**Helicobacter pylori** and cholesterol gallstone formation in C57L/J mice: a prospective study

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Maurer, Kirk J., Arlin B. Rogers, Zhongming Ge, Ashley J. Wiese, Martin C. Carey, and James G. Fox. *Helicobacter pylori* and cholesterol gallstone formation in C57L/J mice: a prospective study. *Am J Physiol Gastrointest Liver Physiol* 290: G175–G182, 2006. First published August 18, 2005; doi:10.1152/ajpgi.00272.2005.—Recently, we demonstrated that cholesterol gallstone-prone C57L/J mice rarely develop gallstones unless they are infected with certain cholelithogenic enterohepatic *Helicobacter* species. Because the common gastric pathogen *H. pylori* has been identified in the hepatobiliary tree of cholesterol gallstone patients, we wanted to ascertain if *H. pylori* is cholelithogenic, by prospectively studying C57L infected mice fed a lithogenic diet. Weanling, *Helicobacter* spp.-free male C57L mice were either infected with *H. pylori* SS1 or sham dosed. Mice were then fed a lithogenic diet (1.0% cholesterol, 0.5% cholic acid, and 15% dairy triglycerides) for 8 wk. At 16 wk of age, mice were euthanatized, the biliary phenotype was analyzed microscopically, and tissues were analyzed histopathologically. *H. pylori* infection did not promote cholesterol monohydrate crystal formation (20% vs. 10%), sandy stone formation (0% for both), or true gallstone formation (20%) compared with uninfected mice fed the lithogenic diet (10%). Additionally, *H. pylori* failed to stimulate mucin gel accumulation in the gallbladder or alter gallbladder size compared with uninfected animals. *H. pylori*-infected C57L mice developed moderate to severe gastritis by 12 wk, and the lithogenic diet itself produced lesions in the forestomach, which were exacerbated by the infection. We conclude that *H. pylori* infection does not play any role in murine cholesterol gallstone formation. Nonetheless, the C57L mouse develops severe lesions of both the glandular and nonglandular stomach in response to *H. pylori* infection and the lithogenic diet, respectively.

### Gallstones

Gallstones are an exceptionally common cause of morbidity worldwide, and cholelithiasis with or without cholecystitis is the most common gastrointestinal disease requiring in-patient treatment in the United States (45). Despite five decades of intense basic, clinical, and epidemiological research (6, 11, 27, 41, 42), there is currently no definitive nonsurgical treatment for the management of gallstones; therefore, the disease is a serious surgical and economic burden with the median per patient cost exceeding 10,000 US dollars (45).

The term gallstones or cholelithiasis is a generic description encompassing both pigment and cholesterol gallstones (41). Cholesterol gallstones are the most common gallstones encountered in the Western world (41). These stones result from liver-induced cholesterol supersaturation of bile and phase separation of cholesterol-rich liquid crystals and solid crystals with subsequent crystal agglomeration and stone growth within the gallbladder in a mucoglycoprotein gel (8, 9, 41). Cholesterol gallstone formation is a polyfactorial disease with heterogenous contributions from both genetics and environment (41). Specifically, genetic susceptibility to cholesterol gallstones is, with very rare exceptions, inherited as a polygenic trait (6, 10, 11, 27, 41, 42, 54). Cholesterol gallstone susceptibility genes require environmental triggers including diet, obesity, estrogenic drugs, and other complex and unknown factors to express the cholesterol gallstone phenotype (41).

The C57L/J mouse is studied extensively as a model to investigate cholesterol gallstone genetics and pathogenesis (25, 42, 51, 52, 54). When fed a lithogenic diet containing 1.0% cholesterol and 0.5% cholic acid for 8 wk, this animal historically develops cholesterol gallstones with an 80% prevalence rate (25, 51). Our initial studies with this mouse model were conducted at facilities where the mice were enzootically infected with *Helicobacter* spp. (33). Recently, we (33) demonstrated that, in the absence of infection with specific enterohepatic *Helicobacter* spp., the prevalence of cholesterol gallstones in mice despite them being fed a lithogenic diet for 8 wk was much less than what we reported previously, approximating 10%.

We and others (3, 15, 30, 32, 38, 50) have found serological and molecular evidence in humans that enterohepatic *Helicobacter* spp. may be associated with several chronic hepatobiliary diseases. Others (7, 39, 47) have reported the identification of *H. pylori* in hepatobiliary tissues from patients with benign and malignant hepatobiliary disease. In some of these studies, bile procurement and sample processing had the potential to bias the results toward identification of *H. pylori*. For example, endoscopic retrograde cholangiopancreatography was utilized to collect bile samples from patients with known gastric *H. pylori* colonization, leading to potential contamination with *H. pylori* from the gastric mucosa (7). Moreover, in some cases, *H. pylori* was identified on the basis of sequencing segments of its 16S rRNA gene (47). However, this method is unsatisfactory for speciating *Helicobacters* due to the high 16S rRNA sequence homology among organisms in this genus (40). Moreover, in vitro studies have revealed that *H. pylori* is sensitive to bile and is chemotactically repelled by solutions of...
both conjugated and unconjugated bile salts (22, 55). Because of these equivocal findings in humans and our recent identification of a number of enterohpatic Helicobacter spp. that promote murine cholelithogenesis, we wanted to ascertain whether H. pylori infection exhibited the ability to induce cholesterol gallstones in the C57L mouse model (33).

MATERIALS AND METHODS

Animal Sources and Husbandry

All animal protocols were reviewed and approved by the Massachusetts Institute of Technology Committee on Animal Care. Three-to four-week-old male Helicobacter spp.-free C57L/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were divided into three groups for study. In the first group, mice remained uninfected and were fed a standard lithogenic diet containing 1.0% cholesterol, 0.5% cholic acid, and 15% dairy fat (25). The second group was infected with H. pylori SS1 (28) and fed a rodent chow diet (n = 5), and the third group was infected with H. pylori SS1 and fed a lithogenic diet (n = 10). Group numbers were chosen based on a 90–95% power to statistically detect similar changes in the gallstone prevalence rate compared with changes noted in our initial study (33). Mice were housed in polycarbonate microisolator cages under specific pathogen-free conditions (free of Helicobacter spp., Citrobacter rodentium, Salmonella spp., endoparasites, and known murine viral pathogens) in an Association for the Assessment and Accreditation of Laboratory Animal Care-accredited facility. Mouse rooms were kept at constant temperature and humidity on a 12:12-h regular light-dark cycle, and mice received food and water ad libitum. Animals were fasted for 12 h before CO2-induced euthanasia. Mice were fed a standard rodent chow (Purina Mills; St. Louis MO) containing <0.05% cholesterol until 8 wk of age. At this time point, mice were either continued on a standard rodent chow or converted to the lithogenic diet.

Helicobacter pylori Infection

H. pylori SS1 (28) was grown for 24–48 h in Brucella broth (Becton Dickinson; Franklin Lakes, NJ) containing 5% heat-inactivated fetal calf serum. Broth cultures were centrifuged at 8,000 rpm for 20 min at 4°C, and the bacterial pellet was resuspended in Brucella broth at a turbidometric (optical density = 660 nm) reading of between 0.6 and 1.2. Four- to five-wk-old mice were infected by oral gavage with 0.2 ml of resuspended bacteria (n = 15) or sham dosed with 0.2 ml Brucella broth (n = 10) three times over a 5-day period. At monthly intervals thereafter, mice were given two more doses of H. pylori SS1 by gavage using the same protocol.

Bile Analyses and Tissue Processing

Mice were euthanized with CO2 in the fasted state at 16 wk of age. A ventral midline incision was made, and the gallbladder was removed intact. Full gallbladders were weighed, and their contents were examined under direct and polarized light microscopy by a microscopist (K. J. Maurer) blinded to sample identity. Bile was analyzed by Fisher’s exact test using the same software. Mouse stomachs were removed aseptically and opened longitudinally along the greater curvature. Approximately 30 mg of glandular stomach were flash frozen in liquid N2 and stored at −80°C for subsequent quantitative PCR. Three 10-mg segments of the liver from different liver lobes were collected aseptically and frozen at −20°C for subsequent PCR.

Histopathological Examinations

At necropsy, the liver, gallbladder, stomach, duodenum, pancreas, and ileoceccolonic junction were collected, trimmed, and fixed in 10% neutral buffered formalin. Tissues were processed routinely, paraffin embedded, cut into 4-µm-thick slices, and stained with hematoxylin and eosin. Additional stomach and gallbladder sections were stained with Alcian blue/periodic acid Schiff (PAS; pH 2.5) for acidic and neutral mucins (18). Tissues were evaluated by a comparative pathologist (A. B. Rogers) blinded to sample identity. Gastritis was scored on an ascending 0–4 scale using previously defined criteria (17). Inflammation of the squamous forestomach was scored using criteria similar to those for the glandular compartment. Epithelial hypertrophy/hyperkeratosis of the squamous stomach was scored as follows: 0, normal thickness; 1, two times normal thickness; 2, three times normal thickness; 3, four times normal thickness; or 4, greater than four times normal thickness. The presence or absence of intraepithelial microabscesses was also recorded. Mean histological grades were compared between multiple groups by Kruskal-Wallis one-way ANOVA followed by Dunn’s posttest using Prism 3.0c for Macintosh (GraphPad). Direct comparisons were made with the Mann Whitney U-test. The prevalence of microabscesses was evaluated using Fisher’s exact test. P values of ≤0.05 were considered statistically significant.

Real-Time Quantitative PCR

Chromosomal DNA from broth-grown H. pylori SS1 and total DNA from mouse stomachs was prepared using the High Pure Template Preparation Kit according to the instructions of the supplier (Roche Molecular Biochemicals; Indianapolis, IN). To quantify colonization levels of H. pylori strain SS1 within the gastric mucosa, a real-time quantitative PCR assay was developed based on the nucleotide sequence of the H. pylori ureB gene in the ABI Prism TaqMan 7700 sequence detection system (A/B Applied Biosystems; Foster City, CA). Two primers (forward: 5’-CAAAATCGCTGGGATTGGGT-3’ and reverse: 5’-CTTCACCCGCTAAGCGCTCA-3’) and an internal probe (5’-AAACAGATCGCAAATGGCTTTAAAACA-3’) were designed to hybridize within the 100-bp region (nucleotides 273–373) of the single-copy ureB gene (AF508016) of H. pylori SS1 using Primer Express software (Applied Biosystems) (40). Quantitative PCR conditions were as described previously (20). The specificity of these oligonucleotides for H. pylori was tested using DNA isolated from H. felis (ATCC49179), H. mustelae (ATCC43772), and “H. helminthini.” To generate a standard curve, serial 10-fold dilutions (from 5 × 105 to 5) of H. pylori SS1 genome copies, estimated from an average mass value (1.66 Mb) obtained from the two published H. pylori genomes, were used (2, 49). Copy numbers of gastric mucosal H. pylori SS1 DNA in mice were then calculated and normalized to micrograms of murine chromosomal DNA determined by quantitative PCR using a mammalian 18S rRNA gene-based primers and probe mixture (Applied Biosystems) as described elsewhere (53).

Liver PCR

Livers were harvested, and DNA was extracted using the Roche DNA High Pure Template Preparation Kit (Roche Molecular Biochemicals) per the manufacturer’s instructions. Two rounds of PCR amplification were performed. The first round of amplification used the genus-specific primer set C97 and C05, which amplifies an amplicon of ~1,200 bp (16, 19). PCRs were performed using 5 µl template DNA and “PuRe Taq Ready To-Go PCR beads” (Amersham Biosciences; Uppsula, Sweden) with previously described conditions (16, 19). After this, a nested amplification was performed using the genus-specific C97 and C08 primer sets, which amplify an amplicon...
of ~400 bp (16, 19). This reaction used 1 µl template DNA from the first reaction and followed the conditions described previously (16, 19). Included as a positive control was known H. pylori SS1 colonized mурine gastric tissue, and proven uninfected mouse tissue was used as a negative control.

RESULTS

Biliary Phenotype

Regardless of the infection status, the gallbladder bile of all mice fed the lithogenic diet displayed cholesterol-phospholipid liquid crystals, indicating phase-separated supersaturated bile (Fig. 1). Mice fed a chow diet did not develop liquid crystals in bile, confirming the well-known requirement for a modified diet to supersaturate gallbladder bile and induce both liquid and solid crystal phase separation in this mouse model (33, 51). H. pylori-infected mice fed the lithogenic diet developed more cholesterol monohydrate crystals (20%) and true cholesterol gallstones (20%) compared with control animals (10% for each), but these changes were not statistically significant (P = 1.0; Fig. 1). Moreover, no differences in sandy stone formation were noted (0% for both groups; Fig. 1). Mucin gel scores and normalized gallbladder weight for animals fed the lithogenic diet were significantly greater than those fed a standard chow diet (P ≤ 0.05); however, neither differed among animals fed the lithogenic diet regardless of infection status (P > 0.05; Fig. 2). H. pylori-infected animals fed a chow diet did not develop mucin gel formation (score of 0; Fig. 2) and exhibited normalized gallbladder weights comparable with uninfected mice fed a chow diet (Fig. 2) based on our previous study (33).

Real-Time Quantitative PCR

To validate the specificity and sensitivity of the quantitative PCR assay for H. pylori, H. pylori SS1 DNA in parallel with the DNA templates from three gastric Helicobacters (H. felis, H. mustelae, and H. “heilmanii”) was detected using the primers and probes designed for the ureB gene. The quantitative PCR assay detected a minimum of five copies of the H. pylori SS1 genome (Fig. 3, lane 6), whereas there was no amplification from 10 ng (approximately equal to 5 × 10⁵ genome copies based on the size of the H. pylori genome) of DNA from H. felis, H. mustelae, or H. “heilmanii” (Fig. 3, lanes 7–9). The mean number of H. pylori organisms per microgram of host DNA in the gastric corpus of chow-fed animals was 2.5 × 10⁵, whereas in lithogenic diet-fed infected animals these values were reduced by ~1 log unit (P < 0.05) with 6.0 × 10⁴ organisms/µg host DNA (Fig. 4).

Liver PCR

Livers from H. pylori-infected animals were uniformly negative on initial PCR screening. Subsequently, nested amplification also failed to amplify any H. pylori DNA from either the infected group fed chow or the infected group fed the lithogenic diet (data not shown). This result contrasts markedly with the positive PCR results from the livers of enterohepatic Helicobacter-infected C57L mice in our earlier study (33).

Histopathology

Liver and gallbladder. Livers of C57L mice fed the lithogenic diet demonstrated a lobular pattern of hepatocellular microsteatosis concentrated in acinar zones 2 and 3 (mid-zonal and centrilobular, respectively; Fig. 5a). In contrast, macrosteatosis characterized by medium to large round clear cytoplasmic lipid vacuoles was mild, patchy, and mostly prominent in the perportal regions. Mild to moderate portal mononuclear cell infiltration was noted in a subset of mice fed the lithogenic diet, but its occurrence was recorded equally in H. pylori-infected and uninfected animals (Fig. 5a). Small lipogranulomata comprised of macrophages with phagocyted lipofuscin-like material were sometimes seen (Fig. 5a). Mild and inconsistent gallbladder lesions were evident in some mice.
fed the lithogenic diet. Microscopic findings included eosinophilic and lymphocytic cholecystitis (Fig. 5b), small islands of mucous metaplasia, and scattered epithelial cell hyalinosis with rare intraluminal crystals (data not shown). Unlike the severe gallbladder lesions in mice fed the lithogenic diet infected with specific endenterohepatic Helicobacter spp. (33), gallbladder lesions in the present study were inconsistent, mild, and unassociated with H. pylori infection status. Moreover, there were no hepatobiliary lesions in H. pylori-infected mice on the chow diet. The duodenum, pancreas, and ileocecal junction were within normal limits in all mice regardless of diet or infection status.

**Stomach.** Two distinct histopathological patterns were produced in the stomach: one associated with H. pylori infection and the other with the lithogenic diet. Compared with uninfected mice fed the lithogenic diet (Fig. 5c), H. pylori-infected mice fed either chow or the lithogenic diet developed moderate mixed mononuclear and granulocytic cell proliferative gastritis of the cardia and corpus with atrophy of oxyntic glands and mucous metaplasia (Fig. 5d). No appreciable intestinal metaplasia or dysplasia were evident. With the use of staining by hematoxylin and eosin, the mucous metaplasia of the oxyntic gland was characterized by a foamy change in the cytoplasm of parietal cells (Fig. 5e). Mucous metaplasia was confirmed by Alcian blue/PAS staining at pH 2.5, demonstrating transformation of surface mucins from the neutral gastric type (red) to the acidic intestinal type (blue). Additionally, there was heavy production of mixed mucins in the parietal cell zone, with intestinal-type mucins concentrated at the upper and lower boundaries of the cellular columns and gastric-type mucins in the middle (Fig. 5f). Compared with uninfected controls, scores of the lesions in the glandular stomach incorporating all criteria were significantly increased in H. pylori-infected mice regardless of diet ($P \leq 0.05$; Fig. 6). However, there were no differences in mean scores between infected groups fed different diets except for an additive effect of the lithogenic diet on mucous metaplasia ($P \leq 0.05$; Fig. 6).

The second pattern of gastritis, associated with the lithogenic diet, affected the anterior squamous compartment (forestomach). Histological changes consisted of moderate mixed inflammation and edema of the lamina propria and submucosa and hypertrophy of the squamous epithelium with orthokeratotic hyperkeratosis and frequent intraepithelial microabscesses (Fig. 5h). Microabscesses, generally 1–2 mm, were composed of degenerate neutrophils and epithelial cells, often with a central core of keratotic debris (Fig. 5h). In contrast, no squamous defects developed in H. pylori-infected mice fed the chow diet (Fig. 5g). However, in mice fed the lithogenic diet, there was a statistically significant additive effect of H. pylori infection on the degree of squamous hyperkeratosis ($P \leq 0.05$; Fig. 7) and inflammation ($P \leq 0.05$). H. pylori-infected mice fed the lithogenic diet were twice as likely to develop microabscesses of the squamous stomach as uninfected mice (80% vs. 40%, respectively), although this difference did not reach statistical significance ($P = 0.17$).

**DISCUSSION**

The literature ascribing a putative role for H. pylori in causing human hepatobiliary disease has been both confusing and inconclusive (4, 7, 13, 39, 47). In this study, we demonstrated that H. pylori, unlike some enterohepatic Helicobacter spp. (33), does not promote cholesterol cholelithogenesis in the C57L mouse model. Moreover, H. pylori does not promote any of the preliminary stages (cholesterol monohydrate crystals, sandy stones) in the physical chemistry of cholelithogenesis. One might argue that a doubling of the prevalence of choles-
terol gallstone formation in infected mice would be important when dealing with a large population (such as humans with *H. pylori*); however, because *H. pylori* does not increase gallbladder mass (a surrogate marker of gallbladder hypomotility) or mucin gel accumulation (infected animals had, in fact, less mucin gel accumulation than uninfected animals), which are markers of cholelithogenesis, it is likely that this increase is merely due to population variation (as our statistical analyses indicate). In addition, the PCR results indicate that *H. pylori* does not colonize the murine hepatobiliary tree. This finding is not surprising because to date there is not a single report of successful bacterial isolation of these organisms from the liver or biliary tree of experimentally infected animals and only a single communication (44) reporting the isolation of *H. pylori* from a human liver damaged by Wilson’s disease.

In the past, authors claimed to have identified *H. pylori*-like organisms in hepatobiliary tissue by 16S rRNA amplification and sequencing, (47). Unfortunately, speciating *Helicobacters* based on sequencing a small (400–1,000 bp) segment of the 16S rRNA gene is fraught with error, because this region is highly conserved among *Helicobacter* spp. (40). Highlighting this concern, Avenaud and colleagues (4) identified organisms that appeared to be *H. pylori* based on sequencing of the 16S rRNA gene from the liver of humans. On the basis of stringent followup tests, these investigators discovered that these organisms were a previously unidentified (and still unclassified) *Helicobacter* sp. that is phylogenetically related to, but not, *H. pylori* (4).

We hypothesize that the presence of *H. pylori* in the biliary tree and liver of human patients with cholesterol gallstones is either a secondary colonizer (due to biliary changes from gallstones, cholestasis, or chronic cholecystitis) or the DNA denotes one or more related *Helicobacter* spp. Perhaps, in the presence of disease induced by other *Helicobacter* spp. or other...
factors, the biliary microenvironment changes to allow for secondary invasion by *H. pylori*. For example, we (33) demonstrated previously that some cholelithogenic *Helicobacter* spp. increase gallbladder mucin gel accumulation significantly as well as contribute to mucinous metaplasia of the gallbladder in C57L mice fed the lithogenic diet. An increase in mucin gel accumulation or a change in mucin species may promote attachment of *H. pylori* to the biliary epithelium. Interestingly, others have noted that the type of mucin produced in the stomach is pivotal for *H. pylori* colonization, and, in fact, some normal gastric mucins are bactericidal (24). Consistent with a possible alteration of the biliary microenvironment leading to secondary invasion by *H. pylori*, Myung and colleagues (39) found that patients that are PCR positive for *H. pylori* exhibited a significantly lower biliary pH than those that were PCR negative. Further evidence for secondary invasion by *H. pylori* was recently demonstrated by multiplex PCR in an Iranian population (14). In this work, *H. pylori*-like DNA could be amplified from bile of gallstone patients with histological evidence of chronic cholecystitis but not from the bile of asymptomatic gallstone patients (14). These authors did not attempt to culture for bacteria other than *Helicobacter* spp. However, others (21, 37, 46) have cultured the biliary tree and bile from humans and experimental animal models with cholecystitis and demonstrated numerous bacterial species that are considered part of the normal distal gastrointestinal tract and nasopharyngeal microbiota. These organisms often colonize the biliary tree secondarily to biliary disease, especially after obstructive cholestasis or sphincter of Oddi ablation. It is likely that *H. pylori* behaves in a similar manner and may colonize the biliary tree due to impaired biliary drainage, changes in mucus content or character, and other biochemical alterations affecting the chemistry and concentration of biliary lipids. To validate or disprove these hypotheses, a thorough chemical and physical-chemical analysis of bile in the presence and absence *H. pylori* DNA in the hepatobiliary tree would be necessary.

In planning the present experiments, we were concerned that the lithogenic diet would inhibit *H. pylori* colonization, because in vitro studies have demonstrated bactericidal effects of bile acids on *H. pylori* (22). Consistent with this notion, there was a significant log unit decrease in the present work in the number of *H. pylori* organisms in the stomachs of lithogenic diet-fed infected mice compared with the chow-fed group (Fig. 4). Interestingly, there was no significant change in the extent or severity of glandular gastritis between the two infected groups. In fact, in both lithogenic diet-fed and chow-fed mice, a robust gastritis was noted. Historically, the *H. pylori* SS1 strain causes minimal lesions in susceptible mouse strains at 3 mo postinfection, and C57BL/6 mice, which are characterized as a susceptible strain, often require 6 mo of *H. pylori* infection to demonstrate moderate gastric inflammation (28). The C57L mouse appears to be exquisitely susceptible to *H. pylori*-induced gastritis (regardless of the type of diet fed) and displays similar lesions and scores to INS-GAS hypergastrinemic mice at this early time point (17). This observation merits further investigation in view of the known fact that INS-GAS mice progress to adenocarcinoma after 6 mo of colonization with *H. pylori* (17).

In addition to infectious gastritis, noninfected mice that ingested the lithogenic diet developed lesions of the squamous forestomach. These lesions included hypertrophy, hyperkeratosis, and inflammation with microabscess formation of the epithelium. Furthermore, some of these diet-induced lesions were exacerbated significantly by infection with *H. pylori*.  

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**Fig. 6.** Comparison of lesion grades in the glandular stomach of C57L mice. Mean grades of all lesion criteria were increased by *H. pylori* infection compared with sham inoculation (*P* ≤ 0.05). An additive effect of the lithogenic diet with *H. pylori* infection was observed in the case of mucous metaplasia (†*P* ≤ 0.03).

**Fig. 7.** Comparison of the lesion grades in the squamous stomach of C57L mice. The lithogenic diet induced squamous hypertrophy and hyperkeratosis, inflammation, and intraepithelial abscesses. *H. pylori* infection increased the severity of lesions in mice fed the lithogenic diet (†*P* ≤ 0.05). In contrast, *H. pylori*-infected mice fed the chow diet did not develop lesions in the squamous forestomach. *Significance compared with either infected group (*P* < 0.05).
Moreover, the squamous epithelial lesions are reminiscent of the lesions caused in part by diet and stress in the pars esophagaea of swine, which consist of hyperkeratosis and ulceration (12). Swine stomachs are commonly colonized by H. suis; however, the contribution of these helicobacters to gastric disease is not completely understood (26, 43). Like the inbred mice in this study, we propose that in domestic swine, dietary composition induces lesions in the pars esophagaea and that H. suis exacerbates these lesions. The lesions in the squamous stomach of mice demonstrate that under modified dietary conditions, H. pylori can exacerbate disease in areas in close anatomic proximity to where these organisms colonize typically, i.e., the glandular stomach. The squamous forestomach of the mouse is lined by stratified squamous epithelium (29) and hence is a nonglandular zone, which is essentially an extension of the mouse esophagus separated anatomically from the glandular gastric compartment by the “limiting ridge” (29). These concepts raise the possibility that this mouse model could be used to study the contributions of diet and H. pylori infection to chronic esophageal diseases such as esophagitis and even esophageal cancer.

Any explanation for the synergism of diet and H. pylori infection in causing lesions of the glandular stomach in C57L mice, most notably metaplasia, must be speculative. The cholic acid triglyceride and cholesterol components of the diet may either singly or together be contributing to gastric lesions. For example, cholic acid promotes azoxymethane-induced aberrant crypt foci in rats (5, 48). However, analysis of the forestomach of bile acid-fed rats did not demonstrate any notable lesions (56). Interestingly, in humans, diets high in fat and cholesterol (so-called “Western diets”) correlate with the risk for esophageal cancer in both mice (31, 34, 35). Additionally, cholesterol free radicals are produced in the glandular squamous cell carcinoma, and common gastric adenocarcinoma, gastric cardia adenocarcinoma, esophageal squamous cell carcinoma, and common gastric adenocarcinoma (31, 34, 35). Additionally, cholesterol free radicals are believed to promote a variety of diseases in mice and humans including atherogenesis (1, 23, 36). It is reasonable, therefore, to hypothesize that gastric inflammation from H. pylori may promote oxidation and free radical derivatives of cholesterol or fatty acids in humans consuming high dietary levels of these lipids and that these oxidized products could further contribute to chronic gastric and potentially esophageal diseases.

In summary, H. pylori infection in this prospective study does not contribute to cholesterol gallstone formation in the C57L mouse model. We believe that the purported suggestions in the literature that H. pylori causes cholesterol gallstones in humans are suspect and that, in parallel with many other bacteria, H. pylori is likely to be a secondary colonizer of the hepatobiliary tree after complicated gallstone disease and/or iatrogenic interventions. In addition, the C57L mouse is highly susceptible to H. pylori-induced gastritis and dietary-induced nonglandular lesions of the squamous forestomach, an anatomic region analogous in structure and function to the esophagus. This mouse strain may provide an important new model to study the effects of diet and chronic H. pylori infection on the development of chronic gastric and esophageal inflammation and perhaps neoplasia.

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