Bacteria rapidly colonize and modulate healing of gastric ulcers in rats

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Departments of Pharmacology and Therapeutics and Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1; and Department of Pediatrics, Louisiana State University Medical Center, New Orleans, Louisiana 70112-2822

Elliot, Susan N., André Buret, Webb McKnight, Mark J. S. Miller, and John L. Wallace. Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G425–G432, 1998.—The stomach is generally regarded as an environment that is not conducive to bacterial colonization. In this study, we examined the possibility that this changes significantly when an ulcer has formed and that colonization of ulcers interferes with the normal healing process. Gastric ulcers were induced by serosal application of acetic acid. The relationship between ulcer healing and bacterial colonization was examined. The effects of antibiotics, induction of Lactobacillus colonization, and selective colonization with an antibiotic resistant strain of Escherichia coli on ulcer healing were examined. Within 6–12 h of their induction, gastric ulcers were colonized by a variety of bacteria, with gram-negative bacteria predominating. Suppression of colonization with antibiotics resulted in marked acceleration of healing. Induction of Lactobacillus colonization also accelerated ulcer healing. The beneficial effects of antibiotics were reversed through selective colonization with antibiotic-resistant E. coli. Bacterial colonization occurred irrespective of the method used to induce the ulcer. This study demonstrates that colonization of gastric ulcers in rats occurs rapidly and significantly impairs ulcer healing. This effect appeared to be primarily attributable to gram-negative bacteria.

acid secretion

THE STOMACH AND UPPER intestine are normally sterile, with low levels of flora only being present for a short period of time after meals. A notable exception is the colonization by Helicobacter pylori of the upper gastrointestinal tract of a significant portion of the human population. This bacterium is specially adapted to survive in the low-pH environment of the stomach through its ability to generate a local microenvironment with a more tolerable pH (18).

An association between bacterial colonization of the stomach and gastric ulcers was suggested more than a century ago (1, 23). Considerable data have been reported to support such an association. For example, Saunders (30) isolated an α-streptococcus from peptic ulcers in humans, whereas Letulle (17) demonstrated that oral or parenteral administration of Staphylococcus pyogenes to guinea pigs resulted in the development of gastric ulcers. However, a clear consensus on the contribution of these bacteria to ulceration was never reached, and since the discovery of the association between H. pylori colonization and peptic ulcer disease, the possible contribution of other bacteria to this disease has essentially been supplanted. It remains possible, however, that bacteria other than those of the Helicobacter genus could contribute to gastric ulceration if they were capable of colonizing the stomach, or more specifically, the ulcer site. In experimental models, bacteria appear to play an important role in exacerbating mucosal injury induced in the stomach or small intestine by nonsteroidal anti-inflammatory drugs (15, 24, 29).

In the present study, we tested the hypothesis that bacteria selectively colonize sites of ulceration in the rat stomach and have a negative impact on the healing of those ulcers. The specific questions addressed in this study were as follows: 1) How quickly does bacterial colonization occur after ulcer induction? 2) Are certain species of bacteria more prevalent than others? 3) Is bacterial colonization specifically localized to the ulcer or is it more widespread? 4) Do changes in gastric acid secretion occur that are permissive to bacterial colonization of the stomach? 5) Can altering the nature (i.e., species) of bacterial colonies influence the rate of gastric ulcer healing?

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 175–200 g were obtained from Charles River Breeding Farms (Montreal, PQ, Canada) and housed in polycarbonate cages. The rats had free access to standard pellet chow and tap water throughout the experiment with the exception of some experiments (see below) in which the animals were deprived of food, but not water, for 18–24 h before the experiments were performed. All experimental protocols described below were approved by the Animal Care Committee at the University of Calgary and are in accordance with the guidelines of the Canadian Council on Animal Care.

Induction of ulcers. Ulcers were induced using a model (8) modified from that described by Okabe and Pfeiffer (22). Briefly, while rats were under halothane anesthesia, a midline laparotomy was performed and the stomach gently exteriorized. The barrel of a 3-ml syringe, which had been cut and filed smooth, was placed on the serosal surface of the stomach in the corpus region. Acetic acid (0.5 ml of 80% vol/vol) was instilled into the barrel of the syringe and allowed to remain in contact with the stomach for 1 min, after which time it was aspirated and the area rinsed with sterile saline. The area exposed to acetic acid was 60 mm². After the different treatment regimens described below, gastric ulcer area was determined as follows. The rats were killed by cervical dislocation, and the stomach was removed and

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pinned out on a wax block. A paper grid with an area of 25 mm² was placed alongside the ulcer, which was then photographed. We then determined the ulcer area by planimetry, using ×5 enlargements of the photographs. The area of ulceration in pixels was converted to square millimeters, using the paper grid as a reference. All planimetric determinations were performed using coded photographs so that the observer was unaware of the treatment the rats had received. Previous work using this ulcer model (8, 22, 32, 33) has revealed that ulcers induced by acetic acid are characterized by a thick layer of granulation tissue at the base and glandular disorganization at the ulcer margins. The ulcers involve the full thickness of the mucosa and penetrate into the muscularis mucosa. Perforation was not observed with this model.

To histologically evaluate the ulcer site for damage and the presence of bacteria, we induced ulcers in a separate group of rats (n = 6) as described above. Seven days after ulcer induction, the rats were killed by cervical dislocation. The stomach was removed, rinsed with sterile PBS (pH 7.4), pinned out on a wax block, and submerged in Carnoy’s fixative for 2–4 h. Tissue samples were also taken from a group of control rats, or rats with no ulcers (n = 5), for comparison. Tissue samples of the ulcers and healthy tissue were embedded in plastic using a commercially available kit (JB-4 embedding kit; Polysciences, Warrington, PA). Thin sections (1–1.5 µm) were stained with methylene blue–fuchsin (basic) and examined under a light microscope.

Bacterial colonization of gastric ulcers. To determine the time course of bacterial colonization of acetic acid-induced ulcers, we killed rats at various time points after ulcer induction (1 h to 21 days; n = 4–6 rats/group) and performed a laparotomy on each, using aseptic technique. The stomach was removed, opened along the greater curvature, and rinsed with sterile PBS. Tissue samples (~150 mg) were obtained from the ulcer and from a contralateral site from each stomach. The samples were placed into tubes containing sterile PBS and weighed. The tissues were homogenized (Polytron homogenizer; Brinkmann Instruments, Rexdale, ON, Canada), diluted serially, and plated onto MacConkey agar or tryptic soy agar. The MacConkey and tryptic soy agar plates were incubated for 18–24 h at 37°C under aerobic conditions. Plates containing between 20 and 200 colony-forming units (CFU) were analyzed to determine bacterial levels, and the results are expressed as CFU per gram of tissue.

To eliminate the possibility that bacteria were being introduced to the animals during the ulcer-induction procedure itself, a separate group of rats (n = 6) underwent sham ulcer induction. The sham ulcer-induction procedure was identical to the ulcer-induction procedure described above, except that distilled water, rather than acetic acid, was instilled into the barrel of the syringe and allowed to contact the serosal wall of the stomach. Seven days after the sham ulcer induction, the rats were killed by cervical dislocation. Gastric tissue samples were taken from the approximate site where the barrel of the syringe had been placed for the sham ulcer induction. These samples were processed and plated as described above for determination of bacterial numbers.

To determine if the bacterial colonization of gastric ulcers was unique to the acetic acid model, we induced ulcers in animals by two other methods; namely, cryostatically and with a nonsteroidal anti-inflammatory drug. In the first case, rats were fasted overnight with free access to water. While rats were under halothane anesthesia, a midline laparotomy was performed and the stomach gently exteriorized. The base of a cuvette (diameter = 7.65 mm), which had been allowed to equilibrate in liquid nitrogen and was about one-fourth full of liquid nitrogen, was placed on the serosal surface of the stomach at approximately the center of the corpus. The cuvette remained in contact with the stomach for 30 s, after which time it was refilled and the process repeated twice. The abdominal incision was then closed with sutures, and the rat was allowed to recover. Seven days after ulcer induction, the rats (n = 5/group) were killed by cervical dislocation and tissue samples of the ulcers were taken. The tissue samples were processed and plated for bacterial level determination as described above.

For the ulcers induced with a nonsteroidal anti-inflammatory drug, a slightly modified version of the model originally described by Satoh et al. (28) was used. Rats were deprived of food, but not water, for 18–24 h. At the end of this period, the rats were permitted access to food for 2 h. After this refeeding, the rats (n = 5–8/group) were given either vehicle (2 ml/kg 5% sodium bicarbonate) or naproxen (80 mg/kg; 2 ml/kg) orally. The rats were then fasted for an additional 24 h. At the end of this second fasting period, the rats were killed by cervical dislocation. Tissue samples of the ulcers, or of corresponding areas from vehicle-treated rats, were processed and plated as described above for the determination of bacterial levels.

To histologically evaluate the ulcer site for damage and the presence of bacteria, we induced ulcers in a separate group of rats (n = 6) as described above. Seven days after ulcer induction through serosal application of acetic acid. Each test sample (serosal and muscularis mucosa) was aspirated with a 20 ml of saline (37°C) to remove any residual matter. The rats were allowed to refeed ad libitum in their home cages for 20 ml of saline (37°C) to remove any residual matter. The rats were then fasted for an additional 24 h. At the end of this second fasting period, the rats were killed by cervical dislocation. Tissue samples of the ulcers, or of corresponding areas from vehicle-treated rats, were processed and plated as described above for the determination of bacterial levels.

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period, perfusate was collected for three 30-min periods. After the first period (basal secretion), a bolus dose of 20 µg/kg pentagastrin was administered intravenously, followed by a continuous infusion of 20 µg·kg⁻¹·h⁻¹ for 60 min. Per fusates were collected, and the concentration of acid in each sample (expressed as μeq/30 min) was determined by titration to pH 7.0 using a Metrohm automated titrator (Brinkmann Instruments, Rexdale, ON, Canada).

To visually determine if there was an alteration of pH at the ulcer site, which may have facilitated bacterial colonization, Congo red dye (25 mg/ml; 1 ml) was orally administered to rats 1 or 7 days after ulcer induction. Congo red is a pH-sensitive dye that appears red at a pH > 5 and blue at a pH < 3 (31). Thirty minutes after dosing, each rat was killed and the stomach removed. The stomach was cut along the greater curvature, rinsed with saline, pinned out on a wax block, and photographed for subsequent evaluation by an observer unaware of the treatments the rats had received.

Effect of antibiotic treatment. The effects of daily treatment with the broad-spectrum antibiotics streptomycin and penicillin were assessed. Ulcers were induced in the rats as described above. On the seventh day after ulcer induction, a 7-day treatment period was initiated during which streptomycin (168 mg/ml) or penicillin (84 mg/ml) was administered orally twice daily. The vehicle for the antibiotics was distilled water (0.5 ml). Control rats were treated with the same volume of the vehicle. Fourteen days after ulcer induction, the rats were killed by cervical dislocation, the stomach was removed and photographed for ulcer area determination, and tissue samples were taken for bacterial culture. In a separate experiment, the effect of treatment with the combination of streptomycin (336 mg/ml; 0.25 ml) and penicillin (168 mg/ml; 0.25 ml) was determined. Control rats received the same volume of vehicle. As before, ulcers were induced in the rats, and on the seventh day after ulcer induction, rats began receiving twice daily oral dosing with the combination of antibiotics or vehicle. After the 7-day treatment period, the rats were killed, the ulcer area assessed, and tissue samples for bacterial culture collected.

Selective colonization with Escherichia coli. After an overnight fast, ulcers were induced in the rats with acetic acid, as described above. Immediately thereafter, the rats received a combination of 4 mg/ml streptomycin and 4 mg/ml bacitracin in their drinking water. On the third day after ulcer induction, one group of rats (n = 10) was given 1 ml of MacConkey broth containing 10⁷ CFU of a streptomycin-resistant strain of E. coli (E. coli C-25) orally (19). This treatment was repeated every 12 h for 7 days. A control group of rats (n = 10) received 1 ml of the MacConkey broth orally at the same times. The rats in the different treatment groups were housed separately to avoid coprophagous ingestion of E. coli C-25. On the tenth day after ulcer induction, the rats were killed by cervical dislocation. Five rats from each group had their stomachs removed, cut along the greater curvature, and pinned out on a wax block for ulcer area determination.

In a separate experiment, the effect of treatment with the combination of 4 mg/ml streptomycin and 4 mg/ml bacitracin was assessed. Ulcers were induced in the rats as described above. Immediately thereafter, the rats received a combination of 4 mg/ml streptomycin (336 mg/ml; 0.25 ml) and penicillin (168 mg/ml; 0.25 ml) was determined. Control rats received the same volume of the vehicle. Fourteen days after ulcer induction, the rats were killed by cervical dislocation, the stomach was removed and photographed for ulcer area determination, and tissue samples were taken for bacterial culture.

Effect of lactulose. Lactulose (4-O-β-D-galactopyranosyl-D-fructose) has been shown to promote the growth of lactobacilli, especially Lactobacillus acidophilus (27). To determine if adding lactulose (5% vol/vol; Lactulax, Pharmascience, Montreal, PQ, Canada) to the drinking water would influence the gastric levels of Gram-positive bacteria and if this would subsequently alter the rate of ulcer healing, we performed the following experiment. The rats were divided into four groups (n = 5–10). One group received lactulose in the drinking water for 2 wk and was killed on the day of ulcer induction. Gastric tissue samples were taken for bacterial level determinations. A second group of rats received lactulose for 2 wk before ulcer induction and continued to receive it for 10 days after induction. A third group of rats received the 5% lactulose solution in the drinking water for days 3–10 after ulcer induction, and control rats, which had ulcers, received regular drinking water throughout the experiment. On the tenth day after ulcer induction, the rats were killed, and ulcer area and bacterial levels were determined as described above.

Statistical analysis. All data are expressed as means ± SE. Comparisons among groups of data were made using a one-way ANOVA and a Newman-Keuls test. For comparisons of bacterial colonization at ulcer sites vs. contralateral sites, a paired Student’s t-test was used. With all analyses, P < 0.05 was considered significant.

Materials. Pentagastrin, Congo red dye, streptomycin sulfate, penicillin G sodium, and bacitracin were obtained from Sigma Chemical (St. Louis, MO). E. coli C-25 was generously provided by Dr. E. Deitch (New Jersey Medical School). Methylene blue and fuchsin (basic) were obtained from BDH (Edmonton, AB, Canada). The bacterial culturing materials were obtained from Becton-Dickinson (Cockeysville, MD). All other materials were obtained from VWR (Edmonton, AB, Canada).

RESULTS

Bacterial colonization of gastric ulcers. Application of acetic acid to the serosal wall of the stomach reproducibly induced gastric ulcers. Histologically, the ulcers were confirmed to penetrate into the submucosa. There was a dense layer of mucus, cellular debris, and granulation tissue in the ulcer bed. The area of the ulcers averaged ∼100 mm² 1 day after induction and gradually decreased, at a fairly constant rate, over the course of the next 20 days (Fig. 1). Histological examination of the ulcer bed revealed considerable levels of colonization by a variety of bacteria. Both rods and cocci were evident. Bacterial colonization of adjacent, normal tissue was occasionally observed, but appeared to be much less dense than that seen in the ulcer bed. In these cases, the bacteria were restricted to the luminal surface of the epithelium (no invasion into the mucosa or into the glands).

Fig. 1. Spontaneous healing of gastric ulcers in rats over a 3-wk period. Groups of rats (n = 5 each) were killed at various times after ulcer induction, and ulcer area was measured planimetrically.
Culturing of tissues taken from rats with ulcers confirmed the extensive colonization of the ulcer bed by various bacteria. In the stomachs of rats without ulcers, the levels of total aerobes ranged from $10^3$ to $10^4$ CFU/g of tissue. Gram-negative bacteria constituted a small portion (~5%) of total aerobes. Significant colonization of the stomach occurred within 6 h of ulcer induction. The levels of colonization (total aerobes) increased to a peak level of $10^9$–$10^{10}$ between 12 and 24 h after ulcer induction and remained at or near those levels for the following 7 days (Fig. 2). Gram-negative bacteria accounted for the majority of the total aerobes during this period (Fig. 2). There was a gradual decline in bacterial counts thereafter, with the levels at day 14 postulcer induction not differing significantly from those in the normal rat stomach. As the numbers of total aerobes declined, an increasing preponderance of gram-positive bacteria became evident.

As suggested by the histological studies, colonization occurred principally at the ulcer site, with significantly greater numbers of bacterial colonies (~10,000-fold increase in CFU) than on the contralateral side of the stomach (Fig. 3). In some rats with ulcers, there were also increased numbers of bacteria on the contralateral side relative to rats without ulcers.

Sensitivity testing revealed that the predominant bacteria at day 1 after induction of ulcers were E. coli, Streptococcus, and Enterococcus (Fig. 4). Lactobacillus, which was present in the stomach of all healthy controls, was absent one day after ulcer induction. By day 7, E. coli was still one of the major bacterial species present, Enterococcus had increased, and Streptococcus had declined. By day 14 after ulcer induction, a point at which healing had reduced the mean ulcer size by ~65% from that a week earlier, E. coli was still evident, but Streptococcus and Enterococcus were rare. The most striking change, however, was the preponderance of Lactobacillus colonizing the ulcer site at this time point.

To determine if bacterial colonization was a feature unique to ulcers induced by acetic acid, we also performed studies in which ulcers were induced in the rat stomach by application of a cryoprobe or by naproxen administration. In both cases, a significant ($P < 0.05$) increase in colonization by aerobes was observed ($\sim 10^{8.1}$ CFU).
and $10^{6.5}$ CFU/g for cryoprobe application and naproxen administration, respectively) relative to a control group in which ulcers were not induced ($10^{3.6}$ CFU/g).

To determine the influence of coprophagy and feeding on bacterial colonization of gastric ulcers in rats, we performed a series of experiments in which ulcers were induced in three groups of rats and the levels of bacterial colonization were determined 18 h later. One group was housed in standard cages, and the rats were not fasted. The other two groups were housed in wire mesh-bottomed cages (to reduce coprophagy). Both groups were fasted before ulcer induction, and in one of these groups the fasting continued for the 18-h period after ulcer induction. In all three groups, significant increases in bacterial colonization of the ulcers occurred relative to the levels seen in the healthy rat stomach. In the two groups that were fed after ulcer induction, the levels of total aerobes in the stomach were significantly higher in the group that continued to be fasted ($\sim 10^6$ vs. $\sim 10^8$ CFU/g; $P < 0.05$). In the latter group, the stomach was empty at the time of death. Thus it appeared that fasting reduced the levels of colonization of the ulcers somewhat. However, even when food was withheld and steps were taken to prevent coprophagy, substantial colonization of gastric ulcers was still observed.

Changes in gastric acid secretion. Congo red staining of the stomach from rats killed 1 or 7 days after induction of a gastric ulcer revealed dark blue staining of all tissue with the exception of the ulcer itself, which stained pink. This indicated that the surface of the normal gastric tissue had a pH of $< 3$, while the ulcer bed had a pH of $> 5$. To further investigate potential changes in gastric acid secretion associated with the presence of an ulcer, perfused stomach preparations were used (in vivo) in which basal and pentagastrin-stimulated acid secretion was measured. Figure 5 summarizes the results of these experiments. On day 1 after ulcer induction, no effect on basal or pentagastrin-stimulated acid secretion was observed relative to controls (no ulcers). However, on day 3 postulcer induction, a significant suppression of pentagastrin-stimulated acid secretion ($\sim 55\%$) was observed. On day 10 postulcer induction, acid secretion was not significantly different from that observed in controls.

Effects of antibiotics on ulcer healing and colonization. Treatment for 1 wk with streptomycin or penicillin alone did not significantly affect the level of total aerobic bacterial colonization of gastric ulcers (range of $10^5$ to $10^6$ CFU/g in all 3 groups). Treatment with these antibiotics also did not affect the rate of healing of the ulcers (mean area of $17.7 \pm 3.1$ mm$^2$ in vehicle-treated rats vs. $17.8 \pm 3.3$ and $15.2 \pm 3.6$ mm$^2$ in streptomycin- and penicillin-treated rats, respectively). In contrast, however, when the two antibiotics were both administered, a marked reduction ($\sim 68\%$) in the extent of bacterial colonization was observed ($P < 0.05$) and this was accompanied by a significant acceleration of ulcer healing relative to the control group (mean ulcer area of $3.2 \pm 1.8$ mm$^2$; $\sim 82\%$ reduction in ulcer size vs. in vehicle-treated rats; $P < 0.05$).

Colonization of ulcers with E. coli C-25. Rats receiving bacitracin and streptomycin in the drinking water and treated daily for a week with bacteria-free culture broth had significantly lower levels of bacterial colonization of their ulcers and significantly enhanced ulcer healing relative to untreated controls (Fig. 6). In contrast, rats receiving the same antibiotics but treated daily with the streptomycin-resistant strain of E. coli
(C-25) had significantly higher levels of bacterial colonization and significantly larger gastric ulcers compared with the group receiving culture broth (Fig. 6). Indeed, in the rats receiving E. coli C-25, the levels of colonization and the size of ulcers did not differ significantly from those in untreated controls.

Effects of lactulose. Addition of lactulose to the drinking water for 2 wk led to a significant increase in total aerobes in the stomach (~40-fold), while the gram-negative bacteria were almost completely eradicated (Fig. 7). In rats in which lactulose was given for 2 wk, ulcers induced, and lactulose administration continued for 10 days, the size of ulcers was significantly reduced compared with rats not receiving lactulose. Treatment with lactulose only on days 3–10 after ulcer induction did not significantly affect ulcer size (42.6 ± 13.7 vs. 61.5 ± 9.6 mm² in controls).

**DISCUSSION**

Within a few hours of ulcer induction, the stomach of the rat is changed from an environment that is unsuitable for significant bacterial colonization to one in which profound colonization occurs. This colonization occurred principally at the site of the ulcer. Colonies were found within the cellular debris and mucus overlying the ulcer bed, a site of relatively high pH (>5) compared with normal tissue. While gram-positive bacteria accounted for ~95% of the bacteria found in the stomach of normal rats, with Lactobacillus being the predominant variety identified, ulcer induction resulted in a rapid shift to a preponderance of gram-negative bacteria. No single bacterial species predominated in the early period after ulcer induction. As the ulcers healed, however, the numbers of total aerobes and the proportion of the total made up of gram-negative bacteria declined. As was the case in the healthy rats, Lactobacillus was the predominant bacteria colonizing the stomach 21 days after induction of ulcers.

The presence of bacteria at the site of ulcers in the stomach clearly affected the natural history of the ulcer, in that reduction of bacterial numbers with antibiotics led to a significant acceleration of ulcer healing. Moreover, during wide spectrum antibiotic therapy (provided in the drinking water) inoculation with an antibiotic-resistant strain of E. coli (C-25) had significantly higher levels of bacterial colonization of the stomach compared with rats not receiving lactulose. Treatment with lactulose only on days 3–10 after ulcer induction did not significantly affect ulcer size (42.6 ± 13.7 vs. 61.5 ± 9.6 mm² in controls).

**Fig. 7. Effects of addition of lactulose to the drinking water on bacterial colonization of the stomach (top) and on healing of gastric ulcers (bottom).** Rats were given lactulose-supplemented drinking water (or just water) for 2 wk. Tissues for assessment of bacterial colonization were taken at the end of the 2-wk period. In other rats, ulcers were induced and lactulose administration continued for an additional 10 days. Ulcer areas were determined on the tenth day after ulcer induction. *P < 0.05 compared with group receiving only water. Each group consisted of at least 5 rats.
intestinal tract. For example, we observed a marked increase in bacterial numbers within the small intestine after induction of injury with diclofenac (24). This increase in bacterial load occurred subsequent to significant increases in intestinal permeability. As in the case of gastric ulcers, bacteria appear to contribute to the injury induced in the small intestine by nonsteroidal anti-inflammatory drugs, because a reduction of the severity of injury could be observed after treatment with antibiotics (15).

Another key question raised by this study is: How do the bacteria that colonize gastric ulcers interfere with ulcer healing? The increased preponderance of gram-negative bacteria during the hours after ulcer induction and the beneficial effects observed after induction of a gram-positive bacteria (Lactobacillus) suggest that endotoxin may be contributing to the chronicity of the ulcers. Another possibility we considered was that bacterial colonization of the stomach might increase acid secretion. Indeed, it has been suggested that H. pylori may contribute to the pathogenesis of duodenal ulceration by increasing gastric acid secretion (9). Beneficial effects of inhibitors of acid secretion have been documented in this ulcer model (32). However, acid secretion actually decreased on day 3 after ulcer induction (a time when colonization was at peak levels) and normalized thereafter. This observation is consistent with those of others using the same model of gastric ulcer in rats (12, 16).

The vast majority of recent research related to bacterial colonization of the stomach is focused on H. pylori, and this is justified given the overwhelming evidence that infection of the stomach by this bacterium is a major risk factor for peptic ulcer disease and possibly other disorders (5). There is good evidence that H. pylori is specially adapted so that it can survive in the harsh environment of the stomach, as well as evidence that some strains of H. pylori carry virulence factors that may promote ulceration (2, 16). It is not clear if preexisting injury to the gastric mucosa is a permissive factor for colonization of the stomach by H. pylori, in the manner that induction of gastric ulcers in the rats allows for rapid bacterial colonization, although this has been previously suggested (3, 20). However, studies performed in the rat have demonstrated that, while H. pylori is not able to colonize or injure the normal stomach, it could colonize the stomach if given to rats twice daily for a week after an ulcer had been induced (26). Moreover, both intact H. pylori and bacteria-free filtrates were capable of delaying gastric ulcer healing in rats (26). These results have been extended by Li et al. (18), who showed a similar delay of ulcer healing in rats inoculated with a vacA− and cagA− strain of H. pylori. The results of the present study demonstrate that these effects are not unique to H. pylori, because the bacteria that spontaneously colonized the ulcerated stomach and a streptomycin-resistant strain of E. coli were capable of significantly delaying ulcer healing. Thus, although H. pylori is undoubtedly the major microbial culprit in the pathogenesis of peptic ulcer disease, the results of the present study suggest that other bacteria (and possibly other microbes) are capable of influencing the natural history of ulcers. In this regard, it is noteworthy that Streptococcus and Candida have been reported to colonize human gastric ulcers and to contribute to the chronicity of those lesions (1, 14, 23, 25, 30). Although eradication of H. pylori has a profound effect on the healing and recurrence of ulcers, one cannot exclude the possibility that some of the beneficial effects of treating peptic ulcer disease with a combination of wide spectrum antibiotics may be attributable to eradication of bacteria other than H. pylori.

In summary, the present study demonstrates a rapid colonization of gastric ulcers in rats by a variety of bacteria. This colonization occurs predominantly at the ulcer site and has a clear detrimental effect in terms of the healing of the ulcer. Gram-negative bacteria are likely to be responsible for the observed delay in ulcer healing, whereas gram-positive bacteria may actually promote ulcer healing. These studies suggest that bacteria other than H. pylori have the capacity to significantly influence the natural history of an ulcer and that ulcers represent an environment conducive to bacterial growth.

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