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An Animal Model of Gastric Ulcer Due to Bacterial Gastritis in Mice

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Abstract. Conventional female BalbC mice were inoculated with *Gastrospirillum*-like bacteria in mouse gastric homogenate or in 5.0-μm filtrate of gastric homogenate. The bacteria were originally isolated from cheetahs with gastritis. The mice were killed 6 months, 7 months, or 1 year after inoculation. All mice became infected with *Gastrospirillum*-like bacteria that were confined to the gastric mucosa. Control mice, given either sterile Brucella broth, 0.22-μm filtrate of infected gastric homogenate, or uninfected gastric homogenate did not become infected with bacteria. Lesions in infected mice included severe lymphoplasmacytic gastritis (26/26 infected mice), gastric epithelial hyperplasia (25/26 infected mice), and gastric ulceration (11/26 infected mice). Neutrophilic inflammatory cell infiltrates were inconsistent. None of the uninfected control mice had *Gastrospirillum*-like bacteria, gastritis, gastric epithelial hyperplasia, or gastric ulceration. These results implicate *Gastrospirillum*-like bacteria from cheetahs in the pathogenesis of gastric ulceration. This model will be useful in investigating the mechanisms of gastric ulceration associated with bacterial gastritis.

Key words: Animal model; BrdU; gastric ulcer; gastritis; *Gastrospirillum*; *Helicobacter pylori*; mouse; rodent; stomach.

Gastric bacteria, including "*Gastrospirillum*" sp. and *Helicobacter* sp., are common in many animal species including human beings,[31] dogs,[14] cats,[13,23] cheetahs,[8] ferrets,[10,11] nonhuman primates,[1] and pigs.[26] These microorganisms have been associated with chronic active gastritis and peptic ulceration in human beings,[31] lymphofollicular gastritis in cats and dogs,[13,14,23] and chronic gastritis in ferrets,[10,11] cheetahs,[8] pigs,[26] and nonhuman primates.[1] In human beings, infection with *Helicobacter pylori* is common and has been implicated as a significant risk factor for peptic ulcer[31] and gastric adenocarcinoma,[24] but the mechanisms by which bacteria predispose to these lesions are not known. Experimental models of disease in gnotobiotic piglets,[15] gnotobiotic dogs,[27] ferrets,[12] and mice[16] have been developed, and have proven useful in elucidating some aspects of the pathogenesis of naturally occurring bacterial gastritis. For example, experimental infection of gnotobiotic piglets with *H. pylori* has been used to identify both motility[5,6] and urease[4] as bacterial virulence factors. Experimental infections of gnotobiotic mice with *Helicobacter felis*[16] and of immunized gnotobiotic piglets with *H. pylori*[3] have produced neutrophilic gastritis, which may be helpful in elucidating mechanisms of neutrophilic inflammation in human beings with *H. pylori*. Experimental infection of conventional mice with *H. felis* has proven useful in developing potential vaccination protocols.[2] In spite of these successes, mechanisms whereby bacterial infec-

tion causes ulceration have not been examined in an animal model of infection. Gastric ulcers have been described in naturally occurring infection of ferrets with *Helicobacter mustelae*[11] and cheetahs with *Helicobacter acinonyx* and *Gastrospirillum* sp.,[8] but ulceration in an experimental animal model has not yet been demonstrated.

Previous studies showed that female BalbC mice infected with gastric bacteria isolated from cheetahs developed severe chronic gastritis that was strikingly similar to the natural disease in cheetahs.[7] In that study, gastritis was present by 1 month after infection, and continued to increase in severity with time. Gastric mucosal hypertrophy and gastric ulcers were not present until 7–12 months after infection. The purpose of the present study was 1) to further characterize the chronic gastritis and gastric ulceration due to prolonged bacterial gastritis in mice, and 2) to determine the morphologic relationship between epithelial damage and bacterial colonization, lymphocytic and neutrophilic inflammation, and epithelial hyperplasia.

Materials and Methods

Bacteria

The bacterial inoculum consisted of a Brucella broth homogenate of murine gastric homogenate containing *Gastrospirillum*-like bacteria from cheetahs.[8] These bacteria were long (7–10 μm) tightly coiled spirals either with or without superficial helical filaments. Because they could not be cul-

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Table 1. Gross lesions in mice infected with *Gastrospirillum*-like bacteria from cheetahs.

Group	n	Mean stomach weight (g)	No. with grossly detectable hypertrophy	No. with grossly detectable ulcers
Unfractionated gastric homogenate				
Infected homogenate, 6–7 months after inoculation*	8	0.306 ± 0.085†	8/8	2/8
Infected homogenate, 1 year after inoculation	10	0.349 ± 0.039†	10/10	3/10
Uninfected homogenate, 1 year after inoculation	7	0.216 ± 0.013	0/7	0/7
Sterile Brucella broth				
6–7 months after inoculation*	8	0.216 ± 0.013	0/8	0/8
1 year after inoculation	8	0.248 ± 0.021	0/8	0/8
Fractionated gastric homogenate, 6–7 months after inoculation*				
0.22-μm filtrate	8	0.203 ± 0.013	0/8	0/8
5.0-μm filtrate	8	0.273 ± 0.036†	7/8	0/8

* In these groups, four mice were killed 6 months after inoculation and four mice were killed 7 months after inoculation. Because there were no differences in these groups they have been combined. Stomach weights were determined for only the mice killed 7 months after inoculation.

† Significantly different from Brucella broth control group, $P < 0.05$.

tured, they could not be definitively identified, but bacteria of similar morphology, variously referred to as "*Gastrospirillum hominis*" and "*Helicobacter heilmannii*," have been shown to be closely related to *H. felis* and *H. pylori*.[29] In this discussion, these spiral bacteria will be referred to as *Gastrospirillum*-like bacteria. It should be understood, however, that the inoculum likely contained a mixture of strains of closely related bacteria in the genus *Helicobacter*, all characterized by a spiral morphology and a habit of growth deep within the gastric glands. Previous results have indicated that these *Gastrospirillum*-like bacteria colonize the gastric glands of these mice and can be consistently passed in mice.[7,8] Naive mice do not have *Gastrospirillum*-like gastric bacteria prior to inoculation with infected gastric homogenate. Because *Gastrospirillum*-like bacteria cannot be cultured in vitro, passage in mice is the only means of propagating them.[7,8] The mice in the present study were given cheetah bacteria that had been passed twice in mice.

Control inocula

In addition to the gastric homogenate from *Gastrospirillum*-infected mice (infected gastric homogenate), four control inocula were used: 1) sterile Brucella broth, 2) gastric homogenate from mice that had not been inoculated with *Gastrospirillum*-like bacteria (uninfected gastric homogenate), 3) infected gastric homogenate that had been passed through a sterilizing filter with 0.22-μm-diameter pores (0.22-μm filtrate), and 4) infected gastric homogenate that had been passed through a filter with 5.0-μm-diameter pores (5.0-μm filtrate). Light microscopic examination of the 5.0-μm filtrate of infected gastric homogenate revealed *Gastrospirillum*-like bacteria. The other three control inocula did not have bacteria.

Animals

Female BalbC mice were orally inoculated with infected gastric homogenate or control inocula at 6–8 weeks of age and killed 6 months, 7 months, or 1 year later. The numbers

of mice in each group are shown in Tables 1 and 2. Mice were housed 3–5 per cage and offered commercial unmedicated rodent chow and water ad libitum. Control and inoculated groups were housed separately. At sacrifice, mice were given 5 mg 5-bromo 2'-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO) by intraperitoneal injection, and killed 2 hours later by cervical dislocation. The stomachs were fixed by luminal perfusion with 0.5 ml of 10% neutral buffered formalin, removed, and immersed in neutral buffered formalin for 24 hours. They were then transected longitudinally along the greater and lesser curvatures, trimmed to remove extraneous tissue, examined, drained, and weighed. Longitudinal and cross sections from the mid-fundus and greater and lesser curvatures and the mid-jejunum and colon were embedded in paraffin and cut in 6-μm sections. Replicate sections were stained with hematoxylin and eosin and Warthin-Starry stains. Sections from mice killed 1 year after inoculation were stained immunochemically to detect incorporation of BrdU. All procedures involving animals were approved by the Ohio State University Laboratory Animal Care and Use Committee. Lesions in mice killed 1–7 months after infection have been reported elsewhere.[7]

BrdU immunochemistry

Poly-L-lysine-mounted 6-μm sections were rehydrated and washed in Automation buffer (Biomeda Corp., Foster City, CA). Antigens were unmasked with antigen retrieval solution (Biogenex Co., San Ramon, CA). Sections were immersed in antigen retrieval solution and heated for 5 minutes on high in a microwave oven. Distilled water was added to replace the volume lost during heating, and the sections were heated in the microwave oven again for 5 minutes. They were then held in the hot antigen retrieval solution at room temperature for 15 minutes. All other incubations were performed at room temperature. Sections were removed from the antigen retrieval solution and washed in Automation buffer. They were incubated in 4 M HCl for 20 minutes, neutralized with

Table 2. Histologic lesions in mice infected with *Gastrospirillum*-like bacteria from cheetahs.

Group	n	Gastritis, mean score*	No. with neutrophilic infiltration	No. with histologically detectable ulcers	No. with *Gastrospirillum*-like bacteria
Unfractionated gastric homogenate					
Infected homogenate, 6–7 months after inoculation†	8	3.75 ± 0.89‡	7/8	2/8	8/8
Infected homogenate, 1 year after inoculation	10	5.00 ± 0.00‡	7/10	6/10	10/10
Uninfected homogenate, 1 year after inoculation	7	0.57 ± 0.56	2/7	0/7	0/7
Sterile Brucella broth					
6–7 months after inoculation†	8	0.88 ± 0.84	0/8	0/8	0/8
1 year after inoculation	8	1.38 ± 0.74	4/8	0/8	0/8
Fractionated gastric homogenate, 6–7 months after inoculation†					
0.22-μm filtrate	8	0.63 ± 0.52	0/8	0/8	0/8
5.0-μm filtrate	8	3.88 ± 1.13‡	6/8	3/8	8/8

* Calculated as described in methods.

† In these groups, four mice were killed 6 months after inoculation and four mice were killed 7 months after inoculation. Because there were no differences in these groups they have been combined.

‡ Significantly different from Brucella broth control group, $P < 0.001$.

borax (50 mM boric acid and 10 mM sodium borate adjusted to pH 8.5), twice for 10 minutes each time, blocked for 20 minutes with normal horse serum diluted 1:200 in Automation buffer, and incubated for 1 hour with mouse monoclonal antibody to BrdU (Becton-Dickinson, San Jose, CA) diluted 1:100 in Automation buffer. Sections were washed in Automation buffer, incubated with biotinylated anti-mouse immunoglobulin, and peroxidase labelled with a StrAViGen staining kit according to the manufacturers instructions (Biogenex Co.).

Morphologic evaluation

Severity of gastritis in all groups of mice was evaluated semiquantitatively. Mean gastritis score was calculated according to the density of lymphocytes and plasma cells as follows: 0 = no infiltrates, 1 = mild, multifocal infiltration, 2 = mild, widespread infiltration, 3 = mild, widespread and moderate, multifocal infiltration, 4 = moderate, widespread infiltration, 5 = moderate, widespread and severe, multifocal infiltration. Neutrophilic infiltration was scored as present or absent. Epithelial insults that resulted in a breach of the epithelial basement membrane were scored as ulcers.

In mice killed 1 year after inoculation, severity of gastritis was determined by morphometry. Measurements were made with a digitizing pad and commercially available software (Bioquant IV, R&M Biometrics, Nashville, TN). The severity of gastritis was evaluated in hematoxylin and eosin-stained sections by counting the number of lymphocytes and plasma cells and the number of neutrophils in the fundic mucosa in 3 adjacent 40× fields. The surface area counted was measured, and severity of inflammation was expressed as number of cells/mm² mucosa. Relative mucosal thickness was determined by measuring the surface area of the mucosa (excluding lymphoid follicles and areas containing exclusively inflammatory infiltrate) and dividing by the length of the

muscularis mucosae. Lymphoid follicles were enumerated and their surface area measured. Epithelial proliferation was evaluated in sections stained for BrdU. The labelling index was calculated by counting labelled and unlabelled nuclei in three contiguous well-oriented 40× fields (labelling index = number of labelled nuclei/number of total nuclei). The distance between the most luminal- and most basal-labelled nuclei in three well-oriented glands (the proliferative zone) was measured and expressed as a percent of the total gland length.

The presence of bacteria was evaluated in Warthin-Starry stained sections. Cheetah gastric bacteria were easily distinguished from luminal bacteria by their large size, spiral morphology, and location deep within the gastric glands. The number of bacteria adjacent to gastric ulcers was scored subjectively, +1 to +3, where +1 = rare bacteria, +2 = bacteria easily identified in the section, but widely scattered, and +3 = glands filled with large numbers of bacteria. All histologic sections were scored without prior knowledge of their source.

Electron microscopy

Samples of gastric mucosa from the fundus and antrum of each mouse were fixed in glutaraldehyde (pH = 7.3), postfixed in 1.3% osmium tetroxide, and embedded in Medcast epoxy resin (Ted Pella, Inc., Redding, CA). Ultrathin sections were stained with uranyl acetate and lead citrate and were examined with a Phillips 300 electron microscope. The distance between bacterial spirals (bacterial wavelength) was measured from the top of adjacent spirals and expressed as the mean ± standard deviation of 20 measurements.

Statistics

Inoculated and control groups were compared with the Mann-Whitney U-test. Values were expressed as mean ± standard deviation.
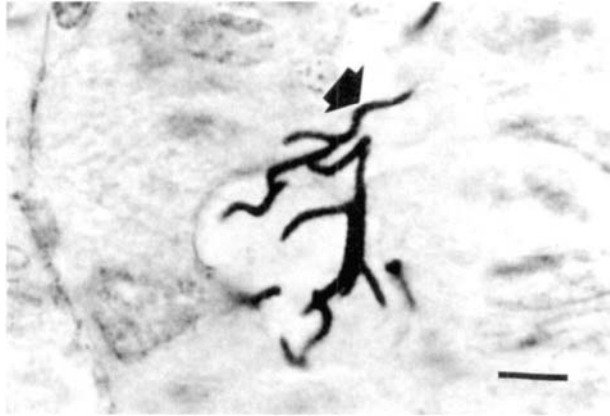
Fig. 1. Warthin-Starry stain of a gastric gland from a mouse infected with gastric bacteria. Dark-staining spiral organisms (arrow) are present within the gland. Bar = 5 μm.

Fig. 3. Stomach from an uninfected control mouse. Thickened rugae are absent. Marker = 1 cm.

Results

Clinical signs

Mice continued to eat well and maintain their weight throughout the experiment. Clinical signs of illness were not observed.

Bacterial colonization

Large spiral *Gastrospirillum*-like bacteria were demonstrated in Warthin-Starry sections of stomach in all mice given either unfractionated infected gastric homogenate or 5.0-μm filtrate of infected homogenate (Fig. 1), but in none of the other groups. Mixed bacterial populations were present in the gastric lumen of all mice, but only spiral bacteria were found in the glands. Spiral bacteria were present in the gastric pits and deep within the glands, and occasionally on the epithelial surface. Large numbers of *Gastrospirillum*-like bacteria were sometimes present packed in the gastric glands and pits.

Gross lesions

Gastric rugal hypertrophy was evident in *Gastrospirillum*-infected mice (Fig. 2), but not in uninfected controls (Fig. 3, Table 1). Rugal thickening tended to be most severe along the greater curvature in the body of the stomach, and was often nodular. In addition, stomachs from the infected mice were significantly heavier than stomachs from the other groups (Table 1). Grossly detectable gastric ulcers were present in some infected mice given unfractionated infected homogenate but not in the other groups of mice (Table 1). These ulcers were 1–3 mm in diameter and were located in the body of the stomach near the lesser curvature (Fig. 4). There were no other gross lesions in the gastrointestinal tract of any mouse.

Fig. 2. Stomach from a mouse infected for 1 year with gastric bacteria of cheetah origin. Nodular thickening of gastric rugae is evident along the greater curvature (arrow). Marker = 1 cm.
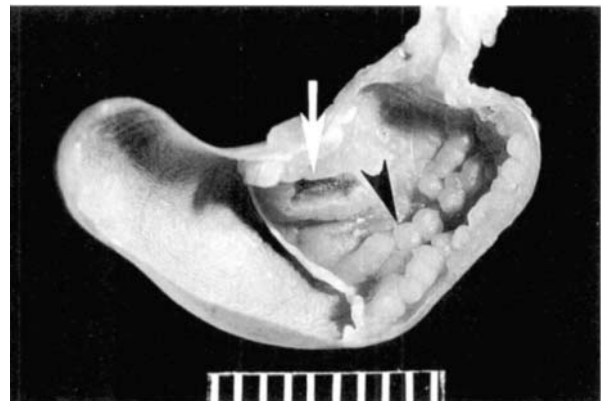

Fig. 4. Stomach from a mouse infected for 1 year with gastric bacteria of cheetah origin. An ulcer is present at the lesser curvature (arrow). There is nodular thickening of the gastric rugae along the greater curvature (arrowhead). Marker = 1 cm.
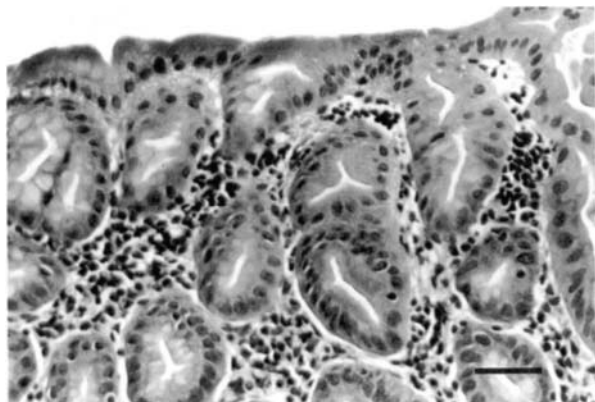
Fig. 5. HE-stained section of fundic mucosa from the stomach of a mouse infected for 1 year with gastric bacteria. Numerous lymphocytes and plasma cells are present in the superficial lamina propria. Parietal cells are not seen due to hyperplasia of mucus neck cells. Bar = 40 μm.

Fig. 7. HE-stained section of the stomach from a mouse infected for 1 year with gastric bacteria. Gastric epithelial hyperplasia is characterized by polypoid projections of disorganized epithelium (arrows). Bar = 300 μm.

Histologic lesions

Moderate to severe chronic lymphoplasmacytic and follicular gastritis was present in all mice given either unfractionated infected homogenate or 5.0-μm filtrate of infected homogenate, but not in the other groups. In infected mice, many lymphocytes and plasma cells were present throughout the glandular stomach in the superficial lamina propria (Fig. 5). Well-developed lymphoid follicles were present in the lamina propria and submucosa throughout the stomach. In mice given Brucella broth, uninfected gastric homogenate, or 0.22-μm filtrate of infected homogenate, only a few widely scattered lymphocytes and no plasma cells or follicles were present (Fig. 6). The mean gastritis score was significantly higher in infected mice than in uninfected mice (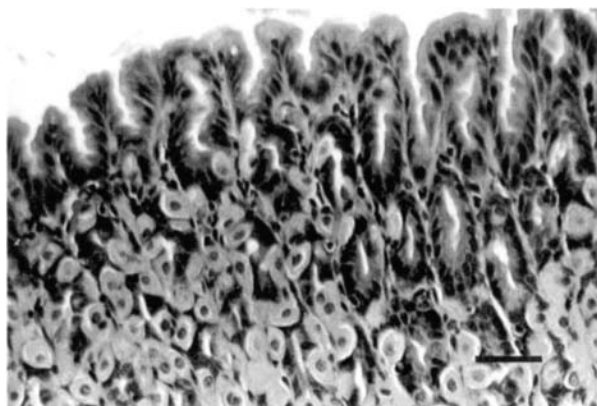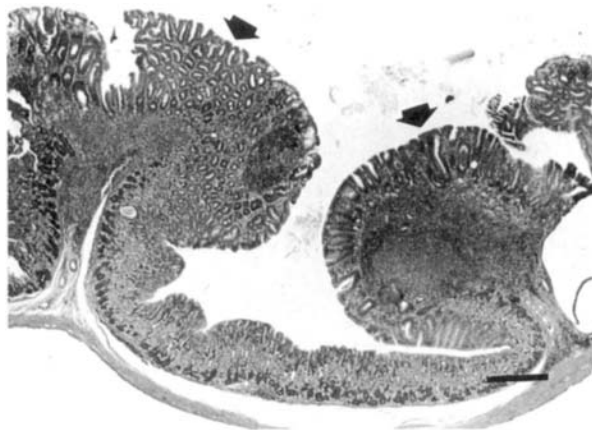Table 2). In mice killed 1 year after inoculation, the number of lymphocytes and plasma cells/mm^2 mucosa in the mice given infected homogenate (1,606 $\pm$ 480) was significantly greater than the number of lymphocytes/mm^2 mucosa in the mice given sterile Brucella broth (398 $\pm$ 121, $P = 0.0005$).

Multifocal accumulations of neutrophils and scattered neutrophils were present in the deep lamina propria of some mice in all groups, but were more common in infected mice (Table 2). In some areas, neutrophils extended into the superficial lamina propria. Neutrophilic gland abscesses were not seen. In mice killed 1 year after inoculation, the number of neutrophils/mm^2 gastric mucosa varied greatly between individual mice. The mean number was greater in inoculated mice (31.6 $\pm$ 42.3) than in control mice (12.6 $\pm$ 28.6) but this difference was not statistically significant ($P = 0.1665$), probably because of the variability between individual animals.

Fig. 6. HE-stained section of fundic mucosa from the stomach of an uninfected control mouse. Parietal cells are prominent, mucus neck cells are within normal limits, and few inflammatory cells are present. Bar = 40 μm.

Fig. 8. HE-stained section of the stomach of an uninfected control mouse. Hyperplastic polyps are not present. Bar = 300 μm.
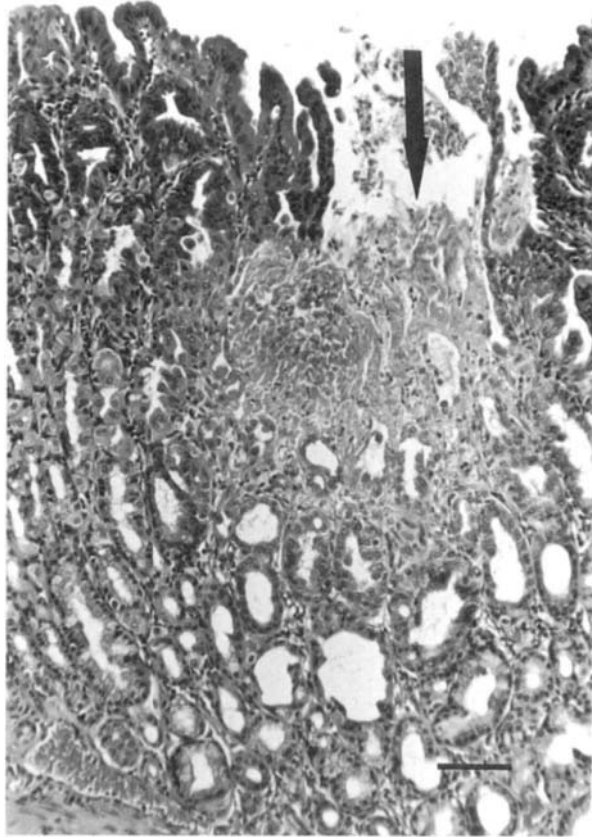
Fig. 9. HE-stained section of the stomach from a mouse infected for 1 year with gastric bacteria. Subacute gastric ulcer (arrow) is characterized by superficial necrosis, dilation of adjacent glands, and early fibrosis. Bar = 30 μm.

Fig. 10. Transmission electron micrograph of a gastric gland from a mouse infected with *Gastrospirillum*-like bacteria. Two morphologic types of bacteria are present: tightly coiled bacteria without surface structures (large arrow), or more loosely coiled bacteria with paired helical filaments (arrowheads). Flagella are present in the section (small arrow). Bar = 1 μm.

In the infected mice there was multifocal gastric mucosal hyperplasia characterized by elongated, often disorganized glands with elongated neck zones. Hyperplasia of mucus neck cells was prominent in areas of intense inflammation (Figs. 5, 7). In some areas, hyperplastic regions appeared to be polypoid (Fig. 7). Hyperplastic polyps were not present in uninfected mice (Fig. 8), and were more prominent in mice killed 1 year after inoculation than in infected mice killed 6–7 months after inoculation. The surface area of gastric mucosa expressed as a percent of the length of the muscularis mucosae was significantly greater in the infected mice killed 1 year after inoculation (42.4 ± 5.4%) than in the uninfected mice (29.4 ± 2.8%, $P = 0.0009$).

Gastric ulcers were evident histologically in infected but not control mice (Table 2, Fig. 9). Of the 16 ulcers identified in 11 infected mice, 15 were located within foci of epithelial hyperplasia. The remaining ulcer was adjacent to a focus of hyperplasia. Seven ulcers were associated with lymphoplasmacytic inflammation, and
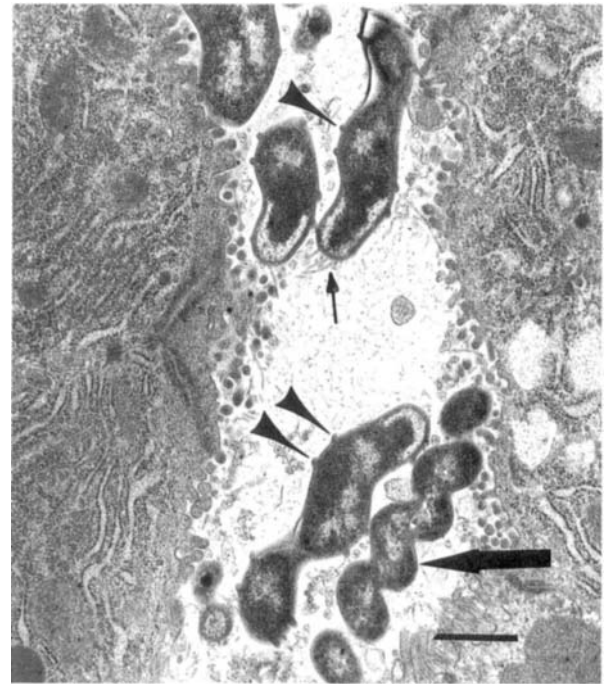
six were associated with lymphoid follicles. The chronicity of the ulcers ranged from acute (sharply demarcated superficial coagulation necrosis and mild deeper epithelial regeneration with no fibrosis) to chronic (loss of superficial mucosa with fibrosis). There was no correlation between the presence or number of neutrophils and gastric ulcers. No gastric ulcers were found in the mouse with the highest neutrophil count (141 neutrophils/mm^2), and no neutrophils were present in the gastric mucosa of two of the mice with ulcers. Where ulcers were present, *Gastrospirillum*-like bacteria were present up to the edge of, but not within the ulcer crater. There was no morphologic correlation between the location of bacteria and the location of inflammation, ulcers, or epithelial hyperplasia. Sections of proximal duodenum, mid-jejunum, and colon were within normal limits in all mice.

Ultrastructural examination revealed two bacterial morphologies (Fig. 10). One type was tightly coiled (wavelength = 0.670 ± 0.106) and had no surface structures. The other was more loosely coiled (wavelength = 1.350 ± 0.178, $P < 0.0001$) and had superficial paired helical filaments. *Helicobacter muridarum*, an occasional gastric contaminant normally found

in the murine intestine,[18] was not observed in any mouse stomach. No other bacterial or viral pathogens were detected ultrastructurally.

BrdU immunochemistry

Uptake of BrdU by proliferating cells was detected in epithelial cell nuclei in the gastric neck region of the stomach, basal epithelial nuclei of the gastric squamous mucosa, epithelial nuclei of the glands of the small intestine, and lymphocyte nuclei in the center of the lymphoid follicles. In addition, scattered lymphocyte nuclei in the gastric lamina propria of infected mice demonstrated uptake of BrdU. The labelling index of gastric glands in areas of histologically evident hyperplasia was significantly greater ($37.5 \pm 6.3\%$) than the labelling index of either uninfected stomachs ($18.9 \pm 4.6\%$, $P = 0.0005$) or nonhyperplastic areas of infected stomachs ($23.0 \pm 11.1\%$, $P = 0.0081$). The width of the proliferative zone in hyperplastic areas ($47.3 \pm 7.9\%$) was also greater than in uninfected stomachs ($17.4 \pm 2.3\%$, $P = 0.0005$), and in nonhyperplastic areas ($17.9 \pm 3.6\%$, $P = 0.0003$). In some hyperplastic areas, the proliferative zone comprised almost the entire length of the gland. In these areas glandular architecture was sometimes sufficiently disorganized to hinder precise measurement of the proliferative zone.

Discussion

The results of this study demonstrate that gastric homogenates containing *Gastrospirillum*-like bacteria are capable of causing gastric ulceration in an experimental model even in the absence of added ulcerogenic cofactors. Although ulcers have been described in naturally occurring bacterial gastritis in human beings,[31] ferrets,[11] and cheetahs,[8] infection of mice by gastric bacteria of cheetah origin represents the first report of experimental production of gastric ulceration by animal inoculation of gastric bacteria. This is significant because the widely varying severity of host response to gastric bacteria in human beings, particularly, has led to the suggestion that bacteria alone do not cause ulceration, but nonbacterial cofactors, such as hyperacidity, stress, nicotine, or alcohol, are necessary for epithelial damage to occur.[9] The results of this study suggest that in the absence of exogenous factors, bacteria in gastric homogenate can be sufficient to initiate ulcerogenesis.

In the present study, two morphologic types of gastric bacteria present in the gastric homogenate were found in infected mice. The predominant type was smooth and tightly coiled and was most similar to an organism found in dogs,[14] pigs,[26] and human beings,[19] and given the provisional name "*Gastrospirillum hominis.*"[19] This organism is most likely a member of the genus *Helicobacter,*[29] but has not yet been cultured in vitro, and therefore cannot be definitively identified. The other type was less tightly coiled and had superficial paired helical filaments, most similar to *H. felis.*[25] It differed from *H. felis* in that it could not be cultured in vitro. Thus, these two morphologic types are similar to both *Gastrospirillum* and *H. felis* in their mode of growth (within the gastric pits and glands) and their morphology, but are similar to *Gastrospirillum* but not *H. felis* in that they do not grow in vitro. It is likely that these two morphologic types of bacteria are closely related to each other, as well as to members of the genus *Helicobacter,* but definitive identification of these bacteria must await in vitro culture.

The mechanisms of epithelial ulceration in bacterial gastritis are not known. It has been suggested that neutrophils and their products contribute to ulcerative effects.[20] In this study, however, the lack of correlation between the presence of neutrophils and gastric ulceration suggests that neutrophils are neither necessary nor sufficient to produce epithelial damage. Neutrophils were present in only some infected mice, and were present in some control mice although generally in lower numbers than in inoculated mice. Furthermore, the number of neutrophils did not correlate with the presence of gastric ulcers. In some mice with large numbers of neutrophils, ulcers were not found. In other mice with ulcers, no neutrophils were present in the gastric epithelium.

Other potential causes of ulceration include the effects of lymphoplasmacytic inflammation and bacterial cell products on gastric epithelial cells. Direct effects of these products could not be demonstrated definitively in these mice. The correlation between lymphoplasmacytic inflammation and ulceration was weak, and there was no morphologic association between gastric ulceration and the number of bacteria adjacent to the ulcer site. In addition, foci of severe inflammation and large mats of bacteria were present in some mice in the absence of morphologic evidence of epithelial damage. Finally, previous studies have demonstrated large numbers of bacteria in mice infected for 1–3 months without ulceration.[7] Thus, any ulcerogenic effect of lymphocytes, bacteria, or their products on gastric epithelium was likely to be indirect.

In this study, gastric ulcers occurred exclusively in or adjacent to hyperplastic epithelial foci. This association suggests a causative relationship between hyperplasia and ulceration. Although epithelial hyperplasia is not routinely described in many reports of bacterial gastritis, mucosal hypertrophy is characteristic of bacterial gastritis in cheetahs.[8] In human beings, *H. pylori* has been associated with hypertrophic gastric polyps[32] and enlarged gastric folds.[22] Thus, gastric mucosal hyperplasia is one possible manifestation of bacterial gastritis, and may lead to gastric ulceration.

Whether gastric epithelial hyperplasia leads to gastric ulceration or both changes are caused by a third factor remains to be determined.

The findings of gastric ulceration, epithelial proliferation, and variable neutrophilic infiltration due to gastric bacteria in BalbC mice in this study are in marked contrast to the effects of *H. felis* in germ-free Swiss-Webster mice. In those mice, *H. felis* caused marked, persistent neutrophilic gastritis.[16] There are several possible explanations for the different lesions found in the two studies. First, it is possible that the different bacterial species used in these studies contribute to differing host responses. In that study, broth-cultured *H. felis* was used as an inoculum. In the present study, the inoculum contained a mixture of morphologic types of *Gastrospirillum*-like bacteria related to *H. felis,* but that could not be grown in culture. It is unlikely that differences between these bacteria and the *H. felis* used in mice in other studies account for differences in lesions seen, however. Other studies have shown that host species have a stereotyped response to different bacterial species. Gnotobiotic puppies respond to both *H. pylori* and *H. felis* with lymphofollicular gastritis.[17,27] Domestic cats respond similarly to both *Gastrospirillum* sp. and *H. acinonyx*.[7] In addition, the lesions described in association with *Gastrospirillum* sp. in human beings are similar to those associated with *H. pylori*.[19,21] Thus, the character of gastritis appears to be more closely correlated with host species than bacterial species.

Several host factors may account for the differences in character as well as the severity of gastritis in different studies. Strain differences may be one factor. Swiss-Webster and BalbC mice may react differently to gastric pathogens. This is consistent with previous studies that showed markedly different responses by individual human beings to *H. pylori* and by individual colonies of ferrets to *H. mustelae*. In humans with *H. pylori* lesions may include mild, chronic superficial gastritis, chronic active gastritis with or without peptic ulceration, and lymphofollicular gastritis, among others.[28] Ferrets with *H. mustelae* may have chronic or chronic active gastritis with or without ulcer,[10,11] or no lesions.[30]

Host immunity may also determine host response. Gnotobiotic piglets, for example, respond differently to *H. pylori* depending on their immune status prior to infection. Naive piglets have gastritis that is primarily lymphocytic and follicular, but prior exposure to *H. pylori* antigens via parenteral immunization induces neutrophilic gastritis with gland abscesses in some piglets.[3] It is likely that the host factors that determine neutrophilic infiltration and gastric ulceration in bacterial gastritis are complex.

Because the cheetah gastric bacteria used in this study could not be cultured, gastric homogenates rather than pure bacterial cultures were used for mouse inoculation. For this reason, other infectious causes of gastritis and ulceration, such as other bacteria and viruses, must be considered. However, gastritis and ulceration only occurred in mice given homogenate containing *Gastrospirillum*-like bacteria. The absence of lesions in mice given 0.22-μm filtrate of infected homogenate suggests that the lesions were not caused by a viral or mycoplasmal agent. The presence of unidentified bacterial agents cannot be completely eliminated by these fractionation experiments, but the large number of *Gastrospirillum*-like bacteria, the absence of other detectable pathogens, the association of these bacteria with lesions in both mice and cheetahs, and the similarity of the lesions to gastritis due to closely related bacteria in other species suggest that the gastric bacteria in the homogenate were the cause of the lesions. It is also unlikely that the bacteria found in the mice were derived from the mice used to passage the bacteria. Uninoculated mice, including the control mice in this study, are routinely free of gastric bacteria. Furthermore, mice inoculated with gastric homogenate from uninfected mice remained free of bacteria and gastritis. Although a related bacterium, *Helicobacter muridarum,* has been described in the murine intestine,[18] this organism was not found in the stomachs of either the control or inoculated mice in this study.

In summary, the results of this study demonstrate that chronic infection of conventional BalbC mice by gastric homogenate containing *Gastrospirillum*-like bacteria results in bacterial gastritis characterized by lymphoplasmacytic inflammation, gastric epithelial hyperplasia, and gastric ulceration. Neutrophilic infiltrates are variable. Epithelial lesions appear to be independent of neutrophilic inflammation, but are associated with gastric epithelial hyperplasia. This model will be useful in determining the pathogenesis of gastric ulceration in bacterial gastritis.

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