Humans infected with *Helicobacter pylori* have abnormally low levels of the antioxidant vitamin C, which protects against the formation of carcinogenic nitrosamines, in gastric juice. Guinea pigs, like humans and nonhuman primates, have a dietary requirement for vitamin C. As such, these species have gastrointestinal vitamin C transport systems not found in other animals. We have developed and characterized a guinea pig model of chronic gastric *H. pylori* infection with the rodent-adapted Sydney strain of *H. pylori*. At 4 weeks post-infection, five of six animals of the infected group and zero of two animals of the control group were positive for *H. pylori* as determined by culture or PCR. At 15 weeks, six of six animals of the infected group and zero of two animals of the control group were positive. *H. pylori*-specific seroconversion was observed among infected animals. There were no histologic abnormalities in the gastric antra or fundi of control guinea pigs. In contrast, there was multifocal, mild to moderate lymphohistiocytic antral gastritis and formation of antral lymphoid follicles in *H. pylori*-infected animals. The lesion distribution in the gastric antra paralleled that observed in *H. pylori*-infected humans. The *H. pylori*-infected guinea pig should prove useful in modeling the interaction of helicobacter and vitamin C in gastric carcinogenesis.

In this report, we describe the development and characterization of experimental *H. pylori* infection in guinea pigs as a preface to investigating the interaction of *H. pylori* and vitamin C transport and metabolism in the stomach.

**MATERIALS AND METHODS**

**Infection protocol.** (i) Animals. Female Hartley strain guinea pigs were obtained from Hazelton Research Products (Denver, Pa.). Two age groups were used: 16 2-week-old 200-g weanling animals and 3 adult females (>1 kg). Animals were maintained in polycarbonate caging and were fed standard guinea pig chow (PML Feeds, St. Louis, Mo.). All animal manipulations were approved by the MIT Institutional Animal Care and Use Committee.

(ii) Bacteria. *H. pylori* Sydney was used (22). Cultures used for dosing guinea pigs were grown under microaerophilic conditions for 24 to 48 h in brucella broth supplemented with 5% fetal calf serum. Broth cultures were examined by phase microscopy and by Gram staining for motility and purity. Bacteria were pelleted at 13,000 × g for 20 min and the pellet was resuspended in freeze medium (brucella broth plus 30% glycerol) at an optical density at 500 nm (OD500) of 1.0 (equivalent to approximately 10^7 CFU/ml).

(iii) Dosing scheme. Guinea pigs were dosed orally with 1 mg of omeprazole per kg of body weight per day starting 1 day before the first *H. pylori* dose and continuing for 1 day after the final dose of *H. pylori*. Twelve weanling guinea pigs were dosed orally with 1 ml of live *H. pylori* suspension (10^8 CFU) every other day for a total of 3 doses. Four control guinea pigs were sham dosed with 1 ml of sterile freeze media. Three adult female guinea pigs were sham dosed with 1 ml of killed *H. pylori* antigen (see below, "Serologic evaluation").

**Microbiological evaluation.** (i) Bacterial isolation from gastric tissue. At necropsy, two 4-mm-diameter punch biopsy samples were obtained aseptically from the antra and bodies of the stomachs. These biopsy samples were homogenized in a sterile tissue grinder, and an aliquot of the resulting shurry was plated on blood agar plates (Remel, Lenexa, Kans.) or Glaxo plates (containing vancomycin, polymyxin B, bacitracin, amphotericin B, and nalidixic acid) (23) for microaerobic isolation of *H. pylori*. The organism was identified by strong catalase, urease, and oxidase reactions and resistance to nalidixic acid and cephalothin. Although growth of *H. pylori* was generally evident within 1 week, plates were maintained for 3 weeks before a determination of no growth was made.

(ii) Bacterial isolation from feces. Fecal samples were obtained at scheduled intervals for *H. pylori* culture. A single fresh guinea pig fecal pellet was suspended in 2 ml of phosphate-buffered saline (PBS). A 100-μl aliquot was used to inoculate a Glaxo plate. Plates were incubated for 3 weeks before a determination of no growth was made.
**TABLE 1. Gastric colonization by H. pylori**

<table>
<thead>
<tr>
<th>Infection and time (wk) after dosing</th>
<th>Culture or PCR</th>
<th>Culture (antrum biopsy)</th>
<th>Culture (fundus biopsy)</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
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<td>4</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>15</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td><em>H. pylori</em></td>
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<td>5/6</td>
<td>5/6</td>
<td>3/6</td>
</tr>
<tr>
<td>4</td>
<td>6/6</td>
<td>1/6</td>
<td>1/6</td>
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<tr>
<td>15</td>
<td>6/6</td>
<td>1/6</td>
<td>1/6</td>
<td>6/6</td>
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</tbody>
</table>

* Colonization was assessed by gastric culture and by PCR of gastric tissue obtained at necropsy. Samples of gastric antrum and fundus were cultured separately.

(iii) DNA isolation for PCR. (a) Gastric tissue. Gastric tissue biopsies obtained at necropsy were ground up and prepared following the protocol for a commercially available DNA preparation kit (Boehringer Mannheim, Piscataway, N.J.). A 22.5-μl sample of the resulting DNA preparation was used for *H. pylori* PCR.

(b) Feces. Half of a guinea pig fecal pellet was suspended in 2 ml of PBS. The suspension was centrifuged at 700 × g for 5 min, and 300 μl of the resulting supernatant was used in a Qiagen kit, following the directions for blood. A 100-μl sample of the resulting DNA preparation was used for *H. pylori* PCR.

(iv) PCR. *H. pylori* PCR was performed as previously described, using the *H. pylori*-specific primers P3 and P4 (24) to perform PCR on DNA isolated from gastric tissue and using both the P3 and P4 primers and the all-helicobacter primers C97 and C98 (26) to perform PCR on DNA isolated from feces. Between 12 and 18 μl of DNA extract was added to a 100-μl final volume) reaction tube containing Taq polymerase buffer (Boehringer Mannheim, La Jolla, Calif.) supplemented with 1 mM MgCl₂ to a final concentration of 3.75 mM, 0.5 μM concentrations of each of the two primers, 200 μM concentrations of each deoxynucleotide, and 200 μg of bovine serum albumin per ml. Samples were heated at 94°C for 4 min, briefly centrifuged, and cooled to 60°C. Taq polymerase (3.2 U) (Pharmacia, Piscataway, N.J.) and 1.25 U of polymerase enhancer (Perfect Match; Stratagene, La Jolla, Calif.) were added, and then an overlay of 100 μl of mineral oil was added. Amplification took place in a thermal cycler under the following conditions: denaturation at 94°C for 1 min, annealing at 56°C (with P3 and P4 *H. pylori*-specific primers) or 59°C (with all-helicobacter primers) for 2 min, and extension at 72°C for 2 min. A total of 35 cycles were performed, followed by a 4-min extension step. A 15-μl sample was electrophoresed through a 6% Visigel separation matrix (Stratagene) and was visualized by staining with ethidium bromide and viewing by UV illumination.

**RESULTS**

Clinical and gross necropsy findings. Guinea pigs did not exhibit clinical signs of gastritis (vomiting, loss of appetite, or weight loss). The weights of infected guinea pigs were not significantly different from the weights of control guinea pigs at either 4 or 15 weeks postinoculation. There were no grossly visible gastric lesions at necropsy in any of the guinea pigs. The
pH of the gastric juice was measured at necropsy and was not significantly different for control and infected guinea pigs (1.70 ± 0.14 versus 1.68 ± 0.13 at 4 weeks or 1.92 ± 0.66 at 15 weeks).

**Gastric colonization.** Colonization was assessed by gastric culture and by PCR of gastric tissue obtained at necropsy. Samples of gastric antrum and fundus were cultured separately; results are summarized in Table 1. Culture and PCR results for individual animals did not always coincide, which may have reflected uneven colonization of the mucosa. At 4 weeks postinfection, five of six animals were culture positive and three of six were PCR positive. At 15 weeks postinfection, two of six animals were culture positive and six of six were PCR positive. Later, it was found that the reduced culture recovery at 15 weeks postinfection was associated with improperly stored culture plates.

**Serology.** As detailed in Fig. 1, two of six guinea pigs were seropositive at 4 weeks and all but one were seropositive at 15 weeks. Titers continued to rise (Fig. 1), suggesting that seropositivity correlated with active infection. To determine whether seropositivity correlated with active infection or merely with exposure to *H. pylori* antigen, three adult female guinea pigs were dosed with killed *H. pylori* antigen, following the same dosing schedule and receiving a similar biomass as the infection group (inset to Fig. 1). One of the three guinea pigs remained seronegative for 5 weeks. The other two guinea pigs were borderline seropositive at 4 weeks but seronegative at 5 weeks postexposure (a decreasing titer), in contrast to the infected guinea pigs, who had rising titers for the duration of their infections (Fig. 1).

**Detection of *H. pylori* in feces.** (i) **Fecal cultures.** Fecal cultures were consistently negative, although samples spiked with approximately $10^4$ CFU of *H. pylori* culture grew readily (data not shown), indicating that this negative result was not due to inhibitory factors in the feces. Thus, live *H. pylori* organisms were not present in the feces at levels detectable by culture.

(ii) **Fecal PCR.** Fecal samples were consistently negative, although signal could be detected in fecal samples spiked with as few as 16 CFU of *H. pylori*.

**Histopathology.** There were no histological abnormalities in any of the control guinea pigs at either 4 or 15 weeks (Fig. 2a). In contrast, moderate multifocal to diffuse antral gastritis was present in the *H. pylori*-infected guinea pigs at both time points (Fig. 2b and c). Lesions were frequently concentrated at the junction between antral and fundic mucosa but were otherwise not seen in fundic mucosa. Inflammation was present throughout the lamina propria, and in some instances it extended through the muscularis mucosa into the submucosa (Fig. 2b). The inflammatory infiltrate was comprised of a mixed population of mononuclear cells, eosinophils, and heterophils (polymorphonuclear phagocytes). At both 4 (Fig. 3a and b) and 15 (Fig. 3c) weeks, lymphoid aggregates and lymphoid follicular structures were present in the antrum (Fig. 3). The inflammation was in general more extensive at 15 weeks than at 4 weeks, with more prominent and more numerous lymphoid nodules and more extensive inflammatory infiltrate. Helical organisms were rarely detected by Warthin-Starry silver staining (data not shown).

**DISCUSSION**

These results demonstrate that guinea pigs can be readily colonized by the rodent-adapted Sydney strain of *H. pylori*. The *H. pylori*-infected guinea pigs in our study had a significant antral gastritis within 4 weeks of infection and were persistently colonized for at least 15 weeks postinfection. Gastritis

![FIG. 2. Experimental *H. pylori* infection causes antral gastritis in the guinea pig. There were no abnormalities in the gastric antra or fundi (not shown) of the control sham-dosed guinea pigs at either 4 or 15 weeks after dosing (a). At 4 weeks (b) and 15 weeks (c) there was a moderate lymphohistiocytic, eosinophilic infiltrate in the submucosa and deep mucosa, with multifocal extensions to the superficial mucosa (arrows). The inset illustrates eosinophils. Active inflammation characterized by heterophilic infiltrate (guinea pig polymorphonuclear phagocytes) was present in the mucosa in scattered foci. At 4 weeks postinfection, the infiltrate was concentrated in the corpus-antrum junction, and at 15 weeks postinfection it extended throughout the antrum. H&E stain was used. Bar = 150 μm.](image)
organisms were apparently present in the fundi, as it was significantly altered in the infected guinea pigs. However, consistent with our finding that gastric juice pH was not significantly altered in the infected guinea pigs, which is not present in the fundi of infected guinea pigs, it was not detectable in the infected guinea pigs, which is consistent with our finding that gastric juice pH was not significantly altered in the infected guinea pigs. However, H. pylori organisms were apparently present in the fundi, as it was cultured from fundic biopsies obtained at necropsy (Table 1). At 15 weeks, the distribution of the gastritis was still antral, but there was additional development of gastric mucosa-associated lymphoid tissue. These lymphoid follicular structures are consistent with chronic helicobacter-induced gastritis in humans and other species. Lymphoid follicles are prominent in the stomachs of ferrets with Helicobacter mustelae (4, 13), dogs with Helicobacter felis (21), cats with H. pylori (17, 24), mice with H. felis (11), macaques with H. pylori (2, 5), gnotobiotic pigs with H. pylori (20), and H. pylori-infected humans with antral gastritis (14, 15).

The gastric inflammatory infiltrate was a mixed population of lymphocytes, macrophages, and heterophils (guinea pig polymorphonuclear phagocyte cells) with prominent eosinophils (Fig. 2b, inset). In humans, it has been observed that infection with H. pylori induces gastric secretion of IL-8 (3). IL-8 is a chemokine that attracts monocytes (1); in guinea pigs, IL-8 also attracts eosinophils (6), so the prominent eosinophilic inflammation is consistent with IL-8 induction. Future studies will examine the cytokine profile directly. The presence of an IL-8 homolog in guinea pigs is a significant advantage over rat and mouse rodent models, which lack an IL-8 homolog (35).

Organisms were seen only rarely in Warthin-Starry silverstained sections of infected guinea pigs. Evaluation of bacterial colonization with silver-stained histologic sections is an insensitive method for detection of H. pylori. In BALB/c mice infected with the Sydney strain of H. pylori, colonization was scored histologically as undetectable to barely detectable, although bacterial colonization was quantitated by culture as 10^{-8} CFU/g (22). In contrast to histological detection of bacteria in Warthin-Starry silver-stained sections, culture and PCR techniques routinely detect 100 or fewer CFU of organisms per g. We estimate that fewer than 10^5 organisms/g of tissue were present in these guinea pigs, but this level of colonization was nevertheless sufficient to produce significant gastritis.

It has been speculated that vitamin C plays a role in the pathogenesis of H. pylori-related disease in humans. Specifically, ascorbic acid is the component of vitamin C that has been shown to inhibit N-nitrosation in vitro and thus to lead to decreased levels of nitrosamines, many of which are potent carcinogens (33). Ascorbic acid has been measured in the gastric juice of normal individuals at levels 3 to 5 times its concentration in plasma, suggesting that there is active gastric secretion of ascorbic acid (25). Interestingly, the total vitamin C and ascorbic acid concentrations (but not plasma ascorbic acid concentration) in gastric juice are significantly lower in individuals infected with H. pylori (28, 29). Gastric juice ascorbic acid levels were shown to increase after intravenous injection of ascorbic acid in human volunteers without H. pylori gastritis, but not in individuals with gastritis (8). Thus, ascorbic acid secretion appears to be impaired in individuals with H. pylori gastritis. Because there is a significant risk factor for gastric carcinoma associated with H. pylori infection, lowered ascorbic acid levels may increase the risk for gastric carcinoma. Correa et al. (8) have hypothesized that ascorbic acid in the normal gastric microenvironment acts as an antioxidant and free-radical scavenger and thus protects against the formation of carcinogenic nitrosamines and oxidative damage to DNA by re-active oxygen species.

In addition to the link between decreased gastric juice ascorbic acid levels and increased risk of cancer, there is evidence that dietary, serum, and gastric juice ascorbic acid levels can influence gastric H. pylori colonization. A recent study examined the effect of diet in a mouse model of H. pylori. In this study, dietary supplementation with vitamin C (not a normal component of mouse diets) resulted in a 75% lower rate of recovery
of H. pylori from gastric cultures of mice experimentally infected with H. pylori than from that mice fed a diet of un-supplemented mouse chow (34). Also, vitamin C directly inhibits the growth of H. pylori on culture media (16). Vitamin C supplementation studies have been performed and a correlation between vitamin C status and severity of gastritis is an active area of research (8, 9). We developed the guinea pig model specifically to study vitamin C because the guinea pig is the only small laboratory animal that lacks gulonolactone oxidase and thus has gastrointestinal vitamin C absorption mechanisms similar to those in humans. We have developed a technique for acquiring gastric juice and measuring gastric vitamin C in guinea pigs and found that guinea pigs concentrated vitamin C approximately fivefold in their gastric juices as compared to their sera (27). Guinea pig gastric juice ascorbic acid concentrations were comparable to those found for human gastric juice ascorbic acid, which ranged from 0.36 to 2.53 mg/dl (28, 30, 31). The guinea pig stomach has also been studied extensively by using in vitro physiology techniques such as measurement of fluxes in Ussing chambers that cannot be studied extensively by using in vitro physiology techniques such as measurement of fluxes in Ussing chambers that cannot be readily adapted to mouse tissues (18, 19, 32). Thus, the guinea pig should provide the ideal animal model to study the roles of H. pylori gastritis and gastric vitamin C transport in the progression of gastritis and to dissect what factors influence the severity of the inflammatory response in the gastric mucosa.

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