

Effect of Purified Lipopolysaccharides from Strains of *Helicobacter pylori* and *Helicobacter felis* on Acid Secretion in Mouse Gastric Glands In Vitro

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Received 28 November 2000/Returned for modification 22 January 2001/Accepted 13 March 2001

As a bacterial product, *Helicobacter pylori* lipopolysaccharide (LPS) can originate in close proximity to parietal cells, but the role of this uniquely structured endotoxin on acid secretion has not been fully investigated and remains unclear. The purpose of this study was to test the direct effect of purified LPS (tested range, 0.1 to 100 µg/ml) from various strains of *H. pylori* and from one *Helicobacter felis* strain on histamine- and carbachol-stimulated acid secretion in vitro using mouse gastric glands and the accumulation of [¹⁴C]aminopyrine. In addition, we investigated whether *H. pylori* LPS can interfere with two native antisecretory substances, prostaglandin E₂ (PGE₂) and somatostatin, which may contribute to bacterial pathogenicity. Except for the LPS from *H. pylori* SS1 (Sydney strain), which gave a statistically significant increase in both histamine- and carbachol-stimulated acid output (38 and 24%, respectively; *P* < 0.05), no effect of the tested LPS was observed on acid secretion. *H. pylori* LPS purified from a patient isolate did not affect the potency or the efficacy of the inhibitory dose response curve to PGE₂ or somatostatin. Bacterial interstrain variation in the direct stimulatory effect of *Helicobacter*-derived LPS on acid secretion was observed, which probably reflects the molecular structure of LPS and the potential to contribute to virulence. Importantly, the data showed that *H. pylori* LPS did not have any direct antisecretory properties. It can be speculated that the acid stimulatory properties of LPS from *H. pylori* SS1 may contribute to the gastric damage observed in the mouse model of *H. pylori* infection.

Helicobacter spp. are known to colonize the stomach of mice with the subsequent development of gastritis. This has led to the development and standardization of a mouse model of *Helicobacter pylori*-induced gastritis (15). Both bacterial factors and host resistance contribute to the severity of *H. pylori*-induced pathology (5). Gastric acid secretion can be beneficial while it acts as a part of the host defense mechanism preventing bacterial infection and hence pathogenicity. However, it also leads to erosive ulcers of the stomach and/or duodenum. Consequently, it also plays a key role in the colonization patterns of *Helicobacter* species, as increased acid secretion tends to keep bacterial colonization in the antral region of the stomach whereas decreased acid secretion permits bacterial colonization and spread throughout the corpus (14, 30). The presence of *Helicobacter* infection can modulate acid secretion by altering the physiology of G cells, D cells, and parietal cells (7). It can do this either by the direct presence of its metabolites or through induction of the inflammatory process as mediated by a wide range of cytokines.

Gram-negative bacterial lipopolysaccharide (LPS) from a number of bacterial species effectively inhibits gastric acid secretion in vivo (1, 6, 26, 28, 32). However, the mechanism of LPS action suggests the involvement of inflammatory products or mediators such as cytokines and prostaglandins (25, 27). Recently, *H. pylori* LPS was shown to inhibit acid secretion in

vivo (22). *H. pylori* LPS has been implicated in the stimulation of pepsinogen and histamine secretion, inhibition of sulfated mucin synthesis, and the production of potentially destructive autoantibodies, which may all contribute to the loss of mucosal integrity (18). In addition, *H. pylori* LPS was shown to bind to the gastric mucosal somatostatin receptor (23). *H. pylori* LPS, as compared to *Escherichia coli* LPS, has a relatively low immunological activity (20) that potentially contributes to the persistence of infection.

The present study focuses on the potential effect of *H. pylori* LPS directly on acid secretion in order to elucidate possible mechanisms by which bacteria can affect parietal cells. *H. pylori* resides in the mucus layer close to the epithelium; therefore, its metabolites originate in relative and, in some circumstances, close proximity to acid-producing cells, but the role of this endotoxin on the secretory properties of parietal cells has not been fully investigated. As the mouse models of *H. pylori* infection have entered the mainstream of research (15), we have adapted and characterized the mouse gastric gland in vitro model of acid secretion. The purpose of this study was to test several purified *Helicobacter*-derived LPS preparations for a direct effect on carbachol- or histamine-stimulated acid secretion and its potential interference with the antisecretory actions of somatostatin and prostaglandin E₂ (PGE₂).

MATERIALS AND METHODS

Purification of LPS from *H. pylori* and *Helicobacter felis*. Bacterial strains of *H. pylori* and *H. felis* were grown on blood agar to produce biomass as described previously (17). Bacteria were harvested in sterile distilled water, centrifuged at 5,000 × g (4°C, 30 min), and washed twice, and the bacterial pellets were

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freeze-dried. After pretreatment of the bacterial biomass with pronase E (8), LPS was extracted by the hot phenol-water technique (17). The LPS preparations were purified by treatment with DNase, RNase, and proteinase K and by ultracentrifugation as described previously (17). Five *Helicobacter*-derived LPS preparations were prepared and tested: water- and phenol-phase LPSs from the type strain *H. pylori* NCTC 11637 (purchased from the National Collection of Type Cultures, London, England), water-phase LPS from the Sydney strain *H. pylori* SS1 (obtained from A. Lee, Department of Microbiology and Immunology, University New South Wales, Sydney, Australia), water-phase LPS from an isolate from a duodenal ulcer patient (*H. pylori* patient isolate [PI]), and water-phase LPS from *H. felis* ATCC 49179 (purchased from the American Type Culture Collection, Rockville, Md.).

Animals. Female BALB/c mice, 6 to 8 weeks old, were obtained from Charles River (St. Constant, PQ J5A 1Y2, Canada), kept under standard housing conditions at 21 to 23°C with a humidity of 40 to 50% and a 12/12 light/dark cycle, and fed Purina Lab Rodent Chow for up to 12 weeks. Ten mice for each acid assay were not fed for 24 h (water, ad libitum) prior to sacrifice by cervical dislocation. Subsequently, the stomachs were quickly removed, opened along the lesser curvature, and placed in oxygenated phosphate-buffered saline buffer, pH 7.3, at 37°C. Utilization of animals was approved by the Animal Research Ethics Board at McMaster University.

Preparation of gastric glands from mice. Preparation of gastric glands was performed according to the method of Berglindh (4) with some modifications. Briefly, the gastric mucosa was scraped off the underlying muscle using a scalpel blade, pooled, and washed twice (approximately 200 g for 5 min) in phosphate-buffered saline. The scrapings were placed in an enzyme solution that contained (per ml) 2 mg of glucose, 1 mg of bovine serum albumin (Sigma A-7888), 0.25 mg of type II-s soybean trypsin inhibitor (Sigma T-9128), and 0.23 mg of type IV collagenase (Sigma C-5138). Since there were discrepancies in the acid-producing capacity of glands depending on the batch of collagenase used, different batches of collagenase were screened. Mouse gastric mucosa was enzymatically digested at 37°C for 45 min in a flat-bottom, covered, 150-ml Erlenmeyer flask and agitated by a magnetic stirrer (around 100 rpm). Subsequently, gastric glands were passed through nylon mesh (500- μ m hole size) in order to separate debris and the undigested remains of the gastric mucosa. Then the preparation was washed three times (approximately 200 g for 5 min) in enzymatic buffer that did not contain collagenase or trypsin inhibitor. Finally, the preparation was resuspended in 50 ml of incubation medium containing 2 mM CaCl₂, 1.2 mM MgSO₄, 2 mg of bovine serum albumin/ml, and 2 mg of glucose/ml.

Measurement of acid secretion in mouse gastric glands. Acid secretion was measured by the accumulation of a weak base, [¹⁴C]aminopyrine ([¹⁴C]AP), as described by Berglindh (4) with some modifications. Briefly, the experiment was carried out in closed 1.5-ml Eppendorf tubes containing 0.5 ml of resuspended gastric glands with added secretagogue (0.01 mM carbachol or 0.1 mM histamine), antisecretory compound (e.g., somatostatin or PGE₂), and doses of test preparations of LPS (for control tubes that did not contain LPS, a corresponding volume of endotoxin-free distilled water was used). For testing basal acid secretion, tubes did not contain histamine or carbachol. Also, 20 μ l (equal to 0.25 μ Ci) of [¹⁴C]AP was added to the tubes and incubated at 37°C for 60 min, with rotation. All tested reagents were coincubated with the gastric glands. The tubes were centrifuged at approximately 1,500 \times g for 5 min, the supernatant was aspirated, and the pellet was washed three times in incubation buffer to minimize any nonspecific aminopyrine retention in the glands. The pellet was transferred to scintillation tubes and solubilized with 1 ml of tissue solubilizer (NCS-2; Amersham) overnight. Subsequently, 50 μ l of glacial acetic acid was added to each tube containing the solubilized pellet in order to neutralize the highly basic tissue solubilizer. After the addition of 5 ml of ACS scintillation fluid (Amersham), radioactivity was determined in a Beckman scintillation counter (LS 5801). The radioactivity of the pellet correlated positively with the amount of acid secreted during 60 min of incubation. The radioactivity accumulated by glands with 0.1 mM dinitrophenol was subtracted from all data to compensate for any nonspecifically trapped [¹⁴C]AP, which accounted for less than 0.5% of the maximal histamine response. Each sample was tested in triplicate within each individual experiment, and each experiment was repeated with different gland preparations. This repetition is expressed by *n* (the number of individual experiments).

Chemicals. *E. coli* LPS and all chemicals were of high purity and were purchased from Sigma unless otherwise stated.

Statistical analysis. The data were calculated as the percentage of the maximal response in AP uptake to various stimulants; *n* represents the number of gland preparations for which each data point was tested in triplicate. The results are expressed as the mean \pm the standard error of the mean of the preparation

results. The significance of differences was tested by Student's *t* test, and differences were considered statistically significant if *P* was <0.05.

RESULTS

Concentration response curves were constructed for carbachol and histamine, with the maximal response being a 3- to 5-fold and 8- to 12-fold increase over basal acid secretion, respectively (data not shown). Maximum acid stimulation occurred at 10⁻⁵ M for carbachol and 10⁻⁴ M for histamine. Each LPS preparation was tested over the range from 0.1 to 100 μ g/ml. For histamine-stimulated acid secretion, none of the tested *H. pylori* LPS showed a direct effect on acid secretion except *H. pylori* SS1 (Fig. 1).

The highest concentration tested, 100 μ g of the LPS of *H. pylori* SS1/ml, caused a significant 38% increase in histamine-stimulated acid secretion. Similarly, for carbachol-stimulated acid secretion, with the exception of the Sydney strain LPS, none of the tested *H. pylori* LPS preparations showed any direct effect on acid secretion (Fig. 2).

Again, only the highest concentration tested, 100 μ g of the Sydney strain LPS/ml, caused a significant 23% increase in carbachol-stimulated acid secretion. No effect on acid secretion was seen with LPS from *Helicobacter felis*. Commercially available *E. coli* LPS did not show any effect on carbachol- or histamine-stimulated acid secretion in the mouse gland preparations when tested over the range from 1.0 pg to 100 μ g/ml (data not shown). Dose response curves were constructed for PGE₂ and somatostatin, and the possible effect of *H. pylori* LPS from a duodenal ulcer patient (PI strain) was tested. PGE₂ caused a dose response-related inhibition of histamine-stimulated acid secretion with the maximum inhibition at 10⁻⁶ M and a 50% inhibitory concentration of 8 \times 10⁻⁸ M (Fig. 3).

The LPS from *H. pylori* PI was coincubated for 60 min with 10⁻⁴ M of histamine and doses of PGE₂ (from 10⁻⁸ to 10⁻⁵ M) and then compared with controls that did not contain any LPS. The LPS of *H. pylori* PI at a concentration of 25 μ g/ml did not cause any significant changes in the efficacy or potency of the inhibitory effect of PGE₂ (Fig. 3). Somatostatin inhibited histamine-stimulated acid secretion in a dose response manner with a 50% inhibitory concentration of around 10⁻⁸ M (Fig. 4). However, when tested with 25 μ g of LPS/ml from *H. pylori* PI this failed to show any significant effect on the potency or efficacy of the somatostatin inhibition. Pretreatment of the glands for an additional 60 min with the tested LPS did not influence any of the reported results (data not shown).

DISCUSSION

The mouse gland preparation enables testing of the potential effect of *Helicobacter*-derived LPS in this in vitro model of acid secretion that lacks the potential involvement of immune inflammatory cells. The model allows testing of substances that act in a more direct fashion on the secretory function of mouse parietal cells. In the present study, only LPS of the Sydney strain of *H. pylori* caused direct stimulation of acid secretion, whereas the other tested preparations did not cause any significant change in acid secretion. A number of earlier reports have shown that LPS from gram-negative bacteria was able to inhibit acid secretion in vivo (1, 28, 32). In particular, these reports focused on the effect of *E. coli*-derived LPS, and its in

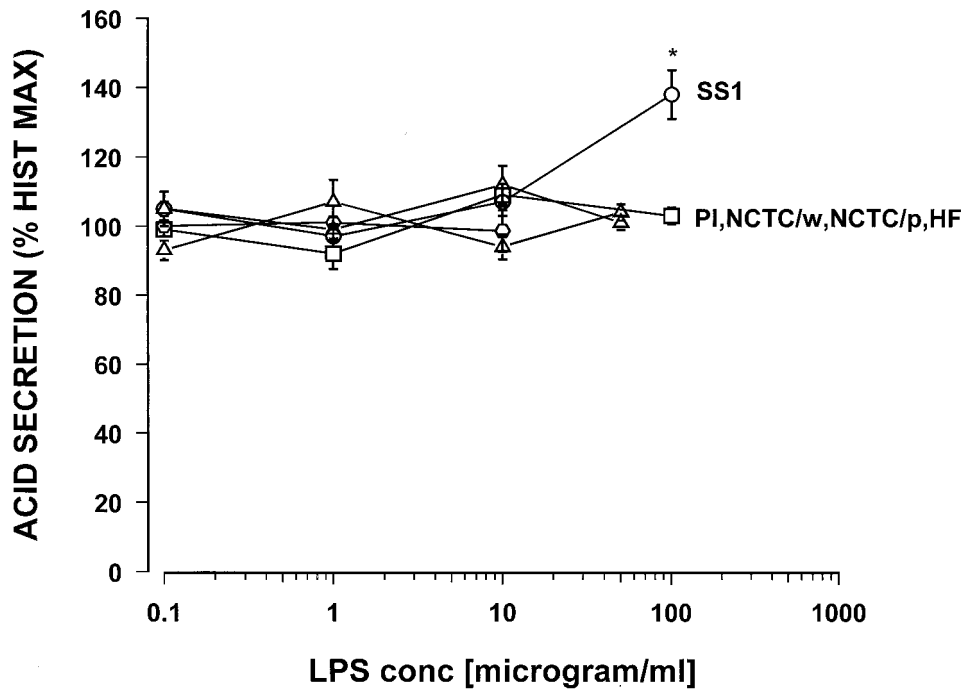


FIG. 1. Effect of various samples of *Helicobacter* LPS on histamine (10^{-4} M)-stimulated acid secretion in the mouse gastric glands as measured by [14 C]AP accumulation. PI, *H. pylori* isolate from a duodenal ulcer patient; NCTC/p, *H. pylori* NCTC 11637 (phenol phase); NCTC/w, *H. pylori* NCTC 11637 (water phase); SS1, the Sydney strain of *H. pylori*; HF, *H. felis*; *, $P < 0.05$. $n = 8$ or 9 .

vivo inhibitory effect has remained undisputed. Also, *E. coli*-derived LPS was shown to exert a lasting inhibitory effect on pepsinogen secretion (28). However, subsequent research revealed that the observed effect was reversible (29) and that the

inhibitory effect of LPS from *E. coli* may have been caused by the involvement of inflammatory cytokines. In particular, interleukin-1 (IL-1) was suspected and this effect was blocked by indomethacin (25, 27). Indeed, administration of IL-1 in vivo

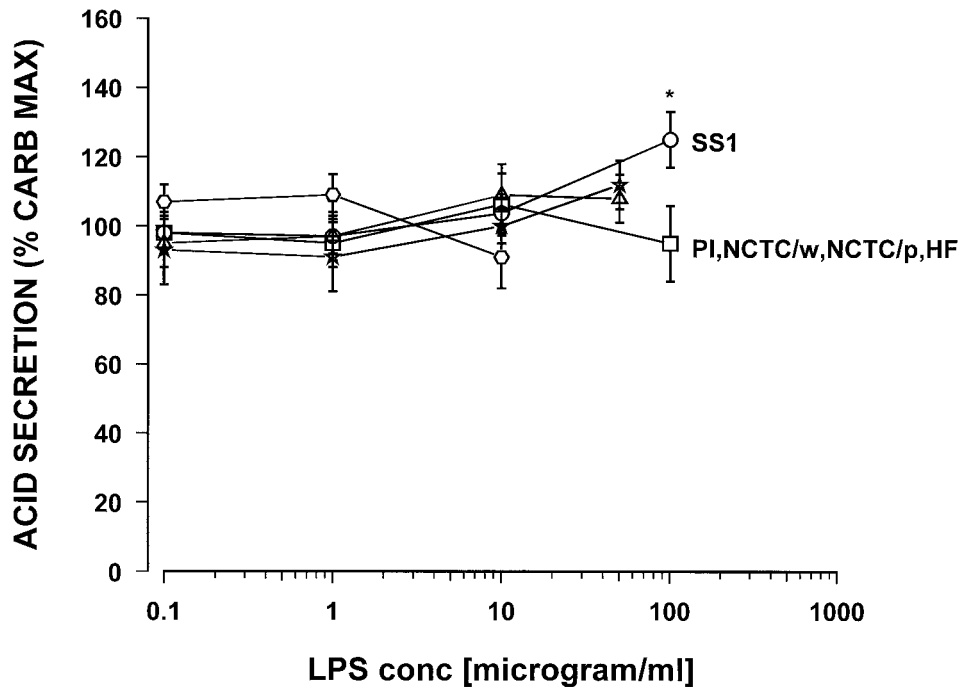


FIG. 2. Effect of various samples of *Helicobacter* LPS on carbachol (10^{-5} M)-stimulated acid secretion in the mouse gastric glands as measured by [14 C]AP accumulation. PI, *H. pylori* isolate from a duodenal ulcer patient; NCTC/p, *H. pylori* NCTC 11637 (phenol phase); NCTC/w, *H. pylori* NCTC 11637 (water phase); SS1, the Sydney strain of *H. pylori*; HF, *H. felis*; *, $P < 0.05$. $n = 7$ or 8 .

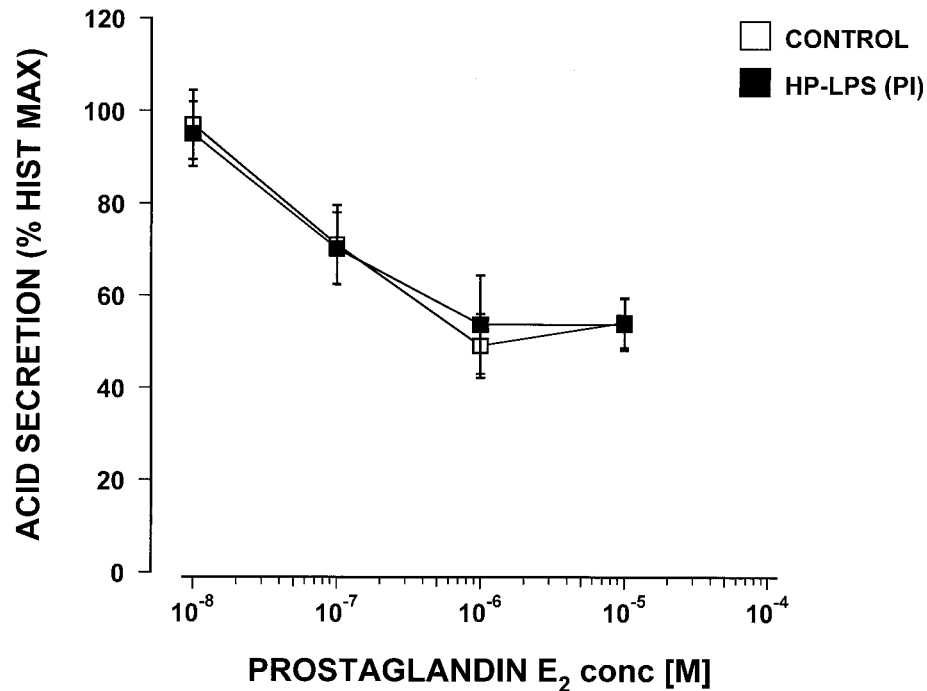


FIG. 3. Effect of the LPS (25 $\mu\text{g/ml}$) of an *H. pylori* isolate from a duodenal ulcer patient (PI) on the inhibition by PGE₂ of histamine-stimulated acid secretion in the mouse gastric glands as measured by [¹⁴C]AP accumulation ($n = 3$).

(13, 24, 31) and, to some extent, in vitro (2) showed its potent antisecretory effects on acid secretion. Both a review of the articles presented in this discussion and studies that show the lack of direct antisecretory effect indicate that *E. coli*-derived

LPS exerts strong antigenic and proinflammatory properties by which it inhibits acid and pepsin secretion. *H. pylori* LPS has, in general, much lower immunological activity than LPS purified from *E. coli* (18, 21). Thus, recently *H. pylori* LPS was shown to

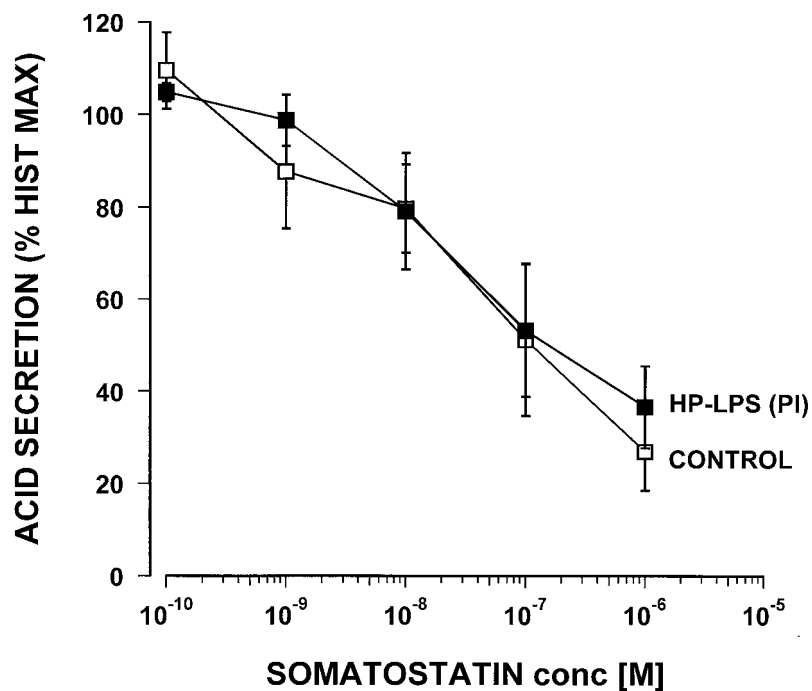


FIG. 4. Effect of the LPS (25 $\mu\text{g/ml}$) of an *H. pylori* isolate from a duodenal ulcer patient (PI) on the inhibition by somatostatin of histamine-stimulated acid secretion in the mouse gastric glands as measured by [¹⁴C]AP accumulation ($n = 4$).

inhibit acid secretion in vivo at a dose over 10,000-fold higher than that of *E. coli* LPS (22), which correlates with its low immunological activity compared to that of *E. coli* LPS (18, 21). It was also suggested that these much lower acid inhibitory properties were the result of structural differences between *H. pylori* and *E. coli* LPS, again consistent with observed structure-bioactivity relationships for immunoactivity (21). However, one could also conclude that the observed antisecretory effect had a similar mechanism involving products of the host immune system, possibly IL-1 and prostaglandins. The antisecretory effect observed with *H. pylori* LPS could not be blocked by indomethacin. Nevertheless, it was also suggested that the inhibitory effect of IL-1 can be prostaglandin independent (31).

As the exact mechanism of the inhibitory action of LPS remains unclear, we tested the hypothesis that the observed effect of LPS could be of a more direct nature and be caused by inhibition of acid at the parietal cell level. However, no direct inhibitory effect by the tested LPS was observed in the present study, even with that derived from *E. coli*. Since these results were observed with an in vitro system, this suggests that the previously reported observed inhibitory effect of *H. pylori*- and *E. coli*-derived LPS on acid secretion stems, indeed, from its proinflammatory properties and is the consequence of the induction of inflammatory mediators. A number of reports have indicated that *H. pylori* sonicates inhibit secretion of parietal cells in vitro and that bacterial factors contributed up to 80% inhibition (3, 10, 11). However, based on the data from our present study, LPS as a possible mediator for the observed inhibition can be excluded. Also, it has been suggested that *H. pylori* LPS may bind to the gastric mucosal somatostatin receptor (23), but we did not find any potential interference with somatostatin receptors on parietal cells and subsequent inhibition of acid secretion. Similarly, *H. pylori* LPS failed to show any interaction with prostaglandin receptors on parietal cells and did not affect acid secretion in this fashion either. Supporting our findings, other studies have concluded that *H. pylori* LPS had no effect on acid secretion in an Ussing chamber study (20, 33). Therefore, it is unlikely that *H. pylori* LPS is directly responsible for the observed hyposecretion of acid in *Helicobacter*-related disease.

Whatever the mechanism of *H. pylori*-related inhibition of acid secretion, it is widely accepted that this effect promotes colonization of the gastric mucosa by *H. pylori* and may contribute to gastric ulcer disease, atrophy, and subsequent progress to cancer (9). In contrast to the described inhibitory properties of *H. pylori* LPS in vivo (22), we have shown that LPS from *H. pylori* SS1 can stimulate acid secretion, and since other LPS preparations did not, this is probably related to differences in the molecular structure of the tested LPS preparations. We have shown that *H. felis* LPS does not affect acid secretion, and although this bacterium colonizes the mouse stomach, development of active chronic gastritis is slow but is present 6 months postinfection (15). The Sydney strain of *H. pylori* was chosen to optimize the mouse model (15), and interestingly, *H. pylori* LPS from this particular strain in our studies had a stimulatory effect on acid secretion. Extensive work on the structure of the Sydney strain LPS (16) may help to explain why this strain is preferred in the mouse model of gastritis. Work on optimizing the mouse model of *H. pylori*-

induced inflammation shows that this *H. pylori* strain can effectively mimic the antral gastritis observed in humans (15). The antral gastritis is generally linked to hypersecretion of gastric acid and tends to evolve into duodenal ulcer disease. It has been proposed that the local acid production will determine the extent of colonization by *Helicobacter* species in the stomach, with decreased acid production promoting the spread of colonization to the acid-producing body of the stomach as opposed to the increased acid production, which tends to restrict colonization predominantly to the gastric antrum (30). These results show for the first time that LPS from *H. pylori* can stimulate acid secretion, which possibly might contribute to mucosal damage of the stomach and duodenum. The second possible mechanism by which *H. pylori* LPS can stimulate acid secretion at the gland level derives from data showing that it can increase histamine release from rat ECL cells (12).

The clinical syndrome of septicemia caused by gram-negative organisms is mainly caused by an immune response to bacterial endotoxin, LPS. Nevertheless, there is no evidence that *H. pylori* infection and its endotoxin cause septicemia or septic shock, supporting the concept that it possesses low immunological activity. In our results the acid stimulatory effect was observed only at the highest dose tested of 100 $\mu\text{g/ml}$, and it is very unlikely that levels of this LPS in plasma can reach these concentrations or be of clinical significance. However, *H. pylori* bacteria can survive in close proximity to the surface of gastric mucosa, and in extreme situations inside gastric glands, local high concentrations of LPS cannot be excluded. The ability of LPS from *H. pylori* SS1 to increase acid secretion may prevent transgastric bacterial invasion and contribute to the predominantly antral colonization observed in BALB/c mice. We have also tested and previously reported the effect of *H. pylori* LPS, including the SS1 strain, on parietal cells obtained from C57BL/6 mice and found no differences, as obtained results were consistent and comparable in both strains of mice (I. T. Padol, A. P. Moran, S. O. Hynes, and R. H. Hunt, Abstr. Gastroenterology, vol. 118, no. 4, abstr. 3976, 2000). Therefore, the stimulatory effect of LPS from the SS1 strain can play only an accessory role in mouse models and the differences in the pattern of colonization are attributed mainly to another mechanism, possibly to the type of immune response evoked. Nevertheless, it seems that the inhibitory effect on acid secretion mediated by bacterium-induced inflammation can be counteracted by the ability of some strains of the bacterium to stimulate the parietal cell to secrete acid. Thus, it is possible that a dynamic balance exists between these processes, and depending on which one dominates, this may determine the pathogenic path for either duodenal ulcer or gastric ulcer.

Based on our data and the available literature, it can be concluded that the *E. coli*-derived LPS inhibits acid and pepsinogen secretion as a consequence of inflammation. In contrast, the LPS purified from the known gastric pathogen *H. pylori* has this antisecretory property greatly impaired and, depending on the strain of the bacterium, is able to stimulate directly both pepsinogen (19, 33) and acid secretion, potentially contributing to gastrointestinal pathology.

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Editor: B. B. Finlay