and immunization of hens

Helicobacter pylori strain no. 130 was used to purify urease. Isolation of this strain from a patient with gastritis, as well as the methods of preparation of the stock culture and mass bacterial propagation, have been described previously [20,25]. The cell biomass of *H. pylori* was collected by centrifugation at 12,000 × *g* for 20 minutes, followed by scraping with a sterile metal spatula, and was then stored at −80°C [20]. Crude urease extract was prepared from the *H. pylori* biomass by aqueous extraction, and two-step affinity purification under mild conditions was performed as described previously [20]. Hy-line hens were immunized by intramuscular injection with the affinity-purified *H. pylori* urease, and defatted egg powder containing anti-*H. pylori* urease IgY was prepared in accordance with a method described in a previous report [23].

Enzyme-linked immunosorbent assay (ELISA)

The binding ability of IgY to *H. pylori* urease was assessed by checking the antibody response, using purified urease as the antigen coating in ELISA antibody titration, based on a previous report [20]. Assay plates were coated with affinity-purified urease and washed with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T). Then, a dilute solution of the defatted powder prepared from the immunized or nonimmunized chickens' eggs was added to each well. After incubation, the unbound material was removed by washing with PBS-T, and bound IgY was detected using peroxidase–rabbit anti-chicken/turkey IgG (Zymed Laboratories, Inc., San Francisco, CA). The color reaction was developed with *ortho*-phenylenediamine dihydrochloride and stopped with 3 N H_2SO_4. The absorbance was read at 490 nm. Wells not coated with the antigen were used as blanks.

Western blot analysis

The IgY contained in the immunized and nonimmunized chickens' egg yolk was subjected to qualitative analysis by Western blotting. IgY isolation from the egg yolk was carried out using the Eggstract® IgY Purification System (Promega Corp., Madison, WI), in accordance with the instructions of the manufacturer. The protein concentrations in the resultant IgY solutions were measured using Smith et al.'s modification [26] of Lowry's method [27].

Gel electrophoresis was performed using the *H. pylori* strains, Sydney strain (SS1) and ATCC43504. The methods of preparation of the stock cultures of these strains and mass bacterial propagation have been described previously

[28]. For Western blotting of the *H. pylori* antigen, the bacteria were suspended in PBS containing protease inhibitors (100 μmol/l phenymethylsulfonyl fluoride, 10 μg/ml aprotinin), and then sonicated on ice in 30 consecutive 0.5-second bursts at 0.5-second intervals, at a power setting of 150 W (Ultrasonic Processor VCX 750, Sonics & Materials, Inc., Newton, CT). The *H. pylori* lysates (10 μg protein/lane) were resolved by sodium dodecyl sulfate–polyacrlamide gel electrophoresis. The gels were transferred to PVDF membranes and the blots were blocked in skimmed milk. The blots were then incubated with the egg-yolk-derived IgY (2 μg protein/ml) as the primary antibody at 4°C. After overnight incubation, they were washed three times with PBS containing 0.1% Tween-20 and incubated with donkey anti-chicken immunoglobulin conjugated to horseradish peroxidase (Affinity BioReagent, Golden, CO) as the secondary antibody. Membranes were developed using the enhanced chemiluminescence plus detection system (Amersham Biosciences, Buckinghamshire, UK).

Animal experiment 1

All the experiments and procedures were conducted after obtaining the approval of the Keio University Animal Research Committee (License No.023009). Fourteen specific-pathogen-free male Mongolian gerbils (5-week-old, MGS/Sea, Seac Yoshitomi, Fukuoka, Japan) were given suspensions of ATCC43504 [10^8 colony-forming units (CFU)/ml, 15 ml/kg] after overnight fasting, while 14 uninfected control animals were given suspension buffer solution alone [29]. Eight weeks after the bacterial inoculation, blood samples were taken from an orbital vessel and the serum level of anti-*H. pylori* IgG was measured using an enzyme-immunoassay (EIA) kit (Determiner *Helicobacter pylori* antibody kit, Kyowa Medex, Tokyo, Japan) modified by changing the original secondary antibody to peroxidase-labeled rabbit anti-mouse IgG (Dako Japan, Kyoto, Japan). Gerbils producing samples with an optical density on anti-*H. pylori* IgG EIA greater than 0.1 at this period were recognized as *H. pylori*-colonized cohorts. Thirteen weeks after the bacterial inoculation, both infected and uninfected control animals were divided into four groups and started on a control chow diet (MF, Oriental Yeast Co., Tokyo; chow group), an IgY-containing diet (25 mg/g; IgY group), a famotidine-containing diet (Yamanouchi Pharmaceutical Co., Tokyo, Japan; 0.16 mg/g; F group), or a diet containing both IgY and famotidine (F + IgY group). Ten weeks later, the gerbils were killed (Figure 1); and their stomachs were excised and opened along the greater curvature and rinsed with saline.

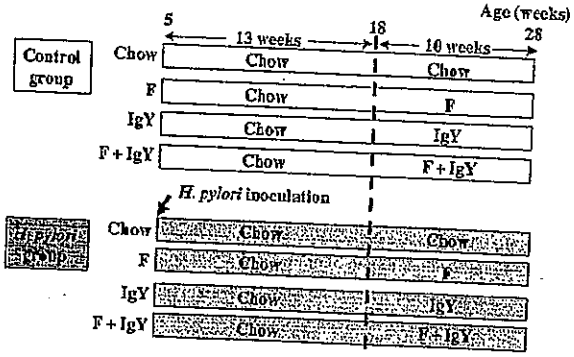

Figure 1 Protocol for animal experiment 1. Five-week-old Mongolian gerbils (MGS/Sea, Seac Yoshitomi, Fukuoka, Japan) were given *H. pylori* suspensions (ATCC43504). Thirteen weeks after the bacterial inoculation, the animals were started on a control diet (Chow group), IgY-containing diet (25 mg/g; IgY group), famotidine-containing diet (0.16 mg/g; F group), or diet containing both IgY and famotidine (F + IgY group). Ten weeks after the start of the diets, the gerbils were examined.

Helicobacter pylori infection was examined by determining the CFU count in a microaerobic bacterial culture. Briefly, the stomach specimens were homogenized in 800 μl of sterilized saline using a plastic pestle. The diluted homogenates were applied to selective agar plates (Columbia *Helicobacter pylori* agar, Becton Dickinson and Company, Franklin Lakes, NJ), and the plates were incubated at 37°C in a microaerobic atmosphere for 7 days. The number of colonies was counted, and the count of viable *H. pylori* was expressed as CFU/g tissue [30].

For measurement of the myeloperoxidase (MPO) activity, an index of tissue-associated neutrophil accumulation, tissue samples of gastric mucosa were collected in microtubes containing 1 ml PBS with protease inhibitors (100 μmol/l phenymethylsulfonyl fluoride, 10 μg/ml aprotinin), and then sonicated on ice. Total protein in the homogenates was measured as described above. The MPO activity was determined by a modification of the method of Grisham *et al.* [31]. Gastric mucosal homogenates were centrifuged at 12,000 × g for 15 minutes at 4°C to pellet the insoluble cellular debris. The pellet was then rehomogenized in a tenfold volume of

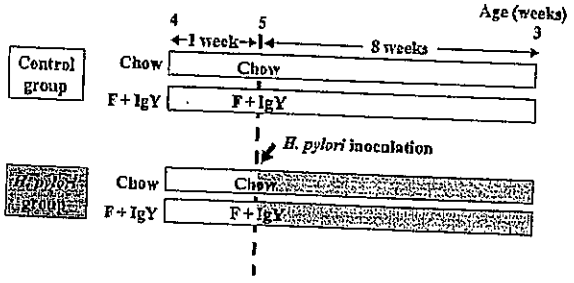

Figure 2 Protocol for animal experiment 2. Four-week-old male Mongolian gerbils were started on a control diet (Chow group) or a diet containing IgY and famotidine (25 mg/g IgY and 0.16 mg/g famotidine; F + IgY group). A week later, the gerbils were given *H. pylori* suspensions (SS1). They were then examined after 9 weeks to determine the infection status.

0.05 mol/l potassium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide. These homogenates were again centrifuged at 12,000 × g for 15 min at room temperature, and the supernatants were reserved. The MPO activity was then assessed by measuring the H_2O_2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine. One unit of enzyme activity was defined as the amount of MPO that caused a change in absorbance of 1.0/minute at 655 nm.

For histological examination, gastric mucosal tissue samples from Mongolian gerbils were fixed in 10% neutralized formalin, and embedded in paraffin. The 4–6-μm paraffin sections were placed on slides pretreated with 0.01% poly L-lysine in aqueous solution. Deparaffinization was performed by heating the sections for 10 min at 70°C. This was followed by a hydration process, in which the slides were passed through the following solutions: twice through a xylene bath for 5 minutes, twice through 96% ethanol for 3 minutes, then once through 90% ethanol for 3 minutes, and once through double-distilled water for 3 min. The thin sections of fixed tissues were then stained with hematoxylin & eosin.

Animal experiment 2

The efficacy of the F + IgY diet in preventing *H. pylori* colonization was determined in Experiment 2 (Figure 2). Six male Mongolian gerbils (4-week-old, MGS/Sea) were fed a control chow diet or the F + IgY diet. A week later, gerbils from both groups were given suspensions of *H. pylori*, strain SS1 (10^8 CFU/ml, 15 ml/kg), after overnight fasting, while the animals marked as controls were given buffer solution alone. Eight weeks after the bacterial inoculation, all the gerbils were killed to evaluate the *H. pylori* infection status and the degree of gastric mucosal inflammation, in the same way as in Experiment 1.

Statistical analysis

All the data were expressed as mean ± SE, and a p-value < .05 was considered to denote statistical significance. The data were analyzed using one-way analysis of variance, followed by Fisher's comparison test.

Results

Characterization of the IgY obtained from the egg yolk of the immunized hens

In the present study, *H. pylori* urease was selected as the target antigen. Defatted egg powder was prepared from the egg yolk of hens immunized with the *H. pylori* urease, and its immunological properties were compared with those of defatted egg powder obtained from nonimmunized hens by ELISA. The relative IgY titer as determined by the ELISA was 6400-fold higher in the former than in the latter, indicating that the egg yolk IgY used in the present study was highly specific for *H. pylori* urease.

Figure 3 shows the electrophoretic profile of the proteins of *H. pylori* strains SS1 and ATCC43504, and Western blotting of the IgY preparation. The IgY preparation from *H. pylori*-urease-immunized hens' egg yolk recognized mainly two proteins, one with a molecular weight of approximately 62 kDa and the other with a molecular weight of 30 kDa, in both strains. On the other hand, no signal was observed with the IgY prepared from the nonimmunized hens' egg yolk. Thus, the egg-yolk-derived IgY used in the present study had specific and potent binding affinity for the 62- and 30-kDa proteins isolated from these two strains of *H. pylori*, corresponding to the B and A subunits of urease, respectively. Furthermore, anti-*H. pylori*-urease IgY showed a higher binding affinity for the B subunit of urease than for the A subunit.

Efficacy of anti-H. pylori-urease IgY in Mongolian gerbils

Two animal experiments using Mongolian gerbils were performed. In the first trial, gerbils infected with *H. pylori* received a control chow

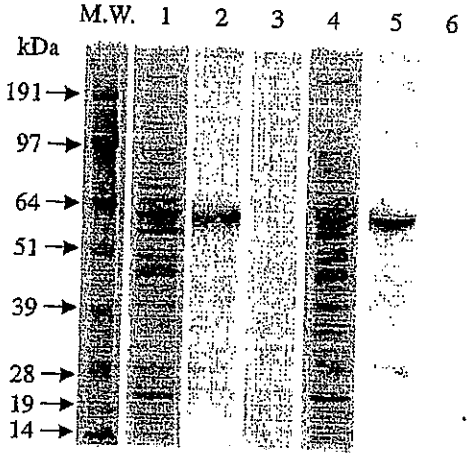

Figure 3 Western blot analysis of IgY derived from immunized and nonimmunized hens. Lanes 1–3; ATCC43504 (10 μg protein/lane): lane 1, electrophoretic pattern; lane 2, Western blot analysis with IgY (2 μg protein/ml) prepared from *H. pylori*-urease-immunized hen's egg yolk; lane 3, Western blot analysis with IgY (2 μg protein/ml) prepared from nonimmunized hen's egg yolk. Lane 4–6; SSI (10 μg protein/lane): lane 4, electrophoretic pattern; lane 5, Western blot analysis with IgY (2 μg protein/ml) prepared from *H. pylori*-urease-immunized hen's egg yolk; lane 6, Western blot analysis with IgY (2 μg protein/ml) prepared from nonimmunized hen's egg yolk.

diet (chow group, n = 3), an IgY-containing diet (IgY group, n = 4), a famotidine-containing diet (F group, n = 3), or a diet containing both IgY and famotidine (F + IgY group, n = 4). There were no significant differences among the four groups with respect to the level of *H. pylori* colonization, and none of the gerbils showed eradication of the organism in Experiment 1 (Table 1). The increase of the MPO activity in the gastric mucosa was significant and more pronounced in the *H. pylori*-colonized cohorts that received the chow diet, F diet or IgY diet, as compared with that in the uninfected control group fed the chow diet. However, in the *H. pylori*-colonized cohorts given the F + IgY diet, the increase in the enzyme activity was attenuated significantly. Histological examination of the hematoxylin & eosin-stained gastric tissues revealed a significant degree of inflammation, with polymorphonuclear and mononuclear cell infiltration both in the gastric corpus and the antral mucosa, in all of the *H. pylori*-colonized gerbils. In the corpus mucosa, however, the inflammatory cell infiltration was remarkably attenuated by the F + IgY diet (Figure 4).

In the second protocol, to determine the efficacy of the F + IgY diet in preventing *H. pylori* colonization, gerbils were started on chow diet (n = 3) or on the F + IgY diet (n = 3) a week prior to *H. pylori* inoculation (Figure 2). Eight weeks after *H. pylori* inoculation, it was found that the serum level of anti-*H. pylori* IgG tended to increase in both the groups (Table 2). However, *H. pylori* colonization was confirmed only in the chow group and not in the F + IgY group, suggesting that the dietary administration of F + IgY might facilitate the excretion of the colonizing *H. pylori* bacteria and prevent persistent infection. Table 2 depicts the mucosal MPO activity 8 weeks after the bacterial inoculation in each group. In the inoculated group on the chow diet, the MPO activity increased significantly to sixfold the level observed in the uninoculated control group. However, no significant increase of the MPO activity was noted in the Hp-inoculated F + IgY group as compared with that in the control group. In the gastric antral mucosa, a significant level of inflammatory cell infiltration was observed in *H. pylori*-inoculated gerbils on chow diets while the histopathological changes were not severe in the corpus mucosa. The gastric wall thickening was also induced in the antrum of *H. pylori*-inoculated gerbils on chow diets (Figure 5A). However, the marked inflammatory cell infiltration in the antral mucosa was not found in *H. pylori*-inoculated F + IgY group, and *H. pylori*-induced thickening

Table 1 Effect of orally administered anti-*H. pylori* IgY on *H. pylori* infection in Mongolian gerbils

Diet	Control group				*H. pylori* group			
	Chow	F	IgY	F + IgY	Chow	F	IgY	F + IgY
H. pylori (Log CFU/g tissue)	ND	ND	ND	ND	4.30 ± 0.61	4.55 ± 0.30	4.69 ± 0.13	4.05 ± 0.54
MPO activity (mU/mg protein)	0.18 ± 0.05	1.22 ± 0.39	0.41 ± 0.10	0.51 ± 0.17	5.89 ± 1.08**	4.75 ± 0.83**	4.78 ± 1.89**	1.92 ± 0.30†‡

Data are expressed as means ± SE.
MPO activity, myeloperoxidase activity; Chow, control chow diet; IgY, IgY-containing diet; F, famotidine-containing diet; F + IgY, diet containing IgY and famotidine; ND, not detected.
**$p < .01$ compared with the control-chow group.
†$p < .05$ compared with the *H. pylori*-F group.
‡$p < .01$ compared with the *H. pylori*-chow or *H. pylori*-IgY group.

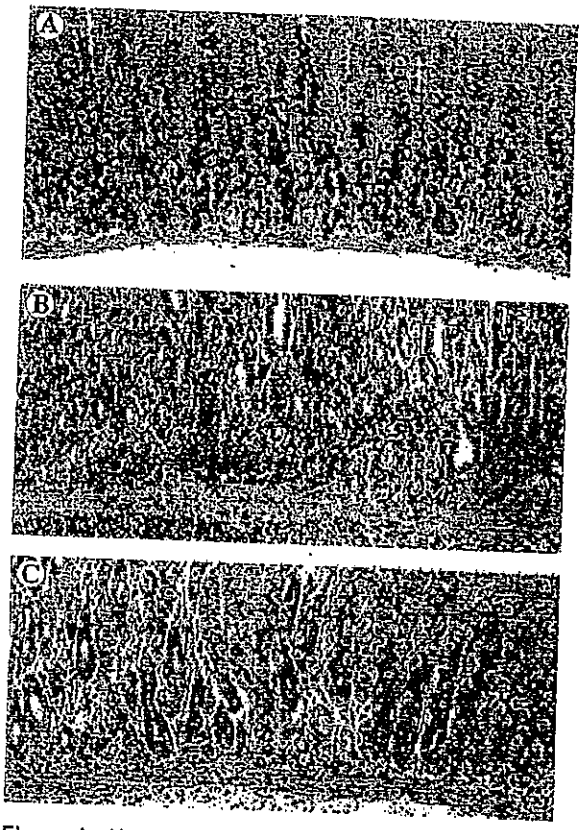

Figure 4 Histological findings of the hematoxylin & eosin-stained gastric mucosal sections in experiment 1. Thirteen weeks after the bacterial inoculation, gerbils were started on a control conventional diet (Chow) or on an IgY-containing diet with famotidine (F + IgY) for 10 weeks. Representative photomicrographs of the gastric corpus in the uninfected control gerbils on a chow diet (A), and in *H. pylori*-infected animals on chow (B) and F + IgY diets (C) are shown (magnification, × 200).

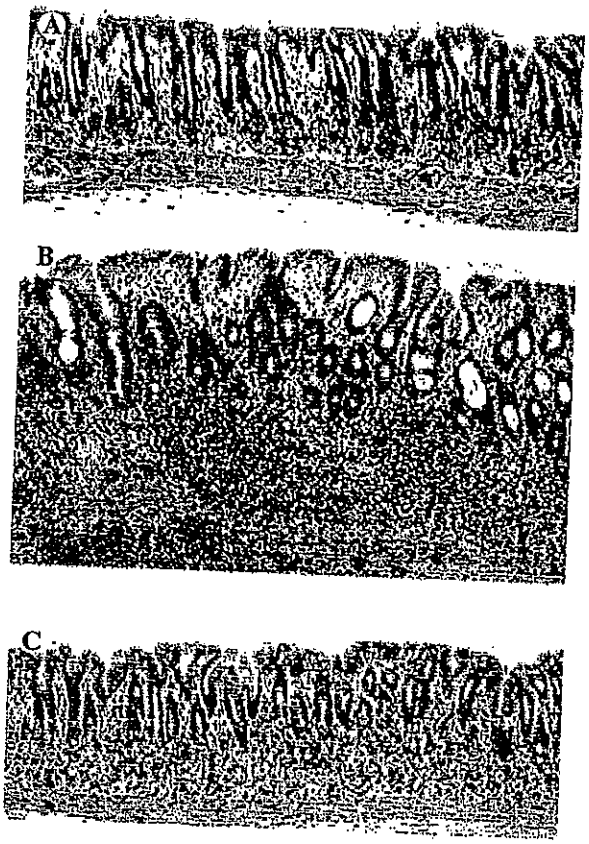

Figure 5 Histological findings of the hematoxylin & eosin-stained gastric mucosal sections in experiment 2. The gerbils were started on a control conventional diet (Chow) or on an IgY-containing diet with famotidine (F + IgY) a week before the *H. pylori* inoculation. Representative photomicrographs of the gastric antrum in the uninoculated control gerbils on a chow diet (A), and in *H. pylori*-colonized animals on chow (B, serum anti-*H. pylori* IgG: positive, bacterial culture: positive) and F + IgY diets (C, serum anti-*H. pylori* IgG: positive, bacterial culture: negative) are shown (magnification, × 200).

of gastric mucosa was diminished by ingestion of F + IgY diet (Figure 5C).

Discussion

We previously evaluated the effect of orally administered anti-urease IgY in asymptomatic volunteers [23]. In that study, the volunteers diagnosed to be *H. pylori*-positive by the ^{13}C-urea breath test (UBT) were orally administered anti-*H. pylori* urease IgY; after 4 weeks, the UBT values had decreased significantly. Furthermore, an *H. pylori*-positive female gastritis patient administered anti-*H. pylori* urease IgY plus lansoprazole for 8 weeks showed a decrease in

Diet	Control group		*H. pylori* group	
	Chow	F + IgY	Chow	F + IgY
Serum anti-*H. pylori* IgG (OD_{450})	0.026 ± 0.003	0.034 ± 0.012	0.150 ± 0.051	0.119 ± 0.042
H. pylori (log_{10} CFU/g tissue	ND	ND	2.47 ± 1.31	ND
MPO activity (mU/mg protein)	0.38 ± 0.06	0.32 ± 0.07	2.39 ± 0.88*	0.91 ± 0.24

Data are expressed as mean ± SE.
MPO activity, myeloperoxidase activity; Chow, control chow diet; F + IgY, diet containing IgY and famotidine; ND, not detected.
*$p < .05$ compared with the control-chow group.

Table 2 Preventive effect of orally administered anti-*H. pylori* IgY against *H. pylori* infection in Mongolian gerbils

the serum pepsinogen (PG) I and UBT values, as well as an increase in the PGI : PGII ratio. These results suggest that anti-*H. pylori* urease IgY may mitigate *H. pylori*-associated gastritis and partially attenuate gastric urease activity. To further confirm the effect of anti-*H. pylori* urease IgY, in the present study, the *H. pylori*-infected Mongolian gerbuils were fed an IgY-containing diet with or without famotidine. Ten weeks after the start of the test diet, although there was no significant difference in the level of *H. pylori* colonization, the gastric mucosal MPO activity had decreased significantly only in the *H. pylori*-colonized gerbils fed the F + IgY diet, as compared with that in the colonized cohorts given a diet containing IgY alone, famotidine alone, or the control chow diet. Subsequently, in a second experiment, another group of gerbils was started on an F + IgY diet or chow diet a week prior to the *H. pylori* inoculation. After 9 weeks, while *H. pylori* colonization was confirmed in the chow group, no *H. pylori* colonization was detected in the F + IgY group, even though the serum anti-*H. pylori* IgG level tended towards an increase in both the *H. pylori*-inoculated groups. Changes of serum anti-*H. pylori* IgG antibodies after inoculation have been reported in gerbils [32], and at 8 weeks after inoculation or later, 100% of gerbils had positive level of serum anti-*H. pylori* IgG titers. In the present study, while the average anti-*H. pylori* IgG level in serum tended to increase in both of the *H. pylori*-inoculated groups, on chow diet and on F + IgY diet, as compared with uninfected control group, one gerbil had a negative IgG level in the *H. pylori*-inoculated F + IgY group. It was reported that not all the gerbils showed a significant reduction of titer even 4 months after treatment with antibiotics, although eradication was complete [33]. These results suggest that while the gastric mucosa was initially challenged by *H. pylori* in both groups, dietary pretreatment with IgY in the presence of famotidine probably caused the bacteria to be excreted, whilst still having positive antibody titers.

Bovine colostral antibodies against *H. pylori* were reported to be useful in the prevention of *Helicobacter* infection in a mouse model [10], and administration of specific colostral antibodies resulted in either eradication of or reduction in the number of bacteria in the infected mice [11]. These efficacies against *Helicobacter* infection of colostral immune preparations containing *Helicobacter*-specific antibodies were observed even in the absence of antacid treatment. Bovine colostrum contains mainly IgG_1, which plays a vital role in passive immunization [34]. Egg yolk from immunized hens contains immunoglobulins capable of specific recognition in abundant, and therefore economically feasible, quantities. However, egg-yolk-derived IgY is relatively unstable under acidic conditions, and passage of the molecule through the low-pH environment of the stomach could readily weaken its overall conformational stability as compared to that of mammalian IgG [35]. In the present study, to reduce the effect of acid on IgY, famotidine was administered concomitantly with the IgY. Oral administration of IgY has been shown by many researchers to be effective in preventing a number of intestinal infections, including those caused by enterotoxigenic *Escherichia coli* [15] and human rotavirus [12–14,16], even in the absence of concomitant antacid administration. These passive protective effects of egg-yolk antibodies against enteric bacterial infections are thought to result from the active antibody escaping the process of degradation by gastric acid. In the case of *H. pylori* infection of the stomach, even if the anti-*H. pylori* urease IgY retaining its original activity can access the bacteria, the antibody is unlikely to be able to bind to the antigen under acidic conditions. Concomitant acid-suppression therapy or a combination of these approaches would therefore be needed for the potential effects of *H. pylori*-specific IgY against *H. pylori* infection.

Affinity-purified *H. pylori* urease was used as the antigen for the IgY preparation in the present study. The same purification procedure provided the fractions containing both the 62 to 65-kDa and 30-kDa proteins corresponding to the B and A subunits of urease, respectively [20]. In the present study, the IgY obtained from the egg yolk of *H. pylori*-urease-immunized hens was confirmed to exhibit specific binding with the *H. pylori* urease subunits, mainly the B subunit, with relatively weaker reactivity with the A subunit. Shin et al. identified the immunodominant proteins of *H. pylori* showing reactivity with *H. pylori*-specific IgY. They reported strong reactivity of the IgY with five proteins, including the A and B subunits of urease [19]. Furthermore, they mapped the urease epitope recognized by the anti-*H. pylori* IgY, and a peptide representing 15 amino acid residues of the B subunit of urease was specifically recognized by the anti-*H. pylori* IgY [36]. Although the role of the B

subunit of urease in *Helicobcter* colonization still remains ill-defined, it was demonstrated that passive protection against *Helicobacter felis* infection could be achieved in a germ-free mouse model by administration of a monoclonal antibody recognizing the B subunit of *H. felis* urease [22]. The results of this study lend support to the contention that the B subunit of urease is an important antigen involved in *Helicobacter* immunity.

Concerning the role of the urease subunits in *H. pylori* colonization, Icatlo et al. have provided in vitro evidence of the high-affinity binding of *H. pylori* urease to diverse types of polysaccharides, including mucin, in an acidic setting [20]. Furthermore, their study using a euthymic hairless mouse model of acute *H. pylori* infection showed that the gastric *H. pylori* load in the test mice could be significantly reduced by oral administration of dextran sulfate, a urease-binding polysaccharide [37]. Co-administration of dextran sulfate with famotidine resulted in a more pronounced reduction of the gastric bacterial load as compared with that observed following monotherapy with dextran sulfate alone. The enhanced activity of the drug combination may be related to the daily pattern of transient acid suppression by famotidine, inducing periodic bacterial convergence to superficial mucous sites penetrated by dextran sulfate from the lumen. The CFU values were counted in mucus and epithelial tissue separately, and the rate of decrease of the epithelium-associated *H. pylori* was higher than that of the mucus-associated bacteria. In the present study, although there was no significant difference in the level of *H. pylori* colonization among the *H. pylori*-infected gerbils, the MPO activity was significantly decreased in the *H. pylori*-infected group receiving the F + IgY diet. The cause of this discrepancy could not be exactly identified. However, the capture of *H. pylori* urease may induce a reduction in the amount of epithelium-associated *H. pylori*. Since some of the cytotoxic effects of *H. pylori* have been reported to be related to bacterial adhesion to the gastric epithelial cells [38–40], the positional change possibly occurring following anti-*H. pylori* urease IgY administration may decrease bacterial adhesion, and this might play an important role in quenching gastric mucosal inflammation.

In conclusion, administration of anti-*H. pylori*-urease IgY in the presence of famotidine resulted in attenuation of *H. pylori*-associated gastric mucosal inflammation in infected gerbils, although there appeared to be no effect on the level of *H. pylori* colonization. Furthermore, the present study demonstrated that dietary administration of anti-*H. pylori*-urease IgY in the presence of famotidine may facilitate the excretion of *H. pylori* from the gastric mucosa and prevent persistent infection in Mongolian gerbils. The encouraging results of this study suggest that the use of this IgY may be a potentially successful novel approach in the management of *H. pylori* infection in humans.

This study was supported by a Grant-in-Aid for Scientific Research C (2) from the Japan Society for the Promotion of Science (JSPS) (15590686, to HS), a grant from Keio University, and a grant from Ortho Corporation Ltd. The authors thank Miss Yuriko Minegishi, Laboratory of Gastrointestinal Ecology & Antibody-Food, Center for Integrated Medical Research, Keio University School of Medicine, for her technical assistance. The authors also thank Dr Yoshikatsu Kodama, Ghen Corporation, Gifu, Japan for providing diets containing anti-HpU IgY. Preliminary data from the present study were presented at the Research Forum, DDW2004, on May 18th, 2004.

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