Gastric Helicobacter Species Infection in Murine and Gerbil Models: Comparative Analysis of Effects of H. pylori and H. felis on Gastric Epithelial Cell Proliferation

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C57BL/6 mice and Mongolian gerbils were infected with Helicobacter felis and Helicobacter pylori SS1 strain to investigate the effects of different Helicobacter species on gastric inflammation and epithelial cell proliferation in different animal models. At 4 weeks, gerbils infected with H. pylori or H. felis developed antral gastritis. Onset of gastritis varied between the models, with mice infected with H. pylori having minimal inflammation at 8 weeks. In mice, H. felis, but not H. pylori, induced significantly increased epithelial cell proliferation in the cardia and corpus at 8 weeks, but no changes were observed at 4 weeks. In gerbils, both H. pylori and H. felis significantly increased antral epithelial cell proliferation at 4 weeks. Epithelial cell proliferation induced by H. felis in gerbils was twice that stimulated by H. pylori. These studies demonstrate host differences in the development of Helicobacter species–induced gastric inflammation and a marked difference in epithelial cell proliferation induced by H. pylori and H. felis in 2 animal models.

Increased proliferation of gastric epithelial cells is considered to be relevant to bacterial-induced gastric neoplasia, and H. pylori infection is associated with increased gastric epithelial cell proliferation in both humans [8] and animals [6]. To date, comparative analysis of the effects of H. pylori and H. felis on gastric epithelial cell proliferation and gastric inflammation in the murine and gerbil models has not been undertaken. Recent studies indicated that short-term infection in Mongolian gerbils is a suitable model for evaluating the effects of putative H. pylori virulence factors [9]. The aims of this study were to use this model [9] to compare the effects of H. pylori (SS1 strain) and H. felis on gastric epithelial cell proliferation in both C57BL/6 mice and gerbils to examine further the relationship between infection and preneoplastic conditions.

Materials and Methods

Bacterial strains. H. pylori strain SS1 (kindly provided by R. Ferrero, Pasteur Institute, Paris) was cultured on 5% (vol/vol) horse blood agar plates at 37°C for 2–3 days in a microaerophilic atmosphere. H. felis strain ATCC 49179 (kindly provided by R. Ferrero) was grown on 5% horse blood agar plates supplemented with 10 μg/mL vancomycin, 2.5 μg/mL amphotericin B, 25 ng/mL polymyxin B and 5 μg/mL trimethoprim at 37°C for 2–3 days in a microaerophilic atmosphere.

Murine and Mongolian gerbil infection with H. felis and H. pylori. Specific pathogen–free 6–8-week-old C57BL/6 female mice (Harlan) and female 6–8-week-old Mongolian gerbils (supplied by MGS/Sea and bred at the University of Leeds) were inoculated 3 times by oral gavage with H. pylori SS1 strain (>10^8 cfu) or H. felis strain ATCC 49179 (>10^8 cfu) suspended in tryptose soya broth (Oxoid) over 5 days.

One hour before the animals were killed, they received an intraperitoneal injection of bromodeoxyuridine (BrdU; 50 mg/kg). At the time of death, gastric mucosal samples were taken for biopsy urease
test, microbial culture, and histologic examination. Culture of *H. felis* from gastric biopsies was on plates, as detailed above. Culture of *H. pylori* from gastric biopsies was on selective plates containing 5% (vol/vol) horse blood agar supplemented with 50 μg/mL amphotericin B, 100 μg/mL vancomycin, 3.3 μg/mL polymyxin B, 200 μg/mL bacitracin, and 10.7 μg/mL nalidixic acid for 5–7 days. Gastric tissue for histologic testing was fixed in 10% (vol/vol) formal saline, and sections were stained with hematoxylin and eosin and modified Giemsa stain for identification of *Helicobacter* species.

**Immunohistological detection of Helicobacter species infection.** Immunohistochemical detection of *H. pylori* and *H. felis* was undertaken using a polyvalent rabbit anti-*H. pylori* antiserum (kindly provided by R. Ferrero), respectively. Bound antibodies were detected with biotinylated swine anti-rabbit immunoglobulins antibody and peroxidase streptavidin complex (Dako). Control animals were inoculated with normal rabbit or preimmune serum. Sections stained with modified Giemsa stain or polyvalent rabbit *H. pylori* and *H. felis* antiserum were used to assess the density of bacterial infection. Bacterial density in gerbils was assessed separately in the corpus and antrum on a scale of 0 to 4. In mice, bacterial density was assessed in both the corpus and antrum on a scale of 0 to 4, and a total density was obtained by addition of the scores. A score of 0 or 4 indicated no detectable organisms or the presence of *Helicobacter* species in all visible gastric pits (≥20 organisms/field of view), respectively [10].

**Epithelial cell proliferation.** The proliferation of gastric epithelial cells was determined by BrdU immunohistochemistry. Sections were blocked with biotin for 15 min (Vector Laboratories) and with 5% (vol/vol) rabbit serum for 10 min before incubation for 1 h with a murine monoclonal antibody to BrdU (Dako). Bound antibodies were detected with a biotinylated rabbit anti-mouse immunoglobulins and peroxidase streptavidin complex (Dako). Epithelial cell proliferation was counted in the cardia, corpus, and antrum of mice, and the corpus and antrum of gerbils. In each area, 10–15 well orientated gastric pits (∼500–1000 cells) were counted. The degree of epithelial cell proliferation was expressed as a labelling index (LI; percentage of stained cells/total cells-per-gastric-pit × 100).

**Statistical analysis.** Data are expressed as mean ± SEM. Comparison of groups of animals was undertaken using a Mann-Whitney *U* test or Fisher’s exact test. *P* < .05 was considered to be significant.

**Results**

**Gastric epithelial cell proliferation in *H. pylori-* and *H. felis-*infected C57BL/6 mice.** C57BL/6 mice were inoculated with either *H. pylori* SS1 strain (*n* = 6) or *H. felis* (*n* = 5). At 8 weeks, all inoculated mice were infected on the basis of results of culture, biopsy urease, and histologic tests. Control mice (*n* = 5) tested negative for gastric *Helicobacter* species infection. Higher levels of gastric inflammation were observed in mice colonized with *H. felis*, particularly in the corpus/cardia region, compared with *H. pylori-*infected mice, which showed no substantial differences relative to uninfected control mice. Mice infected with *H. pylori* had a low bacterial density score (mean ± SEM, 1.00 ± 0), compared with *H. felis*-infected mice (4.00 ± 0.55; *P* < .05, Fisher’s exact test).

Gastric epithelial cell proliferation in mice was determined by BrdU histochemistry (figure 1B). At 8 weeks after infection with *H. pylori*, there was no significant difference in epithelial cell proliferation, compared with uninfected mice (figure 2). In contrast, epithelial cell proliferation was significantly increased in mice infected with *H. felis*, in both the cardia (*P* < .01, Mann-Whitney *U* test) and corpus (*P* < .05). No significant increase in cell proliferation was observed in the antrum of either *H. pylori-* or *H. felis-*infected mice.

**Gastric epithelial cell proliferation in Mongolian gerbils infected with *H. pylori* and *H. felis.** Mongolian gerbils were infected with *H. pylori* (*n* = 8) or *H. felis* (*n* = 5). At 4 weeks after inoculation with *H. pylori*, all gerbils were infected on the basis of results of culture, biopsy urease, and histologic testing. Four of 5 gerbils tested positive for *H. felis* by all 3 methods of detection. Immunohistological labelling with an *H. felis*-specific rabbit antiserum (figure 1G) demonstrated *H. felis* in the antral mucosa of the 4 infected gerbils and in the corpus mucosa of 1 gerbil. Uninfected control gerbils (*n* = 5) tested negative by all tests.

Mongolian gerbils infected with *H. pylori* had a chronic inflammatory response in their antral mucosa at 4 weeks after infection (figure 1D) but no inflammatory changes in the corpus mucosa (figure 1C). In 7 of 8 gerbils, *H. pylori* was restricted to the antral mucosa. The gastric mucosa of uninfected control gerbils was histologically normal. At 4 weeks after infection with *H. felis*, severe antral inflammation was evident in all infected gerbils (figure 1E), with lymphoid follicles in the antral mucosa and inflammatory infiltrates in both the lamina propria and submucosa. One of the Mongolian gerbils also had corpus gastritis at 4 weeks after infection (figure 1F). In the antrum, the density of *H. felis* (3.00 ± 0.41) was significantly greater (*P* < .05), compared with that of *H. pylori* (1.63 ± 0.32).

Gastric epithelial cell proliferation in gerbils was determined by BrdU immunohistochemistry (figure 1A), using identical methodology to the mice. Infection of gerbils with *H. pylori* for 4 weeks resulted in a significantly increased (*P* < .05, Mann-Whitney *U* test) epithelial cell proliferation in the antrum (10.48 ± 1.48), compared with uninfected gerbils (2.85 ± 0.59). No increase in epithelial cell proliferation was observed in the corpus mucosa in *H. pylori* infected gerbils. Gerbils infected with *H. felis* also had significantly increased (*P* < .05, Mann-Whitney *U* test) epithelial cell proliferation in the antrum (22.47 ± 1.26), compared with uninfected control gerbils. An increased LI was also observed in the corpus of the gerbil with corpus gastritis but not in *H. felis*-infected gerbils with no corpus gastritis. Antral epithelial cell proliferation in gerbils infected with *H. felis* was significantly greater (*P* < .02, Mann-Whitney *U* test) than in gerbils infected with *H. pylori* SS1 strain. Because *H. felis* caused enhanced epithelial proliferation in both animal models, a further group of mice (*n* = 5) was in-
Figure 1. Bromodeoxyuridine immunohistochemistry of murine and gerbil gastric mucosa and diagnosis of the gastric mucosa of Mongolian gerbils infected with *Helicobacter pylori* strain SS1 and *Helicobacter felis*. Bromodeoxyuridine immunolabeling of antral mucosa of Mongolian gerbil infected with *H. pylori* strain SS1 for 4 weeks (A) and antral mucosa of C57BL/6 mouse infected with *H. pylori* strain SS1 for 8 weeks (B). Proliferating cells have been detected using a monoclonal antibody to BrdU. Hematoxylin and eosin–stained gastric sections of corpus (C) and antral (D) mucosa of Mongolian gerbil infected with *H. pylori* strain SS1 for 4 weeks; antral (E) and corpus (F) mucosa of Mongolian gerbil infected with *H. felis* for 4 weeks. Note marked infiltration of inflammatory cells into the lamina propria of both *H. pylori* and *H. felis* infected Mongolian gerbils (arrows). G, Immunohistological detection of *H. felis* in gastric mucosa of Mongolian gerbil 4 weeks after infection. Bars: panels A and C–F, 500 μm; panel B, 200 μm; panel G, 5 μm.
Discussion

This study demonstrated host differences in the development of *H. pylori*-induced gastric inflammation and a marked difference in gastric epithelial cell proliferation induced by *H. pylori* SS1 strain and *H. felis* in 2 different animal hosts. Although several studies have examined gastric histopathological changes induced by *H. felis* or *H. pylori* in the murine stomach [1–3, 10], comparative studies on the effects of the 2 Helicobacter species on gastric epithelial cell proliferation in early infection have not been reported. In agreement with studies elsewhere [11], gastric epithelial cell proliferation was significantly increased in the cardia and corpus of *H. felis*-infected mice at 8 weeks postinfection, compared with control mice. Conversely, no increase in epithelial cell proliferation was observed in mice infected with *H. pylori*, compared with uninfected control mice at 8 weeks. These data are the first to compare directly the effect of *H. felis* and *H. pylori* on gastric epithelial cell proliferation in the mouse model. Recently, Suzuki et al. [12] observed no change in gastric epithelial cell proliferation in C57BL/6 mice infected with *H. pylori* SS1 for 18 months.

As described elsewhere [2], C57BL/6 mice developed chronic inflammation in the corpus by 8 weeks after infection with *H. felis*. In contrast, no inflammation was evident in C57BL/6 mice at 8 weeks after infection with *H. pylori* SS1 strain, concurring with an earlier report on the absence of chronic inflammation in C57BL/6 mice at 12 weeks after infection with this strain [13]. However, others have observed gastritis in C57BL/6 mice at 3 months following infection with *H. pylori* SS1 strain [2]. The low level of inflammation and epithelial proliferative response found with *H. pylori* SS1 infection in mice in the present study may be due to the low colonization density of the bacterium. The enhanced inflammation in the corpus mucosa in *H. felis*-infected mice was associated with increased *H. felis* density, particularly in the antrum. Corpus-predominant histopathological changes and antral predominant colonization of *H. felis* in C57BL/6 mice are considered, in part, to represent an autoimmune response [2].

In contrast to the observations in mice, short-term infection in Mongolian gerbils with *H. pylori* SS1 strain resulted in antral predominant gastritis. These observations are in agreement with earlier studies, where antral infiltration by inflammatory cells in Mongolian gerbils was observed as early as 2–4 weeks after infection and increased in severity over time [6]. To date, there have been no reports on *H. felis* infection in Mongolian gerbils. In this study, *H. felis* successfully infected Mongolian gerbils, resulting in severe antral gastritis as early as 4 weeks after infection and corpus gastritis in 1 animal. Of interest, in gnotobiotic rats, *H. felis* also induced antral predominant gastritis at 8 weeks after infection [14]. In the present study, *H. felis* stimulated greater gastric epithelial cell proliferation in the antrum of gerbils than *H. pylori* SS1 strain. This mirrors the results observed with the 2 bacterial species in C57BL/6 mice, although, in mice, the proliferative changes were in the cardia and corpus mucosa, not the antrum.

The density of *H. felis* infection assessed histologically in gerbils was greater than that of *H. pylori* SS1 strain, which may account for the greater epithelial cell proliferative response observed. In contrast to the mouse, where infection can result in changes in cag pathogenicity island (PAI) genotype or related function [15], in the gerbil the function of the cag PAI of *H. pylori* relates to the severity of gastritis [6, 9] and epithelial proliferative responses [6]. *H. pylori* SS1 strain, however, lacks a functional cag PAI and does not induce interleukin-8 secretion in human gastric epithelial cells in vitro [13]. The absence of

![Figure 2. Epithelial cell proliferation in the gastric mucosa of C57BL/6 mice infected with *Helicobacter pylori* SS1 or *Helicobacter felis* for 8 weeks. Gastric epithelial cell proliferation in the cardia, corpus, and antrum of mice infected with *H. pylori* (n = 6) or *H. felis* (n = 5) for 8 weeks was compared with uninfected control mice (n = 4). Mice were injected with bromodeoxyuridine (BrdU) 1 h before being killed, and epithelial cell proliferation was determined immunohistochemically using a monoclonal antibody to BrdU. For each animal, the mean labeling index (LI) percentage (% of stained cells/total cells per pit) was calculated. Data are mean ± SEM. A Mann-Whitney U test was performed to compare *Helicobacter* species–infected groups to uninfected control mice, for each area of the stomach. *P < .05; **P < .01.](https://academic.oup.com/jid/article-abstract/186/9/1348/942410)
cag PAI–related cell signalling may explain the reduced gastric epithelial proliferative response stimulated by *H. pylori* SS1 strain, compared with *H. felis*. The more-severe gastric inflammation and gastric epithelial proliferative responses observed with *H. felis* infection in both mice and gerbils may be due to the increased bacterial density of *H. felis*, compared with that of *H. pylori*.

Recently, Suzuki et al. [12] compared the effects of long-term 18 months infection with *H. pylori* SS1 in gerbils and C57BL/6 mice. The mononuclear cell response was similar in the 2 models, although gerbils had increased infiltration of neutrophils. The present study demonstrates that short-term 4-week infection of gerbils with *H. pylori* SS1 strain results in antral inflammation and epithelial cell proliferation, although mice have no antral inflammation, or increased epithelial cell proliferation at this point in time. This suggests that gerbils respond more aggressively to *H. pylori* infection than mice, emphasizing the importance of host differences in the development of *Helicobacter* species–induced gastric inflammation and epithelial cell proliferation.

Previous studies in gerbils have reported increased antral epithelial cell proliferation at 2–4 weeks after infection with *H. pylori* B128, a strain which induces severe gastritis [6]. However, *H. pylori* strain G1.1, which has a nonfunctional cag PAI [6], induced no changes in gastric epithelial cell proliferation at the same time point. Although the SS1 strain lacks a functional cag PAI [13], it stimulated significant increases in antral epithelial cell proliferation in gerbils in the present study. Other genetic variables between the 2 strains may be important, as may technical differences. The study with *H. pylori* strain G1.1 [6] used proliferating cell nuclear antigen (PCNA) immunohistochemistry to detect cell epithelial cell proliferation rather than BrdU immunolabeling. PCNA does not specifically stain cells in the S-phase of the cell cycle, which might account for differing results. Additionally, the source of Mongolian gerbils differed.

In conclusion, the enhanced gastric epithelial cell proliferative response to gastric *Helicobacter* species infection in Mongolian gerbils observed in this study is likely to be relevant to the ability of Mongolian gerbils to develop gastric cancer with long-term *H. pylori* infection [7]. The elevated gastric epithelial cell proliferation observed with *H. felis* infection in this model suggests that *H. felis* infection in the Mongolian gerbil could also result in the development of gastric cancer. Long-term infection studies with *H. felis* are in progress to test this hypothesis.

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**References**