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Animal Model

Rapid Development of Severe Hyperplastic Gastritis with Gastric Epithelial Dedifferentiation in *Helicobacter felis*-Infected IL-10$^{-/-}$ Mice

Daniel J. Berg,* Nancy A. Lynch,† Richard G. Lynch,† and Dina M. Lauricella*

From the Departments of Internal Medicine and Pathology,† University of Iowa College of Medicine, Iowa City, Iowa*

Interleukin (IL)-10 is a potent anti-inflammatory and immune-regulatory cytokine. Mice deficient in IL-10 production (IL-10$^{-/-}$) develop a spontaneous inflammatory bowel disease, indicating that IL-10 is an important regulator of the mucosal immune response *in vivo*. To study the role of IL-10 in the host response to gastric *Helicobacter* infection, stomachs of IL-10$^{-/-}$ and wild-type mice were colonized with *Helicobacter felis*, as a model of human *H. pylori* infection. Within 4 weeks of *H. felis* infection, wild-type mice develop a mild, focal chronic gastritis. In contrast, *H. felis*-infected IL-10$^{-/-}$ mice develop a severe hyperplastic gastritis, characterized by a dense, predominantly mononuclear cell inflammation of the mucosa and submucosa and epithelial cell proliferation and dedifferentiation. Within 4 weeks of *H. felis* infection, there are striking alterations in the character of the gastric epithelium from IL-10$^{-/-}$ mice, including a profound loss of parietal and chief cells, focal *de novo* production of acidic mucins, and marked epithelial proliferation with disordered epithelial architecture. These findings indicate that, in the absence of IL-10, the inflammatory and immunological responses of the murine host to gastric colonization with *Helicobacter* is a rapidly evolving pathological process with features that mimic those associated with *H. pylori* infection in humans. *H. felis*-infected IL-10$^{-/-}$ mice may provide a model with which to investigate the cellular and molecular changes that stem from gastric infection with *H. pylori*. (Am J Pathol 1998, 152:1377–1386)

Recent epidemiological studies have demonstrated an association between *Helicobacter pylori* infection and the risk of gastric cancer. Increased prevalence of *H. pylori* infection has been strongly correlated with increased incidence of gastric cancer.[1,2] In addition, three independent prospective studies have demonstrated that *H. pylori* infection markedly increases the risk for gastric carcinoma.[3–5] A review of epidemiological and pathological studies has led the International Agency for Research on Cancer (IARC) to declare *H. pylori* a group I carcinogen, a definite cause of human cancer.[6]

To understand the mechanism(s) by which *H. pylori* infection leads to intestinal metaplasia and gastric cancer, it is necessary to use animal models. *H. pylori* has a limited host specificity, and it has been difficult to reproducibly infect laboratory animals with this bacterium[7] (an exception may be the recently described Sydney strain of *H. pylori*[8]). For this reason, laboratory research has frequently utilized non-pylori *Helicobacter* that can infect the stomachs of laboratory animals. A well characterized experimental model of gastric *Helicobacter* infection is *H. felis* infection of mice[9] (Reviewed in Ref. 10). Based on 16 S ribosomal RNA sequence analysis, *H. felis* is genetically quite similar to *H. pylori*,[11] and this organism has been demonstrated to efficiently colonize rodent gastric mucosa.[9,12,13] Mice chronically infected with *H. felis* develop a gastritis that simulates some of the features of human *H. pylori* infection.[12] The *H. felis* model has been useful for study of the immune response to *Helicobacter*[14,15] and for the development of *Helicobacter* vaccines.[16] However, a disadvantage of this animal model is that epithelial changes (if they occur at all) may take up to 1 to 2 years to develop,[17] severely limiting the usefulness of this model for the study of the cellular and molecular changes in gastric epithelium induced by *Helicobacter* infection.

Although the causative role of *H. pylori* in the development of gastric cancer is well accepted, the mechanism(s) by which *H. pylori* infection induces the develop-

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Address reprint requests to Dr. Daniel J. Berg, University of Iowa Hospitals, Department of Internal Medicine, C32-GH, 200 Hawkins Drive, Iowa City, Iowa 52242. E-mail: daniel-j-berg@uiowa.edu.

ment of gastric cancer is not understood. *H. pylori* infection induces chronic inflammation, which may promote carcinogenesis by enhancing gastric epithelial proliferation and increasing genotoxic free radical production.[18] Gastric biopsies from *H. pylori*-infected individuals clearly demonstrate inflammatory cell infiltration as well as increased expression of pro-inflammatory cytokines.[19,20] *H. pylori* infection, however, does not result in unrestrained gastric inflammation, and most *H. pylori* infections are asymptomatic.[21] These observations suggest that *H. pylori*-induced inflammation is a carefully regulated process that, in most cases, prevents deleterious consequences to both host and bacteria.[18] The severity of inflammation induced by *H. pylori* infection is in part linked to the production of bacterial toxins,[22,23] although it is likely that host factors also regulate the inflammatory response to *H. pylori*.

Interleukin (IL)-10, a potent anti-inflammatory and immune-regulatory cytokine, is produced during *H. pylori* infection[19,20] and may down-regulate the host response to gastric *Helicobacter* infection. IL-10 is a potent macrophage deactivator *in vitro*, inhibiting the synthesis of pro-inflammatory cytokines such as IL-1α, tumor necrosis factor-α, IL-6, and IL-8.[24] Furthermore, IL-10 can inhibit, directly or indirectly, the synthesis of cytokines by Th1 T cells.[25] Studies of mice with a targeted disruption of the IL-10 gene (IL-10$^{-/-}$) have allowed further definition of the actions of IL-10 in complex *in vivo* systems.[26,27] In the absence of IL-10, exaggerated immune responses to Th1 T-cell-dependent or macrophage-dependent antigens develop.[27] Significantly, we recently reported that IL-10$^{-/-}$ mice develop an inflammatory bowel disease that is secondary to a dysregulated immune and inflammatory response to normal enteric flora.[28] These studies indicate that IL-10 is an important regulator of the mucosal immune response and suggest that IL-10 may regulate the host response to *Helicobacter*, an important gastric pathogen.

To investigate this, we established gastric infection with *Helicobacter* in IL-10$^{-/-}$ and wild-type mice and examined the gastric immune and inflammatory responses and the alterations induced in the gastric epithelium. We observed that, by 4 weeks, *H. felis*-infected IL-10$^{-/-}$ mice, but not wild-type mice, developed a severe gastritis characterized by a dense mononuclear cell inflammation of the mucosa and submucosa and a striking proliferation of the gastric epithelium with epithelial dedifferentiation.

Materials and Methods

Animals

Healthy 5- to 6-week-old IL-10$^{-/-}$ mice on a 129/SvEv background were used for this study.[28] Wild-type 129/SvEv mice were obtained from Taconic Farms, (Germantown, NY). Mice were maintained in micro-isolator cages in the animal care facility at the University of Iowa.

Bacteria

H. felis (ATCC 49179) was obtained from American Type Culture Collection (Rockville, MD). Bacteria were grown in a biphasic system with the solid phase consisting of tryptic soy agar amended with 7.5% sheep blood and a liquid phase consisting of tryptic soy broth containing 6% bovine calf serum and amended with trimethoprim (5.0 mg/L), polymixin B (2500 U/L), vancomycin (10.0 mg/L), and amphotericin B (5.0 mg/L; all from Sigma Chemical Co., St. Louis, MO). Cultures were incubated at 37°C under a microaerophilic atmosphere created by Campypak microaerophilic gas generators (Fisher Scientific, Pittsburgh, PA). Bacteria were harvested by centrifugation and washed four times in phosphate-buffered saline (PBS) before inoculation into mice. Bacteria were positively identified on the basis of morphology and presence of urease enzyme activity and via polymerase chain reaction amplification of the 16 S RNA gene using *Helicobacter*-specific primers.[29]

Infection with H. felis

H. felis (5×10^8 bacteria in 100 μl of PBS) was instilled by gavage using a 23-gauge feeding needle (Popper and Sons, New Hyde Park, NY). Mice received three inoculations over a period of 5 days. Four weeks after infection, mice were euthanized and tissues harvested for analysis. The presence of *H. felis* infection was verified on histological sections stained using a modified Steiner method (Sigma). Control mice (eight wild type and eight IL-10$^{-/-}$) were treated with identical inoculants of boiled *H. felis*. Twenty-four wild-type mice and twenty-eight IL-10$^{-/-}$ mice were inoculated with *H. felis* for these studies.

Histological Analysis

Tissues from wild-type and IL-10$^{-/-}$ mice were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for light microscopic examinations. The tissue examined consisted of the greater curvature of the stomach, from the gastroesophageal junction to the duodenum. Periodic acid-Schiff (PAS) staining was performed to identify neutral mucins. Acidic mucosubstances were identified using mucicarmine and alcian blue staining (pH 2.5) using standard techniques.[30]

Gastric pH

The pH of gastric contents was determined using pH test papers (Fisher Scientific).

Analysis of Epithelial Cell Proliferation

Two methods, immunohistochemical detection of proliferating cell nuclear antigen (PCNA) and incorporation of bromodeoxyuridine (BrdU) into newly synthesized DNA, were used to assess gastric epithelial cell proliferation in wild-type and IL-10$^{-/-}$ mice 4 weeks after *H. felis* infec-

tion.[31–33] PCNA and BrdU immunohistochemistry was performed using standard immunohistochemical techniques. Biotin-conjugated anti-PCNA was obtained from Caltag (South San Francisco, CA). BrdU and anti-BrdU were obtained from Zymed Laboratories (South San Francisco, CA). The labeling index was determined by calculating the ratio of positive cells to the total number of cells. To quantify the number of positive cells, a minimum of 10 well oriented crypts from the body and the pylorus of each specimen were counted. A cell was scored as positive for PCNA (or BrdU) when the nucleus was stained distinctly brown. For cell kinetic experiments, the glands were divided into three compartments of equal size. The total labeling index and the labeling index per compartment was compared between the study groups using the two-tailed Student's *t*-test for unpaired data.

Results

Characteristics of Inflammation in H. felis*-Infected Wild-Type and IL-10$^{-/-}$ Mice*

To assess the role of endogenous IL-10 in the regulation of the immune/inflammatory response to gastric *Helicobacter* infection, wild-type and IL-10$^{-/-}$ mice were infected by gavage with *H. felis*. As expected, normal uninfected wild-type stomach (Figure 1, A–C) and normal uninfected IL-10$^{-/-}$ stomach (Figure 1F) had minimal infiltration with mononuclear or inflammatory cells. By 4 weeks after infection, a mild to moderate inflammatory response had developed in the mucosa of wild-type mice at the junction of the squamous and glandular epithelium (Figure 1D) as well as in the pylorus (Figure 1E). The infiltrate was primarily mononuclear although significant numbers of neutrophils were present as well as small numbers of eosinophils. No infiltrates were seen in the body glands of *H. felis*-infected wild-type mice. The architecture and cytological character of the gastric epithelium remained normal in wild-type mice infected with *H. felis*.

In contrast, IL-10$^{-/-}$ mice infected with *H. felis* developed severe gastritis. After 4 weeks of infection, the mucosa of IL-10$^{-/-}$ mice was noted to be thickened on gross inspection. Microscopic examination revealed marked changes in the mucosa of *Helicobacter*-infected IL-10$^{-/-}$ mice. Severe inflammatory infiltrates were seen in the pylorus (Figure 1G) and body glands of the infected IL-10$^{-/-}$ mice (Figure 1, H–K). The infiltrate involved the mucosa and lamina propria and extended to the tops of the glandular mucosa. Eight wild-type and eight IL-10$^{-/-}$ mice were evaluated 1 to 2 weeks after *H. felis* infection. Initially, the infection and inflammation were located in the lamina propria of the antrum in both sets of mice (not pictured); however, 4 weeks after *H. felis* infection, inflammation was found diffusely throughout the gastric mucosa in IL-10$^{-/-}$ mice, and the cellular infiltrate extended from the junction of the squamous and glandular epithelium to the duodenum. The infiltrates consisted primarily of large numbers of mononuclear cells but also included neutrophils and eosinophils. Ulcerations and crypt abscesses were present, but uncommon. Mononuclear cells were conspicuous in the lymphatic vessels in the wall of the stomach.

The inflammatory response in wild-type and IL-10$^{-/-}$ mice was dependent upon development of a productive *H. felis* infection. Bacteria with the characteristic long spiral shape could be seen in wild-type and IL-10$^{-/-}$ mice. Silver stains of tissue sections confirmed the presence of *H. felis* in the stomach (Figure 1L). Wild-type and IL-10$^{-/-}$ mice treated with boiled preparations of *H. felis* did not develop inflammation.

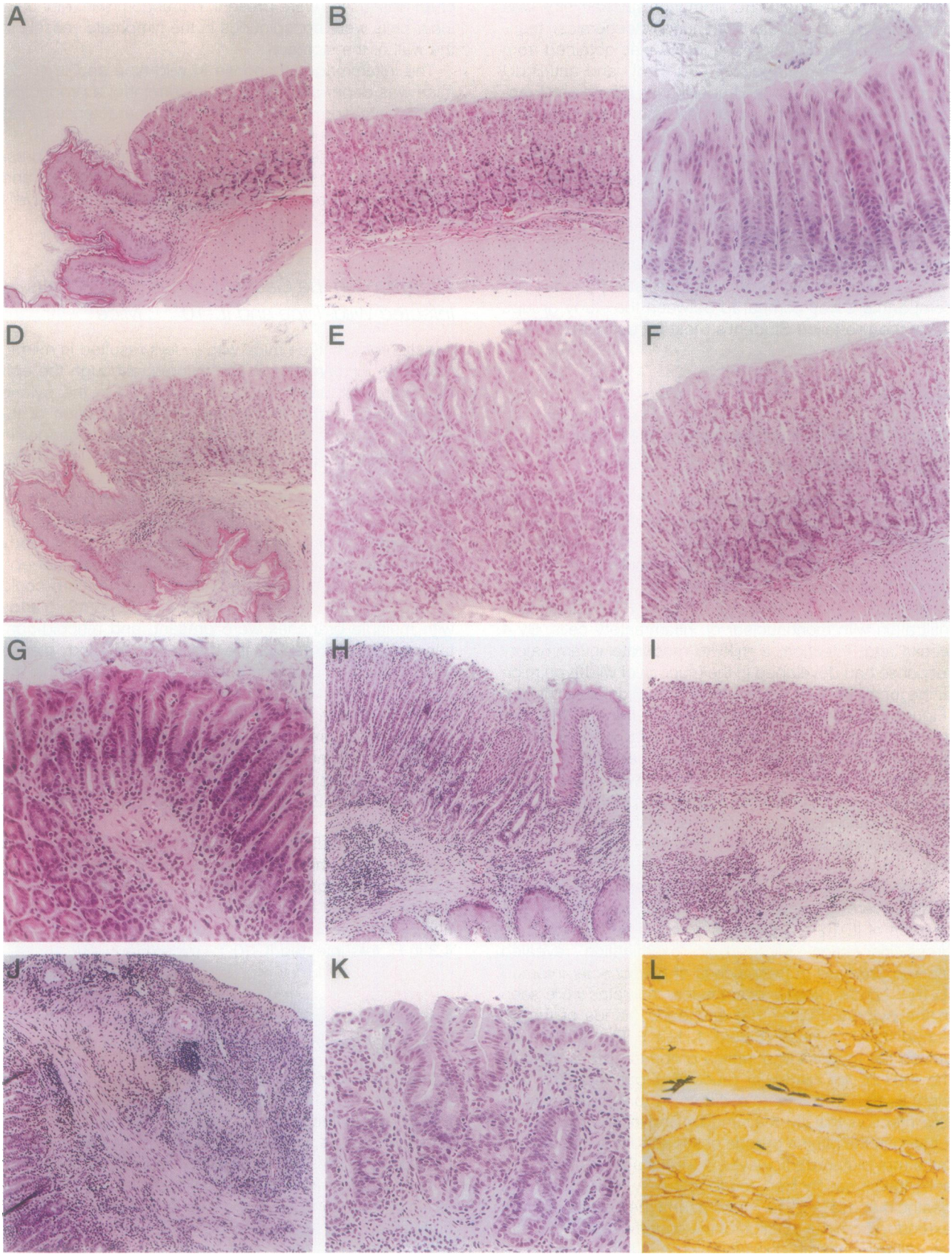
Alterations in the Gastric Epithelium in H. felis*-Infected Wild-Type and IL-10$^{-/-}$ Mice*

Infection of wild-type mice with *H. felis* resulted in mild to moderate inflammation in the pylorus, although the epithelial architecture in both the body and in the pyloric region was normal. In contrast, *H. felis* infection of IL-10$^{-/-}$ mice led to the development of marked changes in the epithelial phenotype. Within 4 weeks of *Helicobacter* infection, IL-10$^{-/-}$ mice had a marked loss of normal differentiation of the gastric epithelium (compare Figure 1F with Figure 1, H, I, and K). There was an increase in the lengths of the gastric pits and glands. The body glands became lined by a layer of simple columnar epithelium, and no discernible differentiation pattern could be seen (Figure 1, H and K; Figure 2A). Abnormal glands with multiple branches were present, and mucoid-containing cells were seen at the bases of some body glands (Figure 2A). A microglandular structure located within the mucosa of an *H. felis*-infected IL-10$^{-/-}$ mouse (Figure 2B) further illustrates the presence of abnormal gastric mucosal architecture. Gastric glands lined with epithelium exhibiting cellular crowding, nuclear pleomorphism, increased nuclear:cytoplasmic ratio, and loss of nuclear polarity (Figure 2C) were also noted in *H. felis*-infected IL-10$^{-/-}$ mice.

A striking finding was the consistent loss of gastric parietal cells, which in some specimens was complete. As a result, the gastric pH increased from an average of approximately 1.8 (control wild-type, control IL-10$^{-/-}$, and *H. felis*-infected wild-type mice) to an average pH of 4.3 in *H. felis*-infected IL-10$^{-/-}$ mice (Figure 3).

Evaluation of Epithelial Cell Proliferation in H. felis*-Infected Wild-Type and IL-10$^{-/-}$ Mice*

Both by gross and microscopic examination it was clear that thickening of the gastric mucosa was present in the *Helicobacter*-infected IL-10$^{-/-}$ mice. To develop more quantitative data, we directly measured gastric epithelial cell proliferation. The nucleoside analogue BrdU (which is incorporated into DNA in cells actively synthesizing DNA) was administered 2 hours before the mice were euthanized, and tissue sections were examined for BrdU-positive cells by immunohistochemistry. The zone of proliferation in normal gastric body glands is located in the base of the gastric pit, which represents the neck region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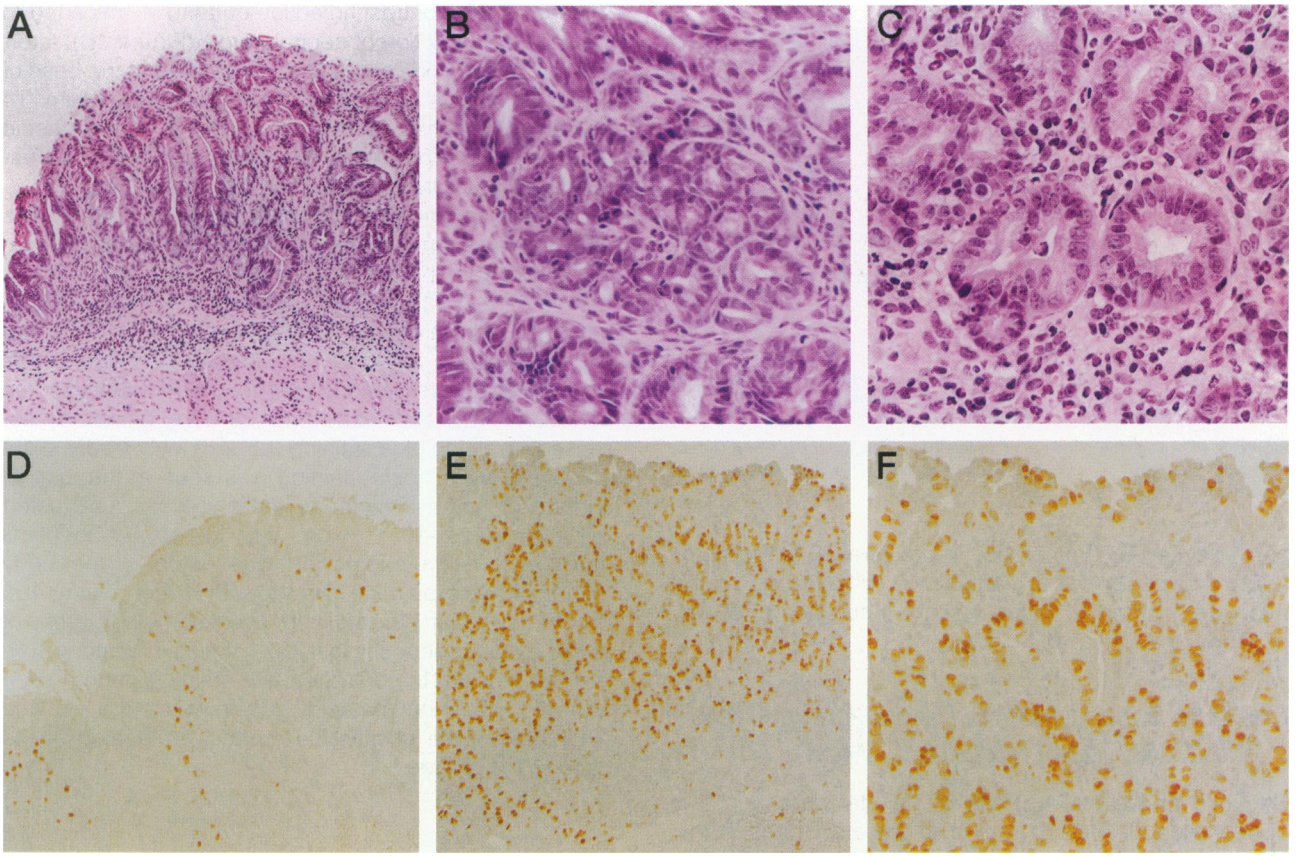

Figure 2. Histopathological analysis of gastric epithelium in *H. felis*-infected wild-type and IL-10$^{-/-}$ mice. The evaluation was performed 4 weeks after inoculation with *H. felis*. **A:** Aberrant epithelium in body glands from an IL-10$^{-/-}$ mouse infected with *H. felis*. Note the lack of cellular differentiation. The glands are tortuous and branched. Magnification, ×25. **B:** A microglandular structure within the body mucosa of *H. felis*-infected IL-10$^{-/-}$ mouse. ×80. **C:** Cross section of gastric body glands in an *H. felis*-infected IL-10$^{-/-}$ mouse. Note the cellular crowding and abnormal glandular structure. ×100. **D:** BrdU staining of wild-type stomach 4 weeks after *H. felis* infection. ×25. The stained cells in the mid-portion of the gland indicate the presence of proliferating cells. The staining pattern is identical to that seen in control wild-type and control IL-10$^{-/-}$ mice (not pictured). **E:** BrdU staining of IL-10$^{-/-}$ stomach 4 weeks after *H. felis* infection. ×25. Note the large number of positively stained (actively proliferating) cells. **F:** BrdU staining of IL-10$^{-/-}$ stomach 4 weeks after *H. felis* infection at a higher magnification. ×50. In some glands, the zone of cellular proliferation extends to the luminal surface.

of the gastric gland. In uninfected wild-type and IL-10$^{-/-}$ mice, BrdU labeling was confined to a small zone in the neck region of the gastric gland, demonstrating normal levels of proliferation (not pictured). Four weeks after infection, wild-type mice appeared to have essentially normal epithelial proliferation in the gastric glands (Figure 2D). In contrast, infected IL-10$^{-/-}$ mice had a marked increase in proliferation in the progenerative zone (Figure 2E). Cells actively synthesizing DNA were found in greater numbers in the neck region of the gland, frequently with extension of the proliferative zone to the surface epithelium lumen (Figure 2F). In some instances, proliferating cells extended to the base of the gland. PCNA staining confirmed the increase in the number of proliferating cells in *H. felis*-infected IL-10$^{-/-}$ mice and the extension of proliferative zone into the upper and lower portions of the body glands. PCNA staining revealed normal appearance of epithelial cell proliferation in wild-type mice 4 weeks after infection with *H. felis*.

The number and percentage of proliferating cells in gastric glands was quantified in wild-type and IL-10$^{-/-}$ mice 4 weeks after *H. felis* infection. As can be seen in Table 1, *H. felis*-infected IL-10$^{-/-}$ mice have a large overall increase in the labeling index. In addition, the proliferative zone is extended in *H. felis*-infected IL-10$^{-/-}$ mice

Figure 1. Histopathological analysis of gastric changes in *H. felis*-infected wild-type and IL-10$^{-/-}$ mice. Histological evaluation was performed 4 weeks after inoculation with *H. felis*. **A:** Junction of squamous and glandular epithelium from an 8-week-old uninfected wild-type mouse. **B:** Gastric body glands from an 8-week-old uninfected wild-type mouse. Note the lack of a cellular infiltrate between the glands. **C:** Pyloric glands from an 8-week-old uninfected wild-type mouse. **D:** Junction of squamous and glandular epithelium from a wild-type mouse, 4 weeks after infection with *H. felis*. Note the mild cellular infiltrate. **E:** Pyloric glands from an *H. felis*-infected wild-type mouse. Note the mild cellular infiltrate between the glands. **F:** Body glands from an 8-week-old uninfected IL-10$^{-/-}$ mouse. Epithelium demonstrates normal differentiation. Note the absence of a cellular infiltrate between the glands. **G:** Pyloric glands from an *H. felis*-infected IL-10$^{-/-}$ mouse. Note the increased cellular infiltrate. **H:** Junction of squamous and glandular epithelium from an *H. felis*-infected IL-10$^{-/-}$ mouse. There is an accumulation of inflammatory cells in the lamina propria between glands as well as in the submucosa. Note the loss of specialized gastric epithelial cells. **I:** Inflammation in an *H. felis*-infected IL-10$^{-/-}$ mouse involving the mucosa and submucosa. **J:** Severe inflammation in an *H. felis*-infected IL-10$^{-/-}$ mouse. Note the lymphoid aggregate. **K:** Mucosal inflammation in an *H. felis*-infected IL-10$^{-/-}$ mouse. Note the loss of normal epithelial differentiation and the presence of abnormal, branched glands. **A:** to **K:** H&E; magnification, ×25. **L:** Modified Steiner stain demonstrating the presence of *H. felis* within the lumen of a gastric gland in an infected IL-10$^{-/-}$ mouse. Magnification, ×164.

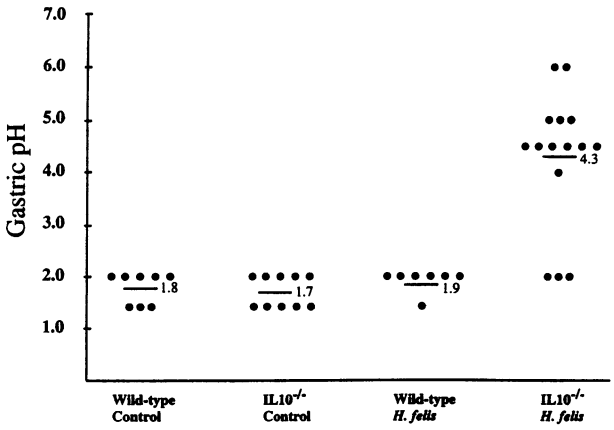

Figure 3. pH of gastric contents of individual control and *H. felis*-infected wild-type and IL-$10^{-/-}$ mice. The average gastric pH is indicated for each experimental group.

as evidenced by an increase in proliferation at the apex (LI-1) and base of the gland (LI-3). Infected wild-type mice were noted to have a slight increase in cellular proliferation of the body glands only.

Characterization of Mucin Production in H. felis*-Infected Wild-Type and IL-$10^{-/-}$ Mice*

Because of the striking loss of parietal cells and the appearance of intestinal cell cytological features in the H&E sections of gastric epithelium from *Helicobacter*-infected IL-$10^{-/-}$ mice, we conducted additional studies to assess the differentiation state of the gastric epithelium. Mucin production by gastric epithelial cells was evaluated in histological sections from uninfected and infected wild-type and IL-$10^{-/-}$ mice. Production of neutral mucin was evaluated with PAS staining and that of acidic mucin through mucicarmine staining and alcian blue staining. In the body glands of uninfected wild-type and IL-$10^{-/-}$ mice, PAS staining was prominent in the surface epithelium (not pictured). This pattern of PAS staining was not altered in *H. felis*-infected wild-type mice (Figure 4A). In contrast, infection of IL-$10^{-/-}$ mice led to increased production of neutral mucin (Figure 4B). Interestingly, the mucin production was located at the base of the body glands rather than the surface epithelium. To further assess the state of differentiation of the gastric epithelium, the production of acidic mucins was assessed. Body glands of control wild-type and IL-$10^{-/-}$ mice were negative for the production of acidic mucins as assessed by mucicarmine staining (not pictured). Similarly, mucicarmine staining for acidic mucin was negative in *H. felis*-infected wild-type mice (Figure 4C). In contrast, focal areas of *H. felis*-infected IL-$10^{-/-}$ mice expressed large amounts of acidic mucin (Figure 4D). Unlike human gastric body glands, which express some acidic mucin in the neck region of body glands as assessed by alcian blue staining (pH 2.5), only a very slight amount of alcian blue staining was seen at the luminal surface of some foveolar epithelial cells in uninfected wild-type and IL-$10^{-/-}$ mice (not pictured). An identical pattern of mucin expression was noted in the body glands of wild-type mice infected with *H. felis* (Figure 4E). In contrast, alcian blue staining revealed multiple areas of acidic mucin production in IL-$10^{-/-}$ mice infected with *H. felis* (Figure 4F). In contrast to the staining pattern seen with wild-type mice, the acidic mucin was found to fill the cytoplasm of the positive cells and was located at the base of the glands.

Discussion

In this study we have used IL-$10^{-/-}$ and wild-type mice to determine whether endogenous IL-10 regulates the immune and inflammatory responses elicited *in vivo* by *H. felis* infection, a model of human *H. pylori* infection. We found that wild-type mice infected with *H. felis* develop, within 4 weeks, a mild gastritis characterized by a predominantly mononuclear cell inflammatory infiltrate. In contrast, *H. felis*-infected IL-$10^{-/-}$ mice develop severe gastric inflammation. A major finding was that, within 4 weeks of *H. felis* infection, IL-$10^{-/-}$ mice had developed

Table 1. BrdU Labeling Indices of Control and *H. felis*-Infected Wild-Type and IL-$10^{-/-}$ Mice

	Total cells counted per mouse	LI% (SD)			
		Total	1 (apex)	2	3 (base)
Wild-type control					
Body	790 (108)	1.43 (0.39)	0.43 (0.41)	4.11 (1.60)	0.12 (0.20)
Pyloric region	498 (79)	11.98 (2.15)	0.15 (0.30)	2.65 (1.95)	28.75 (5.70)
IL-$10^{-/-}$ control					
Body	666 (78)	1.79 (0.35)	0	5.24 (0.71)	0.08 (0.17)
Pyloric region	448 (34)	13.06 (0.96)	0	3.25 (2.66)	30.28 (3.32)
Wild-type *H. felis*-infected					
Body	773 (136)	4.17 (1.41)	1.67 (1.64)	11.28* (4.66)	0.37 (0.76)
Pyloric region	665 (286)	11.84 (4.3)	0.85 (1.70)	5.74 (6.10)	25.89 (10.2)
IL-$10^{-/-}$ infected					
Body	865 (240)	27.35 (5.54)	21.0*† (16.0)	40.32‡§ (8.30)	20.70*† (14.0)
Pyloric region	714 (253)	31.97 (10.91)	15.74*† (14.27)	45.62‡§ (18.0)	31.88 (10.54)

Results are expressed as the mean, with SDs in parentheses. LI%, labeling index.
*$P < 0.05$, as compared with uninfected control.
†$P < 0.05$, as compared with wild-type, infected.
‡$P < 0.005$, as compared with uninfected control.
§$P < 0.005$, as compared with wild-type, infected.

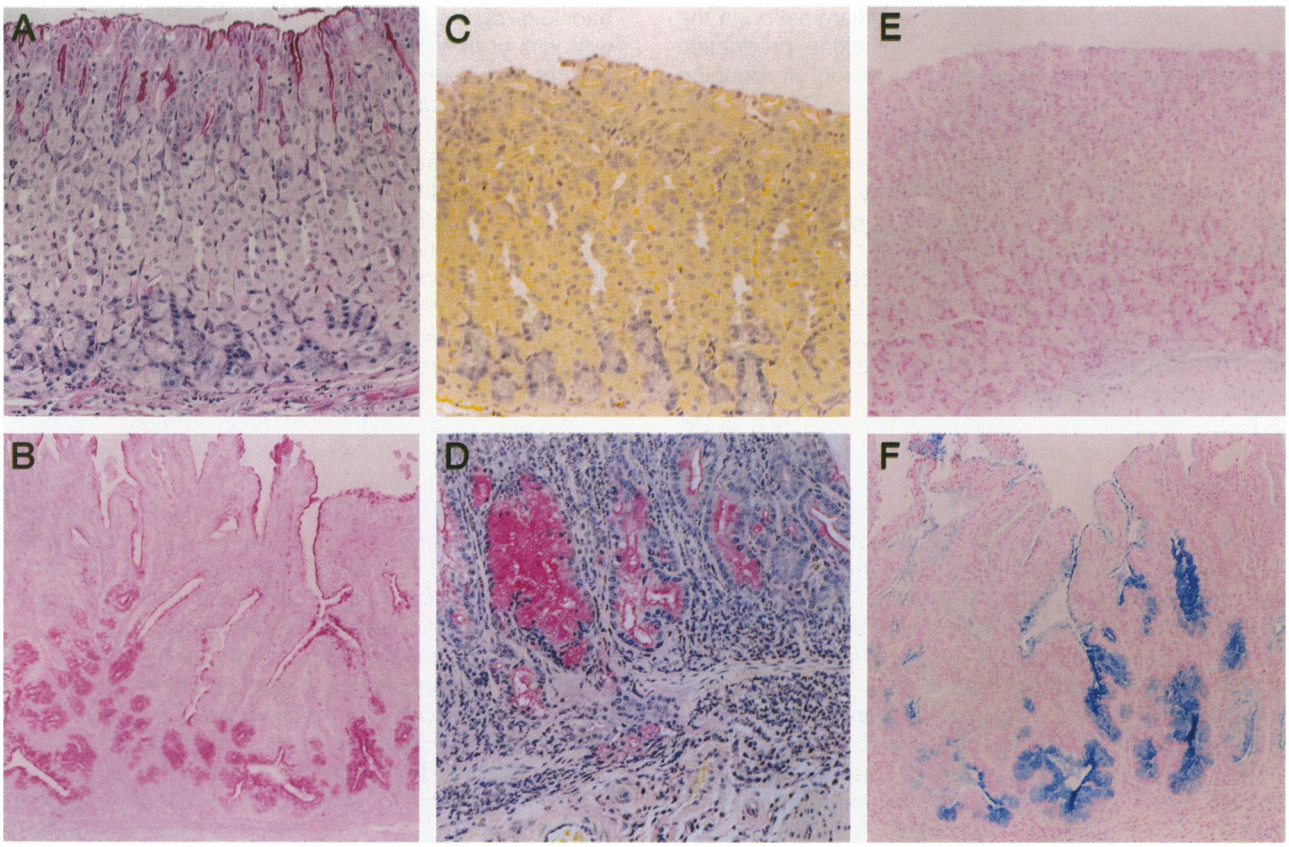

Figure 4. Histochemical analysis of gastric mucin production in *H. felis*-infected wild-type and IL-10$^{-/-}$ mice. Mucin production was evaluated 4 weeks after inoculation with *H. felis*. **A:** PAS staining of body glands of an *H. felis*-infected wild-type mouse. Note positive staining of luminal epithelial cells only; no staining was noted in the base of gland. The staining pattern is identical to that of uninfected wild-type and IL-10$^{-/-}$ mice (not pictured). **B:** PAS staining of body glands of an *H. felis*-infected IL-10$^{-/-}$ mouse. Overall decreased PAS staining was noted in the surface epithelium; however, increased PAS staining, indicating the presence of neutral mucins, was located in the base of the glands. **C:** Mucicarmine staining of body glands of an *H. felis*-infected wild-type mouse. No staining is noted, indicating that acidic mucin is not produced by normal gastric body glands. The staining pattern is identical to that of uninfected wild-type and IL-10$^{-/-}$ mice (not pictured). **D:** Mucicarmine staining of body glands of an *H. felis*-infected IL-10$^{-/-}$ mouse. Increased staining is noted in the base of the glands, indicating the production of acidic mucin. **E:** Alcian blue (pH 2.5) staining of an *H. felis*-infected wild-type mouse. Rare foveolar epithelial cells show slight staining on the luminal edge. Stromal cells within the lamina propria are also lightly stained. The staining pattern is identical to that of uninfected wild-type and IL-10$^{-/-}$ mice (not pictured). **F:** Alcian blue (pH 2.5) staining of an *H. felis* infected IL-10$^{-/-}$ mouse stomach. Large numbers of positively stained cells are located at the base of the glands, indicating an increased production of acidic mucin in the body of the stomach. In contrast to the staining pattern seen with wild-type mice, the acidic mucin was found to fill the cytoplasm of the positive cells and was located at the base of the glands. Magnification, ×50.

alterations in the cytology and architecture of the gastric epithelium that mimic those associated with chronic *H. pylori* infection in humans.

A major aim of this study was to investigate the role of endogenous IL-10 in the immune and inflammatory responses to gastric *Helicobacter* infection. Our finding that *H. felis*-infected IL-10-deficient, but not wild-type, mice develop a severe, dense mononuclear cell infiltrate strongly indicates that IL-10 is a key regulator of the host's response to gastric *Helicobacter* infection.

An important component of the inflammatory response to gastric *Helicobacter* infection is the host's response to *Helicobacter*-derived lipopolysaccharide (LPS). LPS derived from *H. pylori* differs from other bacterial LPSs in both structure[34] and biological activity.[35–37] *H. pylori* LPS is less mitogenic and induces lower cytokine levels *in vitro*[38,39] and has less pyrogenicity and lethality *in vivo*.[38,40] LPS with decreased biological activity may be advantageous for the *Helicobacter* as this would decrease the host inflammatory response, enabling the establishment of a chronic infection. The absence of IL-10, however, likely alters the inflammatory response to *Helicobacter*-derived LPS. We previously demonstrated that IL-10 is a key regulator of the host response to LPS *in vivo*.[41] LPS-stimulated IL-10$^{-/-}$ mice had markedly increased production of tumor necrosis factor-α, IL-6, IL-12, interferon-γ, and IL-1α[41] as compared with wild-type mice, and IL-10$^{-/-}$ mice were also much more sensitive to LPS priming in the Shwartzman reaction, providing additional evidence that IL-10 regulates the host's response to LPS.[41] These data suggest that the severe gastric inflammation in IL-10$^{-/-}$ mice may in part be secondary to an exaggerated inflammatory cytokine response to *H. felis* LPS. The recent finding that C3H/HeJ mice (which are not responsive to LPS) do not develop atrophic gastritis with chronic *H. felis* infection supports this hypothesis.[42] Increased responsiveness to LPS might also be an element in the rapidity with which the IL-10$^{-/-}$ mice respond to gastric colonization with *Helicobacter*.

Recently, we reported that IL-10 is an important regulator of the mucosal immune response in the colon.[28]

IL-10$^{-/-}$ mice develop spontaneous inflammation in the colon characterized by excessive cytokine production from activated macrophages and interferon-γ-producing CD4$^+$ T cells. Transfer of these Th1-type CD4$^+$ T cells from colons of IL-10$^{-/-}$ mice into immunodeficient mice (Rag2$^{-/-}$) led to the development of severe colitis,[28,43] demonstrating that Th1 T cells mediate the colitis in IL-10$^{-/-}$ mice. Although data on the type of immune response to *H. pylori* has been conflicting,[44–48] recent studies of *H. felis* infection in wild-type mice[49] and *H. pylori* infections in humans[50] report the development of a Th1 CD4$^+$ T cell response to gastric *Helicobacter* infection. A potential explanation for the severe inflammation seen in *H. felis*-infected IL-10-deficient mice is that the absence of IL-10 results in an exaggerated Th1 response to the gastric *Helicobacter* infection, similar to the exaggerated Th1 response to enteric antigens in the colon. Although IL-10$^{-/-}$ mice can develop severe colitis, this takes 6 months or greater to develop.[28] In the present study, the stomachs of IL-10-deficient animals were infected with *H. felis* at 4 weeks of age, a time at which there is minimal inflammation in the colon.[28] We observed no discernible impact on the development of colitis in the mice with stomachs that were colonized with *H. felis*. Studies to further characterize the immune response to *H. felis* infection in IL-10$^{-/-}$ mice are ongoing.

Infection of IL-10$^{-/-}$ mice with *H. felis* has strong similarities with *H. pylori* infection in humans. Similar to human *H. pylori* infection, *H. felis* infection and inflammation was initially localized in the antrum of IL-10$^{-/-}$ mice. As has been seen in previous studies of *H. felis*-infected mice,[51] IL-10$^{-/-}$ mice developed inflammatory changes in the body of the stomach. This pattern differs from the usual antrum-predominant gastritis seen in humans, although this type of gastritis can be seen in populations that are prone to gastric cancer. The inflammatory infiltrate in *H. felis*-infected IL-10$^{-/-}$ mice contained a significant component of neutrophils, a feature also seen in human *H. pylori* infection. The major difference in this model from human infection and from previous animal models is the rapidity at which severe inflammation and epithelial alterations developed in the IL-10-deficient animals. *H. pylori* infection is chronic and in most patients does not progress beyond as asymptomatic superficial gastritis.[52] Only with prolonged infection (years in human patients and months to years in standard animal models[53]) does inflammation involve the corpus and lead to significant epithelial changes. The difference in kinetics in the present study is likely due to the absence of IL-10, a pleiotropic factor that regulates production of inflammatory cytokines[24] and chemokines[54] and regulates helper T cell differentiation.[55] Although it is unlikely that *H. pylori*-infected individuals develop severe inflammation due to an IL-10 deficiency, we propose that this model can provide insights into the role of IL-10 in the immune response to *Helicobacter* and can help dissect out the mechanisms by which immune/inflammatory cytokines and cells regulate epithelial differentiation.

A surprising finding in our studies was the rapid development of abnormal gastric epithelium in *H. felis*-infected mice. Four weeks after infection, IL-10$^{-/-}$ mice had loss of the normal gastric epithelial differentiation with loss of parietal cells, marked epithelial proliferation, and the development of a columnar epithelium that produces acidic mucin. The mechanism(s) driving the altered expression of mucins in *Helicobacter*-associated chronic inflammation are presently not known.

Increased epithelial cell proliferation and expansion of the zone of proliferation was a major component of the epithelial phenotype in *H. felis*-infected IL-10$^{-/-}$ mice. Similarly, *H. pylori* infection in humans has been consistently found to cause increased epithelial cell proliferation.[56–58] The mechanism(s) driving the increase in *H. pylori*-related epithelial cell proliferation are not known but may be secondary to epithelial cell damage and cell loss secondary to production of ammonia or cytotoxins by *H. pylori*[59] or by the host immune response.[60] Alternatively, severe inflammation may significantly increase epithelial cell proliferation by altering the local production of epithelial growth factors.[61–63] In addition to increased proliferation, gastric epithelium in *H. felis*-infected IL-10$^{-/-}$ mice demonstrated loss of normal epithelial differentiation with areas of disordered architecture.

In summary, our studies indicate that IL-10 has a pivotal role in the regulation of the inflammatory response to gastric *Helicobacter* infection. Moreover, we have found altered epithelial morphology, increased epithelial proliferation, and acidic mucin production in IL-10$^{-/-}$ mice within 4 weeks of *H. felis* infection. These data indicate that *H. felis*-infected IL-10$^{-/-}$ mice develop an abnormal gastric epithelial phenotype that shows some of the characteristics of *H. pylori*-induced preneoplasia. These data suggest that *H. felis*-infected IL-10$^{-/-}$ mice will be useful for furthering our understanding of the regulation of the immune and inflammatory response to *Helicobacter* and may provide insights into the mechanism(s) by which *H. pylori* infection can promote the development of preneoplastic epithelium.

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