Impaired mucus-bicarbonate barrier in Helicobacter pylori-infected mice

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Henriksnäs, Johanna, Mia Phillipson, Martin Storm, Lars Engstrand, Manoocher Soleimani, and Lena Holm. Impaired mucus-bicarbonate barrier in Helicobacter pylori-infected mice. Am J Physiol Gastrointest Liver Physiol 291: G396–G403, 2006. First published April 13, 2006; doi:10.1152/ajpgi.00017.2006.—To resist the harsh intrinsic milieu, several lines of defense exist in the stomach. The aim of this study was to investigate the effect of the gastric pathogen Helicobacter pylori on these mechanisms in vivo. We used FVB/N mice expressing human α,1,3,4-fucosyl transferase (producing Lewis b epitopes) and inoculated with H. pylori 1. Mice were anesthetized with isoflurane or Hypnorm-midazolam, the stomach was exteriorized, and the surface of the corpus mucosa was exposed. Mucus thickness was measured with micropipettes, juxtamucosal pH (pHjm) was measured with pH-sensitive microelectrodes, blood flow was measured with laser-Doppler flowmetry, and mRNA levels of the bicarbonate transporter SLC26A9 were quantified with real-time PCR. The increase in mucosal blood flow seen in response to luminal acid (pH 1.5) in control animals (140 ± 9% of control) was abolished in infected mice. The firmly adherent mucus layer was significantly thinner in infected mice (31 ± 2 μm) than in control mice (46 ± 5 μm), and no mucus accumulation occurred in infected mice. pHjm decreased significantly more on exposure to luminal acid in infected mice (luminal pH 1.5, pHjm 2.4 ± 0.7) than in control mice (pHjm 6.4 ± 0.5). Despite reduced pHjm, SLC26A9 mRNA expression was significantly, by increased 1.9-fold, in infected mice. The reduction in pHjm might be due to a reduced firmly adherent layer of mucus together with HCO3−. In previous studies in rats, we (28, 35) have shown that the pH at the epithelial surface [juxtamucosal pH (pHjm)] is neutral despite an acidic lumen. Only the firmly adherent layer is important in maintaining pHjm at a neutral level during luminal acid exposure (28). Thus the firmly adherent layer of mucus together with HCO3− secreted from the surface epithelial cells seems to form a pH gradient with a neutral pH next to the epithelial cells even though the luminal pH is acidic (28, 35).

SLC26A9, a cloned member of the SLC26 family of anion exchangers, has been recently shown to mediate Cl−/HCO3− exchange and has been found in the apical membrane of gastric surface epithelial cells (39). In addition to Cl−/HCO3− exchange, SLC26A9 can function as a Cl−-independent HCO3− transporter. Thus, given its localization and functional modes, SLC26A9 may be important in creating and maintaining the neutrality of pHjm. The SLC26A9-mediated Cl−/HCO3− exchange has been shown to be inhibited by NH4+ (6). A recent study (39) from our laboratories has demonstrated that the gastric lumen of rats treated with NH4+ could not maintain neutral pHjm when challenged with acid. Given the abundant amount of ammonium/ammonia that is produced by H. pylori urease activity, it was suggested that H. pylori can disrupt the mucus-HCO3− layer by inhibiting SLC26A9-mediated HCO3− secretion (39).

Gastric mucus blood flow has a vital role in gastric mucosal protection. It maintains the juxtamucosal HCO3− gradient during acid secretion by transporting HCO3− from parietal cells to surface epithelial cells, through which HCO3− is secreted into the mucus gel layer. In addition, adequate blood...
flow is required to maintain the different functions of mucosal cells, including the process of rapid repair. Blood flow is also important in removing backdiffusing hydrogen ions, toxic waste products, accumulated oxygen metabolites, and other inflammatory mediators. We have shown in previous studies (29, 35) that luminal acid alone induces hyperemia without any macroscopic lesions. Furthermore, we have suggested that epithelial inducible nitric oxide (NO) synthase (iNOS) is involved in the hyperemic response to acid, possibly signaling to afferent nerves, leading to a blood flow increase.

The purpose of this study was to investigate how a chronic infection with _H. pylori_ influences gastric mucus thickness and accumulation, pH unnatural, the gastric mucus and a blood flow response to acid, and the expression of the Cl⁻/HCO₃⁻ exchanger SLC26A9, all of which are important factors in the gastric protection barrier.

**MATERIALS AND METHODS**

All experimental procedures in this study were approved by the Swedish Laboratory Animal Ethical Committee in Uppsala, Sweden, and were conducted in accordance with guidelines of the Swedish National Board for Laboratory Animals.

**Mice**

FVB/N mice expressing human α-1,3/4FT were used. These mice produce Leb epitopes in the surface epithelium, as previously described by Falk et al. (10). Breeding pairs of mice expressing the epitope were kindly provided by Per Falk (Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO). The genotype of each mouse was determined by PCR analysis of DNA isolated from tail tissue. Mice expressing α-1,3/4FT were used for both the control and infected groups; 34 female mice and 21 male mice, weighing between 22 and 43 g, were used for the experiments (equally distributed in the experiments).

**Infection**

The strain Hp1, isolated from a Peruvian patient with gastritis, was used. The strain has been shown to infect this line of mice under normal conditions (14). Isolates of Hp1 were cultured on Columbia II agar plates (BBL, Becton-Dickinson; Cockeysville, MD) with 10% horse serum and 8.5% denatured horse blood (chocolate agar) under agar plates (BBL, Becton-Dickinson; Cockeysville, MD) with 10% female mice and 21 male mice, weighing between 22 and 43 g, were used for both the control and infected groups; 34 PCR analysis of DNA isolated from tail tissue. Mice expressing α-1,3/4FT were used. These mice produce Leb epitopes in the surface epithelium, as previously described by Falk et al. (10). Breeding pairs of mice expressing the epitope were kindly provided by Per Falk (Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO). The genotype of each mouse was determined by PCR analysis of DNA isolated from tail tissue. Mice expressing α-1,3/4FT were used for both the control and infected groups; 34 female mice and 21 male mice, weighing between 22 and 43 g, were used for the experiments (equally distributed in the experiments).

**Animal Preparation**

Body temperature was maintained at 37°C by means of a heating pad controlled by a rectal thermostirr probe. A catheter containing heparin (12.5 IU/ml) dissolved in isotonic saline was placed in the carotid artery to monitor blood pressure. The jugular vein was cannulated for the continuous infusion of a maintenance fluid (modified Ringer solution, 0.35 ml/h). In a few experiments, the vein cannulation failed, and the infusion was performed via the arterial catheter through a Y connection.

The preparation of the gastric mucosa for intravital microscopy in mice has been described previously (16). Briefly, exteriorization of the mucosa through a midline abdominal incision was followed by an incision along the greater curvature in the forestomach. The animal was placed on a Lucite table with part of the corpus of the stomach loosely draped over a truncated cone, with the mucosal surface facing upward. A “mucosal chamber” was fitted over the gastric mucosa, exposing −0.13 cm² of the mucosa. The chamber was filled with 3 ml of unbuffered 0.9% saline, maintained at 37°C by means of warm water circulating in a jacket in the bottom of the chamber. The saline was replaced at regular intervals of 10–15 min, and the pH of the solution was measured. The mouse was moved to a stereomicroscope (Leica MZ12, Leica, Heerbrugg, Switzerland), and the gastric mucosa was transilluminated. Before the experiments, animals were allowed to stabilize for 20–60 min after surgery.

**Mucosal Permeability Measurements**

To investigate the effect of _H. pylori_ infection on gastric mucosal integrity, mucosal permeability was determined by measuring the clearance of ⁵¹Cr-labeled EDTA (⁵¹Cr-EDTA, Perkin-Elmer Life Sciences; Boston, MA) from blood to lumen (26).

Fifteen minutes after the completion of surgery, ⁵¹Cr-EDTA (5–7.5 μCi) was injected as a bolus dose (0.03–0.05 ml), followed by a continuous intravenous infusion of ³¹Cr-EDTA (15–60 μCi/ml in Ringer solution) at a rate of 0.35 ml/h. One blood sample (~25 μl) was taken 30 min after the injection of ³¹Cr-EDTA, and a second one was taken 30 min later. After the first blood sample withdrawal, the blood volume loss was compensated for by an injection of 7% bovine albumin in saline (0.05 ml). The luminal solution and blood were analyzed for ³¹Cr activity in a gamma counter (model 1282, Compu- gamma CS, Perkin-Elmer Life Science; Upplands Väsby, Sweden). The clearance value was calculated by dividing each individual effluent counts per minute value by a corresponding plasma counts per minute value and expressed as millimeters per minute per 100 g of wet tissue weight.

**Mucus Measurements**

Micropipettes made of glass tubing [borosilicate tubing, outer diameter (OD) 1.2 mm and inner diameter (ID) 0.6 mm, Haer; Brunswick, ME], pulled to a tip diameter of 1–2 μm and prepared as
previously described (21), were used. The mucus gel thickness was measured by inserting a micropipette from the luminal solution into the gel at an angle of 33–39° to the surface using a micromanipulator (Leitz; Wetzlar, Germany). The mucus thickness was measured by advancement and withdrawal of the tip of the micropipette at five different sites on the mucosa. The mean value of these five measurements was regarded as one measurement. A “digtimatic indicator” (IDC series 543, Mituyo; Tokyo, Japan) was connected to the micromanipulator for measurement of the distance covered by the micropipette. The distance in the vertical direction (90° to the surface) from the luminal surface of the mucus gel was calculated.

After the measurements of total mucus thickness, the loosely adherent layer was removed by gentle suction, allowing the thickness of the firmly adherent layer to be measured (first removal). Fifteen and thirty minutes later, mucus thickness was again measured, whereby the rate of mucus accumulation could be calculated. Finally, the loosely adherent layer was removed again, and the thickness of the firmly adherent layer was measured once more (second removal).

**pH Measurements**

The hydrogen ion concentration in the mucus gel at the epithelial cell surface, i.e., pH$_{m}$, was measured with hydrogen ion-selective microelectrodes as previously described (28). Glass tubing (borsilicate tubing with omega dot, OD 1.2 mm and ID 0.9 mm, Haer) was calibrated before and after the experiments in isoosmolar (310 mosM) solutions with a pH of 1.5–8 at 37°C. Microelectrodes were inserted into the mucus gel at an angle of 30–40° to the molar (310 mosM) solutions with a pH of 1.5–8 at 37°C. The hydrogen ion concentration was measured by inserting a micropipette from the luminal solution into the mucosa gel at an angle of 30–40° to the mucosal surface by means of a micromanipulator (Leitz).

Because an earlier study (28) in the rat has revealed that pH$_{m}$ was not influenced by the loosely adherent mucus layer, and because the position of the electrode was easier to verify if the loosely adherent mucus layer was removed, this was done before the experiments were started. Ten minutes after the electrode had been placed in position, acid (HCl, pH 2) was instilled into the lumen for 10 min, after which it was changed to saline for 10 min, and then acid (HCl, pH 1.5) for another 10 min, followed by 10 min of saline.

**Blood Flow Measurements**

Blood flow was measured with laser-Doppler flowmetry equipment (Periflux instruments PF3 and PF4001, Perimed; Stockholm, Sweden), which has previously been used to study microcirculatory blood flow of the gastric mucosa in the mouse model (16). The laser probe was held in a fixed position in the chamber solution at a distance of 1–2 mm above the mucosa by a micromanipulator. With the type and position of the probe used in these experiments, the laser light most likely penetrated through the entire thickness of the gastric wall (20). However, the recorded blood flow was mainly mucosal, because the amount of backscattered light decreases exponentially with the depth in the tissue.

The continuously measured blood flow is reported as a percentage of that in the control period, i.e., the 10-min period before the HCl application. After the stabilization period, the loosely adherent mucus was removed, and blood flow was recorded for 30 min, after which the same protocol as for pH$_{m}$ measurements was used.

**SLC26A9 Expression**

Surface epithelial cells. Two-thirds of the stomach from H1p-infected and control mice was gently scraped with the blunted end of a scalpel to obtain epithelial cells. The removed material was snap frozen in liquid nitrogen. To confirm that only the uppermost part of the mucosa was removed, the remaining tissue was fixed and stained as described above. The remaining intact one-third of the stomach was used for confirmation of infection (also described above).

**mRNA expression (real-time PCR).** Total RNA was isolated with TRIzol (Invitrogen; Carlsbad, CA) from a surface epithelial cell preparation of five control and three H1p-infected animals. cDNA was synthesized using the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen) with random hexamers according to the protocol from the manufacturer. A Rotor-Gene 3000 (Corbette Research) and detection with SYBR green were used to amplify and analyze generated cDNA. The primers used were glucose-6-phosphate dehydrogenase (G6PDH; Genebank Accession No. BC075663), 5'-ATTGACCACTACCTGGCCAA-3' and 5'-CCCTGATGATCCCAAATTCA-3', and SLC26A9 (Genebank Accession No. AK086815), 5'-TCGTCTTCCCTAGGCCCTGCC-3' and 5'-CCAGTGTGGAGCCATTTCGA-3'.

PCR amplifications were performed in a total volume of 25.5 μl, containing 8 μl cDNA, 0.3 μl each primer, and 12.5 μl SYBR Green PCR Master Mix (Applied Biosystems; Warrington, UK). For each reaction, the polymerase was activated by a preincubation for 15 min at 95°C. Cycling parameters were 95°C for 20 s, 55°C for 30 s, and 72°C for 40 s. The results are presented as threshold cycle (C$_T$) values, i.e., the estimated amplification cycle number when the fluorescence exceeds a specified threshold value. All samples were run in duplicate. C$_T$ values were used to calculate the amount of PCR product compared with G6PDH as a “housekeeping gene” by subtracting the C$_T$ value for G6PDH from the C$_T$ value for SLC26A9 (ΔC$_T$).

**Semiquantitative immunoblot analysis.** A total cellular fraction containing plasma membrane and intracellular membrane vesicles was prepared from pooled gastric surface epithelial cell preparations from three control and three H1p-infected animals. Tissue samples were homogenized in ice-cold isolation solution (250 mM sucrose and 10 mM triethanolamine, pH 7.6) containing protease inhibitors (0.1 mg/ml phenylmethanesulfonyl fluoride and 1 μg/ml leupeptin) using a Polytron System PT1200 CL (Kinematica). The homogenate was centrifuged at low speed (1,000 g) for 10 min at 4°C to remove nuclei and cell debris. The supernatant was centrifuged at 150,000 g for 90 min at 4°C. The pellet containing plasma membrane and intracellular vesicles was suspended in isolation solution with protease inhibitors. Membrane fractions were solubilized at 65°C for 20 min in 5X Laemmli buffer (0.125 M Tris, 0.86 M glycerine, and 1% SDS), and the total protein concentration was measured with a DC protein assay (Bio-Rad; Hercules, CA). One hundred micrograms of total protein per well were loaded onto 10% polyacrylamide minigels (Ready Gel Precast Gels, Bio-Rad), size fractionated under denaturing conditions, and then transferred to a polyvinylidene difluoride membrane (Bio-Rad). The membrane was blocked with 8% milk proteins and then probed with affinity-purified anti-SLC26A9 immune serum. Goat anti-rabbit IgG antibody coupled to hors eradish peroxidase (Bio-Rad) was used as a secondary antibody. Proteins were visualized by enhanced chemiluminescence detection (ECL+; Amersham Biosciences). SLC26A9-positive bands were quantified with Quantity One software (Bio-Rad).

**Chemicals**

The chemicals used were as follows: heparin (Leo Pharma), Hypnorm (0.315 mg/ml fentanyl) citrate and 10 mg/ml fluanisone, Janssen Pharmaceutica; Beerse, Belgium), midazolam (5 mg/ml Dormicium, Roche; Stockholm, Sweden), silicone grease (Dow Corning high vacuum grease, Dow Corning; Weisbaden, Germany), HEPES (Merck; Darmstadt, Germany), tributylchrosoliane (Fluka), and bovine albumin (Sigma-Aldrich Chemie; Steinheim, Germany). DNA was purified and amplified using a DNeasy Tissue Kit and Master Mix

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No ulcers were detected, but small mucosal bleeding was observed in one H. pylori-infected stomach compared with uninfected controls. 

H. pylori-infected mice had a mean arterial blood pressure decreased significantly during the experiments in both groups (from 71 ± 5 to 63 ± 3 mmHg in controls and from 75 ± 5 to 67 ± 5 mmHg in infected mice), with no difference between the groups.

**RESULTS**

**Infection**

No H. pylori was found in the noninoculated mice, but these bacteria were observed in 90% of the H. pylori-inoculated mice. The mouse body weight-to-stomach wet weight ratio did not differ between the groups (control mice 135 ± 8, n = 8, and infected mice 130 ± 6, n = 12). The density of the bacteria was 3.8 ± 0.1 log cfu/stomach (n = 12). With light microscopy, mild to moderate inflammation was noted in the H. pylori-infected stomachs compared with uninfected controls. No ulcers were detected, but small mucosal bleeding was observed in one H. pylori-infected animal after acidic challenge. The gastric permeability (51Cr-EDTA clearance) was significantly higher in infected mice than in control mice (Fig. 1). We did not observe any gender differences in any of the parameters investigated.

**Mucus Measurements**

Results from the mucus measurements are presented in Fig. 2, A and B. The total mucus thickness (both loosely and firmly adherent) did not differ between the controls (n = 5) and infected (n = 6) animals. However, when the loosely adherent layer was removed by gentle suction, a significantly thinner firmly adherent layer was revealed in infected animals. Thirty minutes after the first mucus removal, the mucus thickness had increased significantly in control animals, and the mucus accumulation was calculated to be 9 ± 3 μm or 0.3 ± 0.1 μm/min. In infected animals, no significant mucus accumulation had occurred (3 ± 1 μm or 0.1 ± 0.04 μm/min). The mean arterial blood pressure decreased significantly during the experiments in both groups (from 71 ± 5 to 63 ± 3 mmHg in controls and from 75 ± 5 to 67 ± 5 mmHg in infected mice), with no difference between the groups.

**pH Measurements**

Figure 3 shows the mean values for pHjm in control (n = 7) and infected (n = 5) animals. pHjm was neutral during the control period in both groups. When HCl (pH 2 and 1.5) was applied luminally in the control animals, pHjm decreased significantly to 6.6 ± 0.4 (transiently) and 6.3 ± 0.6, respectively. In infected animals, pHjm decreased significantly to 4.7 ± 0.8 during topical administration of HCl (pH 2) and returned to the control level after removal of the acid. During luminal instillation of HCl (pH 1.5), pHjm decreased continually to 2.4 ± 0.7 and did not return to the control level until 10 min after removal of the acid. There was a significantly greater decline in pHjm in infected animals than in controls during luminal application of HCl at both pH 2 and 1.5. The mean arterial blood pressure decreased significantly during the experiments in both groups (from 67 ± 3 to 61 ± 3 mmHg in controls and from 61 ± 2 to 55 ± 2 mmHg in infected mice). Acid secretion was negligible in both groups.
with stomach. In this study, we have shown that a chronic infection of mice infected with a human strain (Hp1).

Blood Flow Measurements

Figure 4 shows the results for gastric mucosal blood flow, vascular resistance, and blood pressure in control (n = 6) and H. pylori-infected (n = 6) mice during luminal application of acid. In control mice, the blood flow increased significantly during luminal application of HCl (pH 2 and 1.5). This increase occurred despite a reduction in blood pressure. Accordingly, there was a decrease in gastric vascular resistance. In infected animals, neither gastric mucosal blood flow nor blood pressure changed during the experiment. The baseline blood flow value was 301 ± 92 perfusion units/g stomach in controls and 292 ± 43 perfusion units/g stomach in infected animals.

SLC26A9 Expression

The procedure of scraping the mucosa removed only the uppermost part of the mucosa, as revealed histologically (Fig. 5C). The expression of SLC26A9 mRNA in surface epithelial cells from H. pylori-infected mice increased significantly (by 1.9-fold) when measured by real-time PCR. The ΔC_T values are shown in Fig. 5A (control, n = 5, and infected, n = 3). The results of semiquantitative Western blot analysis of SLC26A9 showed the upregulation of the expected ~80-kDa band by approximately fourfold in H. pylori-infected animals (Fig. 5B; control, n = 3, and infected, n = 3).

DISCUSSION

The mucus-HCO_3^- layer is an important protective mechanism in the stomach, and disruption of this barrier might be the way in which H. pylori induces pathological changes in the stomach. In this study, we have shown that a chronic infection with H. pylori affects major mechanisms of gastric mucosal protection. In the presence of such infection, the mucus layer is thinner, the mucus pH at the surface of the epithelial cells is more acidic when the luminal pH is reduced, and blood flow regulation is altered.

In the present study, we used a special mouse model, α-1,3/4FT-expressing mice, infected with a human H. pylori strain (Hp1). α-1,3/4FT mice use human α-1,3/4FT to express Le^b epitopes in their pit region and surface mucus cells (14). Le^b is a blood group antigen that is recognized by an H. pylori adhesin, blood group antigen-binding adhesin (BabA) (19). Thus the presence of Le^b in the host and BabA in the bacteria mediates adherence to the gastric epithelial lining and implies a supposedly more severe infection. Indeed, the α-1,3/4FT mice developed more severe gastritis when infected than their nontransgenic littermates and also develop autoantibodies against parietal cells (14). Gastric permeability was also increased in the infected mice, in conformity with findings in H. pylori-infected patients (11) and animals (34).

A continuous mucus layer covers the mucosa of the stomach. We have previously shown in a rat model (3) and in this mouse model (16) that the mucus layer can be divided into two layers: one that can be removed (designated loosely adherent) and one that cannot be removed by mechanical means without destroying the epithelial layer (the firmly adherent layer). From another study, we (28) have also concluded that it is the firmly adherent layer that is important for the protection against luminal acid, because it is in this layer that the pH gradient is formed. In our unique mouse model in vivo, we can measure the thickness of both the loosely and firmly adherent mucus layers and also follow the mucus accumulation over time. Using this model, we observed here that, when the loosely adherent mucus was removed, the firmly adherent layer in infected mice was significantly thinner than in noninfected controls, but was still a continuous layer. Furthermore, no mucus accumulation was detected during the 30-min measuring period. Clearly, mucus does accumulate also in infected animals, because we found the same total mucus thickness in noninfected mice and infected mice. We have previously shown in a rat model (3) and in this mouse model (16) that the mucus layer can be divided into two layers: one that can be removed (designated loosely adherent) and one that cannot be removed by mechanical means without destroying the epithelial layer (the firmly adherent layer). From another study, we (28) have also concluded that it is the firmly adherent layer that is important for the protection against luminal acid, because it is in this layer that the pH gradient is formed. In our unique mouse model in vivo, we can measure the thickness of both the loosely and firmly adherent mucus layers and also follow the mucus accumulation over time. Using this model, we observed here that, when the loosely adherent mucus was removed, the firmly adherent layer in infected mice was significantly thinner than in noninfected controls, but was still a continuous layer. Furthermore, no mucus accumulation was detected during the 30-min measuring period. Clearly, mucus does accumulate also in infected animals, because we found the same total mucus thickness in noninfected mice and infected mice.
both infected and control animals before the loosely adherent mucus was removed the first time. The accumulation time might, however, be longer in the infected situation.

Mucus accumulation is a result of synthesis, secretion, degradation, and the quality of the mucus. Earlier in vitro and histology studies have shown that H. pylori might reduce the mucus gel thickness in different ways, for example, mucin synthesis might be inhibited (4), MUC secretion reduced (36), or mucins degraded (24). Furthermore, the expression patterns across epithelial cells or the expression of HCO$_3^-$ transporters. In this study, however, we observed increased mRNA expression and protein abundance of an important Cl$^-$/HCO$_3^-$ exchanger, SLC26A9, in gastric surface epithelial cells in H. pylori-infected mice. On the basis of this observation alone, an obvious conclusion would have been that HCO$_3^-$ transport would increase. However, because the in vivo measurements showed reduced pH at the surface of epithelial cells, an inhibition of HCO$_3^-$ transport most probably occurred. Interestingly, we have shown in an in vitro study (39) that SLC26A9 is inhibited by the H. pylori product NH$_4^+$. In the same study, we also found that the ability to maintain neutral pH$_{jm}$ was reduced in rats treated with NH$_4^+$. Because the effect of NH$_4^+$ was immediate and thus independent of gene transcription, we concluded that its inhibitory effect on pH$_{jm}$ was nongenomic. Thus increased expression of SLC26A9 in gastric mucous cells in H. pylori-infected mice is likely a compensatory response to overcome the chronic inhibition of SLC26A9-mediated HCO$_3^-$ secretion by H. pylori.

In this study, acid-induced hyperemia is absent in H. pylori-infected mice. Hence, another important defense mechanism is altered. This finding is somewhat surprising in light of the higher permeability measured in H. pylori-infected mice compared with control mice, which would predict an increase in the hyperemic response. One possibility for the blood flow not to respond to acid would be if the resistance vessels were already maximally dilated. This is, however, not probable because the blood flow levels in control and infected mice were similar. It is also supported by results from studies (9, 33, 38) in which blood flow was either not altered or even reduced in chronically H. pylori-infected mice or humans. Furthermore, in pilot studies in infected animals, we measured an increase in blood flow when the mucosa was challenged with a stronger acid (pH 0.8).

In a recent study, we (29) showed that the hyperemia induced by luminal acid was absent or substantially reduced in iNOS knockout mice. Our results also suggested that iNOS was constitutively expressed in surface epithelial cells. This epithelial NO production might be inhibited by the bacteria residing in the vicinity of these cells. Several suggestions of how H. pylori can reduce the formation of NO have been proposed, including the production of an l-arginine analog,
asymmetric dimethyl arginine (12). An arginase produced by the bacteria has also been suggested as a strategy to reduce NO production, because it will consume the substrate for NOS (13). Because the hyperemia seen on challenge with luminal acid in control mice was abolished in infected mice in our experiments, we may speculate that the bacterium might counteract the acid-induced formation of NO by epithelial iNOS and thereby inhibit the signal for hyperemia. However, a hyperemic response was seen on a higher concentration of acid in mice infected with \textit{H. pylori} (as described above), which might be due to direct submucosal acidification and vasodilation.

In conclusion, we have found in this study that a chronic \textit{H. pylori} infection alters the ability of the mucosa to maintain a neutral pH at the epithelial cell surface. This could be due to the thinner inner, firmly adherent mucus gel layer, an increased hydrogen permeability through the mucus, and/or defective HCO$_3^-$ transport across the epithelium, e.g., through inhibition of the Cl$^-$/HCO$_3^-$ exchanger SLC26A9. The latter is most probably involved because we found increased expression of SLC26A9 mRNA and protein in the superficial mucosa of infected mice. This would have resulted in increased HCO$_3^-$ transport unless the transport was inhibited. In addition, because the hyperemia normally seen in response to luminal HCl was abolished in infected animals, a disturbed signal to the resistance vessels is also suggested. Thus it is apparent that infection with \textit{H. pylori} negatively influences several of the important defence mechanisms in the gastric barrier. It is very likely that the weakened barrier is less resistant to harmful agents, but the exact mechanisms leading to the development of pathological changes by \textit{H. pylori} remain to be further investigated.

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