

Enhanced Disease Severity in *Helicobacter pylori*-Infected Mice Deficient in Fas Signaling

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Received 6 September 2001/Returned for modification 28 November 2001/Accepted 22 January 2002

Recent evidence suggests that immune-mediated gastric epithelial cell apoptosis through Fas-Fas ligand interactions participates in *Helicobacter pylori* disease pathogenesis. To define the role of Fas signaling in vivo, *H. pylori* strain SS1 infection in C57BL/6 mice was compared to that in mice deficient in the Fas ligand (*gld*). *gld* mice had a degree of gastritis similar to that of C57BL/6 mice after 6 weeks (gastritis score, 5.2 ± 0.6 [mean \pm standard error] versus 3.5 ± 0.8) and 12 weeks (4.0 ± 0.7 versus 3.4 ± 0.5) of infection. Bacterial colonization was comparable in each group of mice at 12 weeks of infection (2.1 ± 0.3 versus 1.6 ± 0.3 for *gld* and C57BL/6, respectively; the difference is not significant). Sixty-seven percent of *H. pylori*-infected *gld* mice displayed atrophic changes in the gastric mucosa, compared with 37% of infected C57BL/6 mice, at 12 weeks. In addition, atrophic changes were more severe in *H. pylori*-infected *gld* mice ($P < 0.05$). Splenocytes isolated from *H. pylori*-infected C57BL/6 mice had a twofold increase in production of the Th1 cytokine gamma interferon (IFN- γ) in response to *H. pylori* antigens at both 6 and 12 weeks compared to controls (143 ± 65 versus 69 ± 26 pg/ml and 336 ± 73 versus 172 ± 60 , respectively). In contrast, there was a lack of detectable IFN- γ in *gld* mice infected with the bacterium. *H. pylori*-infected C57BL/6 mice had increased epithelial cell apoptosis compared with sham-infected C57BL/6 mice (35.0 ± 8.9 versus 12.3 ± 6.9 ; $P < 0.05$). Epithelial cell apoptosis did not differ between *H. pylori*-infected and control *gld* mice (5.2 ± 1.6 versus 6.5 ± 2.9 [not significant]). These data demonstrate that mice with mutations in the Fas ligand develop more severe premalignant mucosal changes in response to infection with *H. pylori* in association with both an impaired gastric epithelial cell apoptotic response and IFN- γ production. The Fas death pathway modulates disease pathophysiology following murine infection with *H. pylori*. Deregulation of the Fas pathway could be involved in the transition from gastritis to gastric cancers during *H. pylori* infection.

Chronic infection with the gastric pathogen *Helicobacter pylori* is associated with the development of peptic ulcer disease and gastric cancers (4). The mechanisms involved in disease pathogenesis remain unclear. Alterations in the gastric epithelial cell cycle, including both enhanced proliferation (3) and increased apoptosis of gastric cells, are identified during infection with the bacterium (34). These changes in cell turnover are present in both *H. pylori*-infected children (16) and adults (21, 24). Current evidence indicates that alterations in cell turnover are likely to play a central role in *H. pylori*-mediated disease pathogenesis. An increase in epithelial cell apoptosis is detected in tissue obtained from subjects with active duodenal ulceration compared to those with healing ulcers (18). In contrast, an increase in cell proliferation in the absence of enhanced apoptosis is detected in gastric tissues obtained from patients with the premalignant changes of intestinal metaplasia (33). Similarly, during *H. pylori* infection in gerbils, which has been reported to lead to gastric adenocarcinomas, a transient increase in apoptosis is detected, followed by a persistent increase in proliferation (30).

Recent studies indicate that both host determinants and bacterial factors are involved in mediating such changes in

epithelial cell turnover. Peek and colleagues (29) have demonstrated that infection with *cagA*⁺ *H. pylori* strains is associated with enhanced proliferation without a corresponding increase in apoptosis. Mice deficient in secretory phospholipase A have altered apoptosis in response to infection with the related bacterium *Helicobacter felis* (39). In vitro studies indicate that the bacterium is capable of directly inducing apoptosis of gastric cells (8, 17, 37). Fan and colleagues (7) provided evidence that binding of urease to class II major histocompatibility complex molecules mediates apoptosis in vitro. In addition, *H. pylori* infection increases the sensitivity of gastric epithelial cells to Fas-triggered apoptosis by enhancing Fas receptor expression (17). Inflammatory cytokines present during *H. pylori* infection, such as gamma interferon (IFN- γ), also enhance activation of the Fas signaling pathway in vitro (13). Furthermore, among *H. pylori*-infected children, gastric epithelial cell apoptosis returns to baseline levels only following both eradication of bacterial colonization and resolution of the accompanying inflammatory cell infiltrate (16). These findings suggest that immune-mediated cell death through the Fas pathway could contribute to the apoptosis that is observed during infection in vivo.

To determine if Fas signaling is involved in *H. pylori* disease pathogenesis in vivo, *H. pylori* infection in C57BL/6 mice was compared with infection of an inbred *gld* (for generalized lymphoproliferative disease) strain with alterations in the Fas signaling pathway due to a point mutation in the Fas ligand (25).

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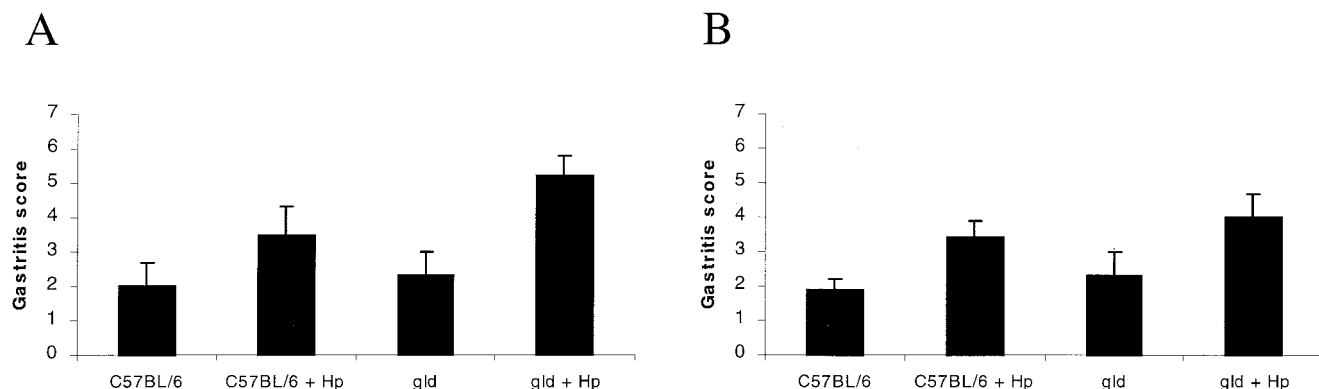


FIG. 1. Quantitation of the degree of gastritis in sham-infected and *H. pylori* (Hp) strain SS1-infected C57BL/6 mice and *gld* mice following 6 weeks (A) and 12 weeks (B) of infection. The degree of gastritis did not differ between groups of mice at 6 and 12 weeks postinfection ($P = NS$). Error bars represent the SE.

Following infection, colonization, mucosal inflammatory changes, gastric cell apoptosis, and cytokine responses were measured.

MATERIALS AND METHODS

Bacteria and growth conditions. The mouse-adapted *H. pylori* strain SS1, originally described by Lee et al. (19) and kindly received from Ken Croitoru (McMaster University, Hamilton, Ontario, Canada), was employed in these studies. Bacteria were grown under microaerophilic conditions on Columbia blood agar plates for 72 h at 37°C, harvested, and resuspended in brucella broth (Difco, Detroit, Mich.) supplemented with 10% heat-inactivated fetal calf serum (Gibco BRL, Grand Island, N.Y.), 10 mg of vancomycin per liter, and 5 mg of trimethoprim per liter (17). Bacteria were grown overnight at 37°C in an Erlenmeyer flask with shaking at 120 rpm, as described previously (17). Cells were then pelleted and resuspended in sterile phosphate-buffered saline (PBS) at a concentration of 10^9 CFU/ml. Before use, the suspension was assessed for motility of organisms under bright-field microscopy and cultured on blood agar plates under microaerophilic conditions to exclude contamination.

Animals. Specific-pathogen-free female mice (6 to 8 weeks old), strains C57BL/6 and B6S^{mn}.C3H-Fas^{gld} (*gld*), were purchased from Jackson Laboratories (Bar Harbor, Maine). Animals were housed in microisolator cages under barrier conditions with free access to commercially available chow and water for the duration of the experiments. All animal experimentation was performed according to institutional guidelines and with approval of the local institutional review board.

Infection of mice with *H. pylori* strain SS1. Mice were inoculated with 10^8 bacteria in 0.1 ml of PBS by orogastric lavage three times within a 5-day interval as described previously (19). A control group for each strain of mice was given 0.1 ml of PBS alone by orogastric lavage under the same conditions.

Groups of 10 to 12 mice from each strain were either sham inoculated or challenged with *H. pylori* strain SS1 on two different occasions. At 6 or 12 weeks following infection, mice were killed by an intraperitoneal injection of chloral hydrate and then necropsied. The stomach was removed en bloc, opened, and rinsed with PBS before assessment of visual evidence of macroscopic changes. Gastric tissue was then cut into strips from the squamocolumnar junction to the duodenum, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. In a subset of mice from each group, the spleen was also removed for isolation of splenic mononuclear cells, as described below.

Following 8 weeks of infection, another group of *H. pylori* SS1-infected *gld* mice ($n = 7$) received eradication therapy consisting of 6.15 mg of bismuth per ml, 50 mg of tetracycline per ml, and 22.5 mg of metronidazole per ml administered by orogastric lavage daily for 7 days (19). Mice were then sacrificed at 21 ($n = 4$) or 42 ($n = 3$) days after infection.

Bacterial colonization. The degree of gastric colonization with *H. pylori* was assessed as described previously (19), with minor modifications. Briefly, Giemsa-stained gastric tissue sections (5 μ m) were examined microscopically under oil immersion and assigned semiquantitative scores for colonization by a blinded observer, as follows: 0, no bacteria detected; 1, mild level of colonization, with bacteria not detected in every gastric crypt; 2, mild level of colonization, with

bacteria detected in the majority of crypts; 3, moderate to heavy colonization in all crypts; and 4, severe colonization, with all crypts densely packed with bacteria.

Determination of apoptotic cells. The terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assay was performed on sections of paraffin-embedded tissue from a subset of mice from each group with a commercially available kit (ApoTag; Intergen, Purchase, N.Y.) according to the manufacturer's directions with minor modifications. Briefly, sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol at room temperature. Triton-X (0.5%) was applied directly onto slides and incubated for 15 min at 25°C. After blocking of endogenous peroxidase by incubation in hydrogen peroxide (1.2%) in methanol for 25 min at 25°C, the slides were washed and an equilibration buffer was applied for a minimum of 10 s. The terminal deoxynucleotidyltransferase enzyme diluted in reaction buffer (1:5) was then applied to the slides and incubated for 1 h in a humidified chamber at 37°C. The stop buffer was then applied for 10 min at 25°C. Peroxidase conjugate was then applied to the sections and incubated for 30 min at 25°C. Detection of peroxidase conjugate was achieved by using a commercially available kit (Histostain Plus; Zymed Laboratories, San Francisco, Calif.). Sections were counterstained with hematoxylin, washed, and mounted with a coverslip.

Apoptotic cells were quantitated by enumerating the number of TUNEL-stained cells in 40 well-oriented gastric glands in antral tissue. Enumeration was performed in a blinded fashion. The apoptotic index represents the mean number of apoptotic cells counted in 40 well-oriented antral glands.

Gastritis score. Giemsa-stained sections of gastric tissue were viewed under bright-field microscopy and graded for the severity of antral gastritis, as described previously (16). Briefly, the presence of an increased number of mononuclear cells, the presence and severity of mucus depletion, and the presence of polymorphonuclear leukocytes within the mucosa were each assessed separately and graded from 0 to 3+ by a pathologist in a blinded fashion with coded slides. Submucosal inflammation was not included in the antral gastritis score.

The presence of atrophic changes, as defined by atrophy of glandular cells and hyperplasia of mucus cells, was determined in a blinded fashion and scored based on the percentage of altered gastric mucosa, as described previously (10): 0, no mucosal alterations; 1, less than 5%; 2, 10 to 25%; 3, 25 to 50%; and 4, 50 to 75%.

Production of IFN- γ and IL-5 by splenic mononuclear cells. Spleens harvested at necropsy were mashed through sterile filter screens into RPMI culture medium to obtain single-cell suspensions. Cell suspensions were incubated in red blood cell lysis buffer for 2 min to lyse erythrocytes. The cells were then washed three times and resuspended in RPMI medium containing 10% fetal calf serum. Viable cells (10^6) were then incubated with sterile *H. pylori* strain SS1 whole-cell sonicate (1 μ g/ml) for 72 h at 37°C in 96-well flat-bottom microtiter plates in triplicate. Supernatants were collected and stored at -70°C until used for cytokine assessment. Production of IFN- γ and interleukin-5 (IL-5) by splenic mononuclear cells was measured in collected supernatants by using commercially available immunoassays (Biosource, Camarillo, Calif.).

Statistics. Results are expressed as means \pm standard errors (SE). Comparison of results between multiple groups was performed using analysis of variance (ANOVA) followed by post-hoc comparisons with the Newman-Keuls or Dun-

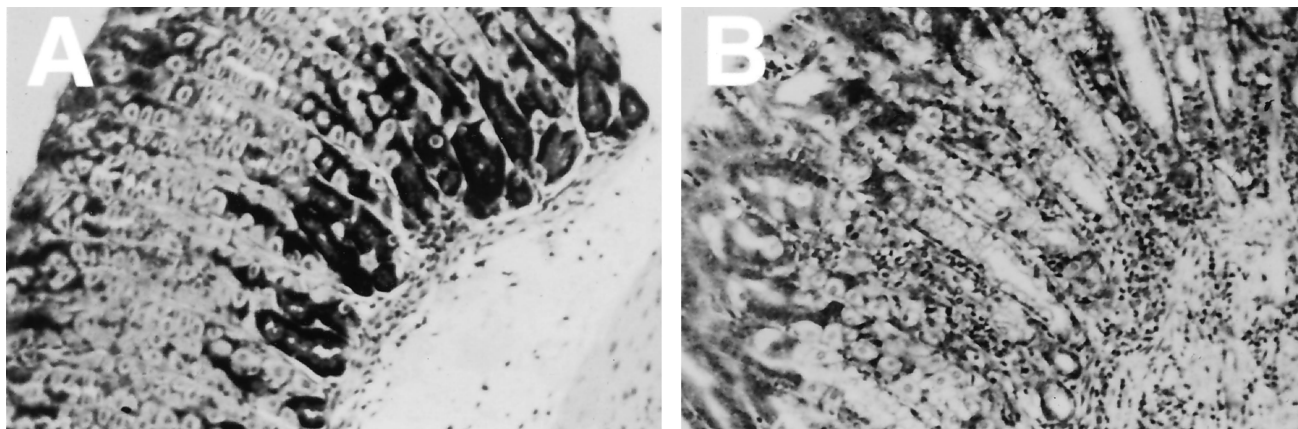


FIG. 2. Photomicrographs of Giemsa-stained gastric tissue sections demonstrating mucosal alterations in response to *H. pylori* strain SS1 infection of C57BL/6 (A) and *gld* (B) mice. Tissues from infected C57BL/6 mice showed a mild inflammatory infiltrate in the lamina propria. In contrast, panel B shows an increase in infiltrating inflammatory cells within the lamina propria extending through the muscularis mucosa in *gld* mice with associated atrophic changes, including loss of parietal cells and mucous cell hyperplasia. Original approximate magnifications, $\times 40$.

can test. For comparisons between two groups, an unpaired Student *t* test was performed.

RESULTS

Effect of Fas ligand deficiency on bacterial colonization. All mice that were challenged with *H. pylori* strain SS1 were colonized when assessed at both 6 and 12 weeks following inoculation. All sham-infected mice remained free of *H. pylori* infection. The majority of C57BL/6 and *gld* mice infected with *H. pylori* displayed mild to moderate bacterial colonization with bacteria detected in most crypts (2.1 ± 0.3 versus 1.6 ± 0.3 ; the difference was not significant [$P = NS$]).

Increased gastric mucosal atrophy in mice deficient in the Fas ligand. At both 6 and 12 weeks following infection, no macroscopic evidence of mucosal ulceration was detected in stomachs obtained from either the C57BL/6 or *gld* mice. However, one of the infected *gld* mice had a thickened gastric wall that corresponded histopathologically to an increase in inflammatory infiltrate predominantly within the submucosa of the corpus region.

Following 6 weeks of infection, when the degree of gastritis was quantitated, *gld* mice demonstrated mucosal inflammation comparable to that in the stomachs of *H. pylori* strain SS1-infected C57BL/6 mice (gastritis score at 6 weeks, 5.2 ± 0.6 versus 3.5 ± 0.8 ; $P = NS$) (Fig. 1). In addition, the degree of gastric atrophic changes were similar in infected wild-type and *gld* mice ($P = NS$). Following 12 weeks of infection, there was no detectable difference in the degree of gastritis between SS1-infected C57BL/6 and *gld* mice (3.4 ± 0.5 versus 4 ± 0.7 ; $P = NS$) (Fig. 1). However, as shown in Fig. 2, atrophic changes, including mucus cell hyperplasia and parietal cell loss, were detected more frequently in the corpus regions of *gld* mice following 12 weeks of infection than in the infected control group of mice (67% of *gld* mice versus 37% of C57BL/6 mice). When the degree of atrophic changes was quantitated, both groups of mice displayed an approximately twofold increase in atrophic changes compared to uninfected mice. However, *H. pylori*-infected *gld* mice displayed more severe mucosal alterations (Fig. 3) ($P < 0.05$, ANOVA).

In the *gld* mice that received eradication therapy 8 weeks after *H. pylori* infection, bacteria were not detected in gastric tissue by using the modified Steiner stain. In addition, in mice that received bacterial eradication therapy, the degree of mucosal inflammation was comparable to that in uninfected mice (gastritis score, 2.0 ± 0.8 versus 2.2 ± 1.1 ; $P = NS$).

Fas signaling mediates an apoptotic response in epithelial cells following infection with *H. pylori*. As shown in Fig. 4, apoptotic epithelial cells were detected in superficial epithelium confined to the upper one-third of gastric crypts by the TUNEL assay in tissues obtained from each of the four groups of mice. Compared to uninfected controls, *H. pylori* strain SS1-infected C57BL/6 mice had increased apoptosis of gastric epithelial cells extending into the neck region (35.0 ± 8.9 versus 12.3 ± 6.9 ; $P < 0.05$) (Fig. 5). In contrast, an increase in the number of apoptotic gastric epithelial cells was not detected in tissues obtained from *gld* mice infected with *H. pylori*. The degrees of apoptosis were comparable in *H. pylori* strain SS1-infected and sham-infected *gld* mice (5.2 ± 1.6 versus 6.5 ± 2.9 ; $P = NS$).

Lack of detectable Th1 inflammatory cytokine response dur-

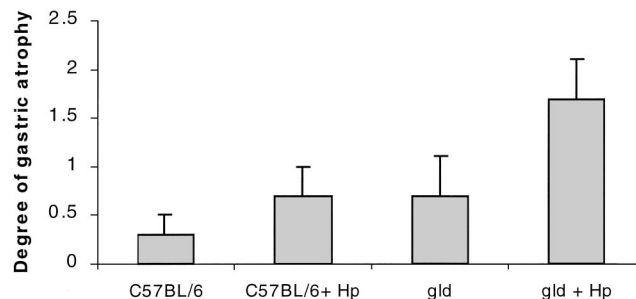


FIG. 3. Determination of severity of atrophic changes in gastric mucosa of sham-infected and *H. pylori* (Hp)-infected C57BL/6 and *gld* mice following 12 weeks of infection. *gld* mice displayed more severe atrophic changes following infection ($P < 0.05$, ANOVA). Error bars represent the SE.

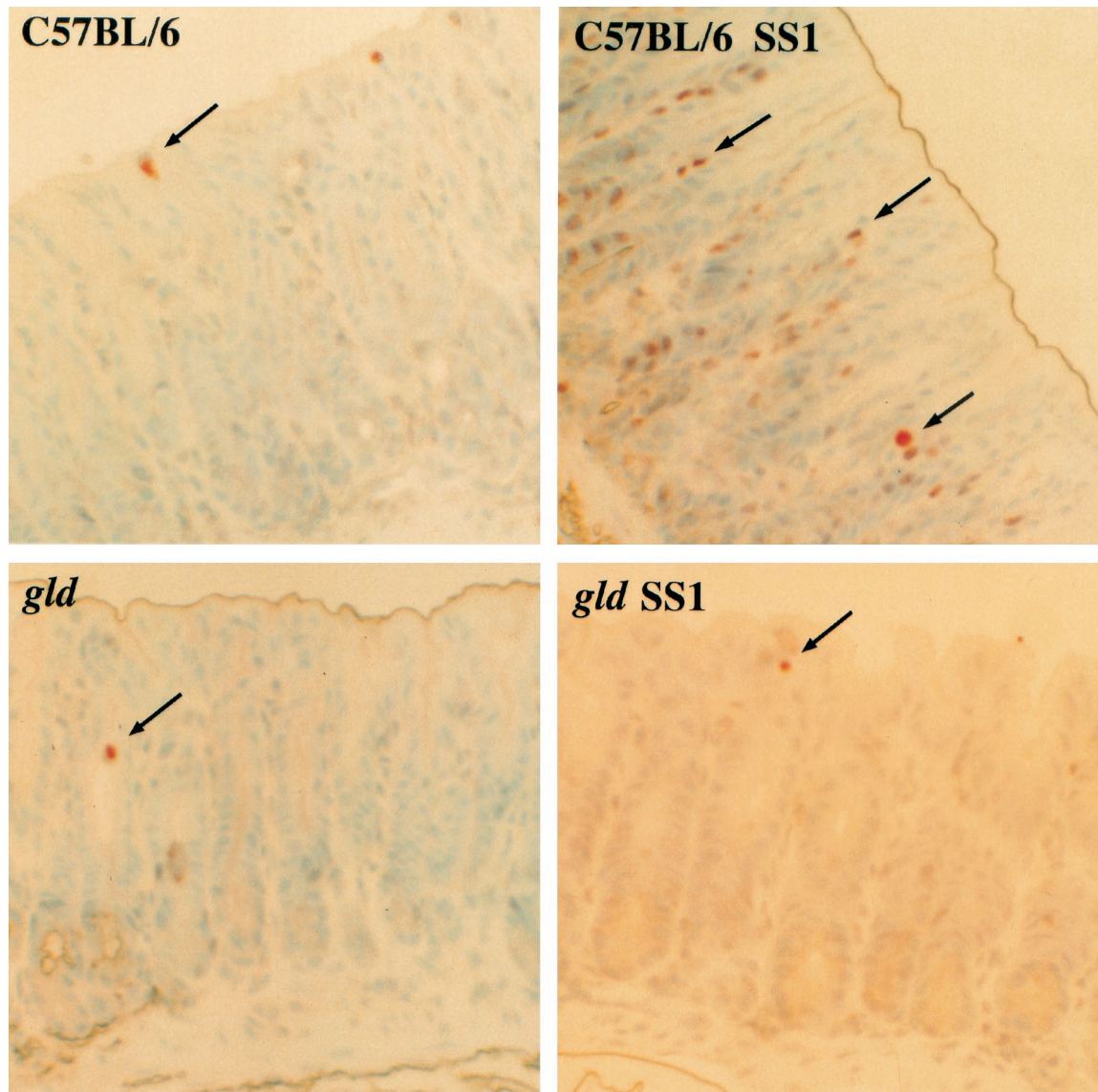


FIG. 4. Photomicrograph demonstrating apoptotic cells (arrows) as assessed by the TUNEL assay in gastric antral tissue from sham-infected and *H. pylori* strain SS1-infected C57BL/6 and *gld* mice. Compared with sham-infected C57BL/6 mice, *H. pylori* SS1-infected C57BL/6 mice had an increase in the number of gastric cells undergoing apoptosis. In contrast, an increase in apoptosis was not detected in infected *gld* mice. Original approximate magnification, $\times 40$.

ing *H. pylori* infection in *gld* mice. C57BL/6 mice displayed an approximately twofold increase in production of the Th1 cytokine IFN- γ following 6 and 12 weeks of infection (143 ± 65 versus 69 ± 26 pg/ml and 337 ± 73 versus 172 ± 60 pg/ml, respectively; $P = \text{NS}$) (Fig. 6). In contrast, production of IFN- γ was undetectable in both sham- and *H. pylori*-infected *gld* mice.

Th2 response to *H. pylori* infection in *gld* mice. Production of the Th2 cytokine IL-5 was minimal in sham-infected C57BL/6 and *gld* mice at both 6 (5.2 ± 2.8 and 4.5 ± 4.5 pg/ml) and 12 (0 and 20.8 ± 16.7 pg/ml) weeks. Levels of IL-5 did not change in response to infection with *H. pylori* in either group of mice (Fig. 7).

DISCUSSION

Fas, or CD95, is a member of the tumor necrosis factor receptor family which when bound by its natural ligand, Fas

ligand, stimulates an apoptotic signal through activation of the caspase cascade (1). Expression of the Fas ligand is found predominantly on activated T lymphocytes (20). The Fas death signal regulates immune responses by deleting autoreactive T cells, triggering activation-induced cell death, and mediating tolerance (20). Expression of the Fas ligand in specific tissues, such as the testes, is implicated in immune privilege of this site (9).

In the gastrointestinal tract, Fas receptor normally is constitutively expressed in epithelial cells. By contrast, there is little or no detectable Fas ligand expression, except on the surface of Paneth cells (23). Current evidence indicates that disruption of Fas signaling is involved in the pathogenesis of intestinal diseases. For example, Fas ligand is highly expressed on infiltrating T lymphocytes in the colons of patients with ulcerative colitis (35). In a murine model of autoimmune gastritis, en-

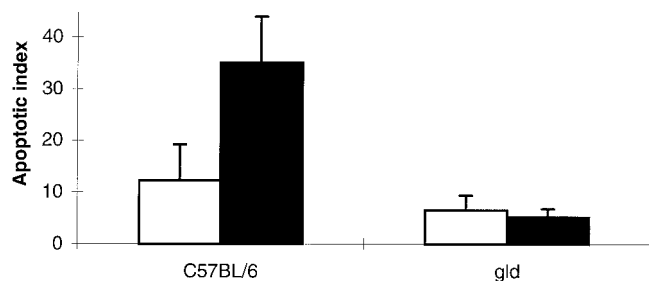


FIG. 5. Quantitation of apoptosis in gastric tissue obtained from sham-infected (open bars) and *H. pylori* (Hp) SS1-infected (solid bars) C57BL/6 and *gld* mice following 12 weeks of infection. C57BL/6 mice had an increase in apoptosis of gastric cells compared to both groups of *gld* mice. C57BL/6 mice infected with the bacteria demonstrated an enhanced degree of apoptosis ($P < 0.05$, ANOVA). Results are expressed as the mean apoptotic index. Error bars represent the SE.

hanced Fas expression is detected on gastric parietal cells in association with the induction of apoptosis (26).

Apoptosis is implicated in the regulation of inflammation in response to bacterial pathogens. The gastric pathogen *H. pylori* induces apoptosis of gastric epithelial cells both in vivo and in vitro (34). In addition, infection with *H. pylori* enhances expression of the Fas receptor on gastric epithelial cells and increases sensitivity to Fas-triggered cell death in vitro (17). An increase in infiltrating Fas ligand-expressing lymphocytes is detected in gastric tissues obtained from *H. pylori*-infected individuals (31). In addition, *H. pylori* infection of T-cell lines in vitro induces apoptotic cell death mediated by the Fas pathway (38). However, the relative contribution of the Fas death pathway in mediating disease during *H. pylori* infection is not clear. This study is the first to directly investigate the importance of Fas signaling during *H. pylori* infection in vivo using a murine model of infection.

Consistent with enhanced programmed cell death observed in humans infected with *H. pylori* (16, 21, 24), C57BL/6 mice demonstrated an increase in apoptosis of gastric epithelial cells during infection. However, in gastric tissues obtained from mice deficient in the Fas ligand, enhanced gastric epithelial cell

apoptosis was not detected. Thus, gastric epithelial cells from mice deficient in the Fas ligand are resistant to *H. pylori*-mediated cell death. Therefore, although *H. pylori* can directly induce apoptosis of gastric epithelial cells in vitro (8, 17), the Fas signaling cascade is the predominant form of cell death which occurs during infection in vivo. Indeed, the Fas pathway may be the preferential cell death pathway utilized by helicobacters. In support of this contention, Houghton et al. (14) recently demonstrated that mice with a mutation in the Fas receptor (*lpr*) show a lack of an apoptotic response during infection with a related gastric helicobacter, *H. felis*.

We have demonstrated that in *gld* mice deficient in the Fas ligand, *H. pylori* infection results in more severe disease than that in infected C57BL/6 mice. Although there was a trend towards increased mucosal inflammation in the infected wild-type and *gld* mice compared with the sham-infected controls, this was not statistically significant. This finding is in agreement with the original study characterizing murine infection with the Sydney strain. Lee et al. (19) described a significant difference in the degree of antral inflammation between control and SS1-infected mice only following 8 months of infection. Atrophic changes characterized by a loss of parietal cell mass and replacement with metaplastic mucus cells are considered premalignant lesions (10). In our study, gastric atrophy was detected more frequently in *gld* mice than in C57BL/6 mice in response to infection. The exact mechanisms underlying the transition from gastritis to atrophic changes during gastric *Helicobacter* infection are not known.

An increase in bacterial colonization due to insufficient Fas-mediated elimination of infected cells is one possible explanation for the enhanced pathological changes observed in *gld* mice. The importance of Fas-triggered cell death in modulating disease severity during infection was recently demonstrated during *Pseudomonas aeruginosa* infection in mice (11). In comparison with wild-type mice, mice deficient in either the Fas receptor or Fas ligand rapidly developed sepsis with increased splenic colonization in association with a reduction in lung epithelial cell apoptosis and markedly decreased survival. Similarly, studies with both *Mycobacterium tuberculosis* (28) and

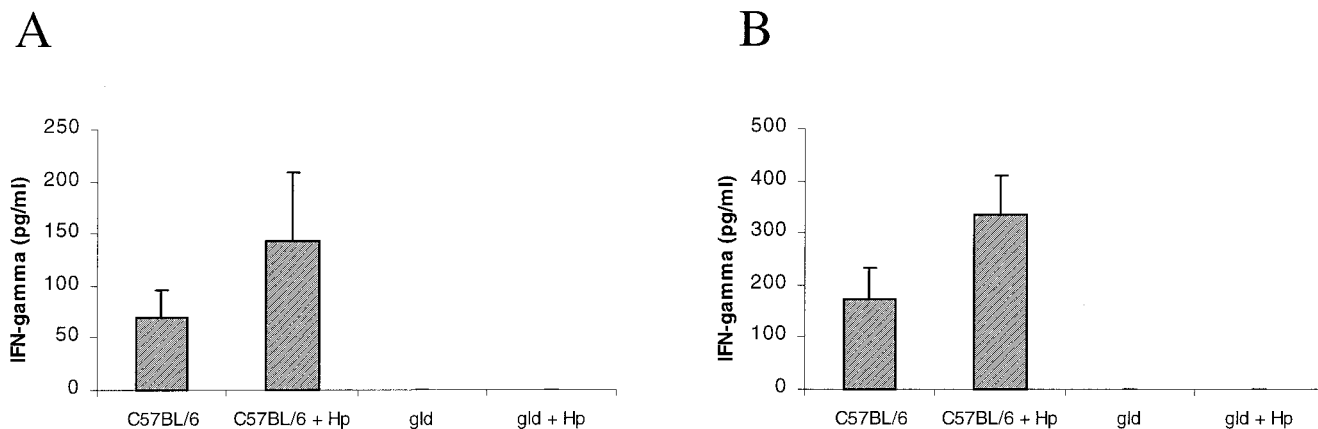


FIG. 6. Th1 (IFN- γ) cytokine profiles in isolated splenocytes stimulated with *H. pylori* (Hp) antigens obtained from sham-infected and *H. pylori* strain SS1-infected C57BL/6 and *gld* mice at 6 weeks (A) and 12 weeks (B) of infection. *H. pylori*-infected C57BL/6 mice displayed a predominant Th1 response. In contrast, *gld* mice lacked an IFN- γ response during infection. Error bars represent the SE.

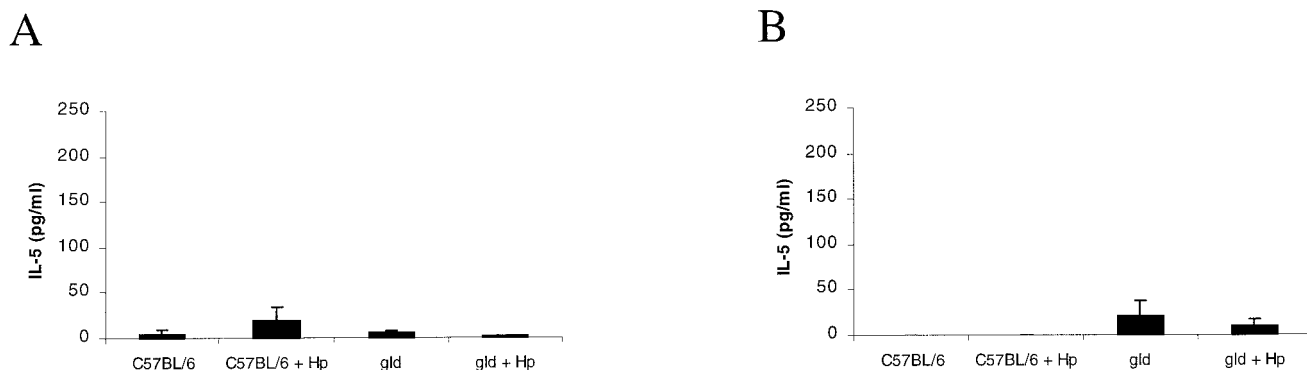


FIG. 7. Th2 (IL-5) cytokine profiles in isolated splenocytes stimulated with *H. pylori* (Hp) antigens obtained from sham-infected and *H. pylori* strain SS1-infected C57BL/6 and *gld* mice at 6 weeks (A) and 12 weeks (B) of infection. Production of IL-5 was minimal in each group of mice. Error bars represent the SE.

Listeria monocytogenes (15) indicate that Fas-mediated cell death is involved in host defense. Fas-triggered apoptosis reduces the viability of *M. tuberculosis* within infected macrophages in vitro (28). Compared to wild-type mice, Fas-deficient mice have elevated bacterial titers in the liver during infection with *L. monocytogenes* (15). However, in our study levels of bacterial colonization were similar in both groups of mice, suggesting that the enhanced degree of mucosal injury was not a result of increased bacterial load.

Previous studies with mice indicate that *Helicobacter* infection is associated with a predominant Th1 cytokine profile (10). In addition, murine studies suggest that levels of Th1 cytokines, in particular IFN- γ , contribute to the degree of gastric injury during *Helicobacter* infection (10). For example, mice deficient in IFN- γ or administered anti-IFN- γ develop less severe inflammation than controls (32). Similarly, Fox et al. (10) recently reported that coinfection with the Th2-promoting helminth *Heligmosmoides polygyrus* during *H. felis* infection in C57BL/6 mice causes a reduction in atrophic changes in association with an enhanced Th2 profile and a reduced Th1 cytokine profile.

To determine if the increased gastric atrophy observed in *H. pylori*-infected *gld* mice was associated with altered Th1 or Th2 responses, we measured the levels of the Th1 cytokine IFN- γ and the Th2 cytokine IL-5 in response to infection in isolated splenocytes. In preliminary experiments we were not able to reliably measure IL-4 production from isolated splenocytes. Therefore, IL-5 was measured as a representative Th2 cytokine. Previous studies have demonstrated that cytokine responses in splenocytes correlate with gastric cytokine responses during *Helicobacter* infection in mice (22). Both groups of mice displayed similar levels of IL-5 before and after infection. However, splenocytes from mice deficient in Fas signaling showed a lack of IFN- γ production in response to infection compared to control mice. This finding suggests that multiple factors, in addition to IFN- γ , are involved in mediating the transition to gastric atrophy during infection in *gld* mice.

Conflicting data exist regarding the stimulation of IFN- γ from unfractionated spleen cells obtained from *gld* mice. For example, Davignon et al. (6) presented evidence that concanavalin A-stimulated unfractionated splenocytes from *gld* mice produce less IFN- γ than stimulated control cells. In con-

trast, Davidson et al. (5) reported that unfractionated lymphocytes from *gld* mice do not spontaneously produce IFN- γ but can produce IFN- γ after concanavalin A stimulation. The majority of lymphoid cells that accumulate in the lymph nodes and spleens of *gld* mice are a unique subset of double-negative T cells (5). When purified, these T cells proliferate weakly and fail to produce cytokines, including IFN- γ , following stimulation with a variety of agents (6). Further studies are required to determine if infection with *H. pylori* enhances the proportion of unresponsive, double-negative T-cell subsets in the spleens of *gld* mice.

Whereas Fas-triggered cell death likely serves as a host defense mechanism, dysregulation of the pathway could result in complications associated with infection. For example, excessive apoptosis would result in disruption of the epithelial barrier and thereby cause the development of mucosal ulceration. Indeed, *H. pylori*-infected subjects with duodenal ulcers have an increase in apoptosis of gastric epithelial cells compared to subjects with healing peptic ulcer disease (18). By contrast, a resistance to apoptosis could promote the development of gastric malignancies (33). In support of this contention, enhanced proliferation in the absence of increased cell death is detected in tissues obtained from patients with the precancerous lesion intestinal metaplasia compared to tissues from subjects with *H. pylori*-mediated gastritis alone (33). In our study the degree of apoptosis did not correlate with the degree of proliferation as assessed by proliferating cell nuclear antigen immunohistochemistry in serial sections (data not shown). Our findings in mice showing that programmed cell death is triggered through Fas and that the absence of Fas signaling enhances premalignant changes suggest that disruption of this signaling pathway could promote carcinogenesis.

Deregulation of the Fas pathway has been implicated in the development of a variety of human cancers, including gastric malignancies (27). Differential expression of the Fas receptor is detected both in cell lines derived from human gastric cancers (12) and in gastric carcinoma tissue specimens (36). Bennett et al. (2) detected enhanced Fas ligand expression in 30 gastric carcinomas and suggested that expression of Fas ligand allows gastric cancers to escape immune surveillance by inducing cell death in infiltrating lymphocytes.

In summary, this study demonstrates the importance of the

Fas death pathway in disease pathophysiology following murine infection with *H. pylori*. Determination of whether chronic infection with *H. pylori*-induced dysregulation of the Fas signaling pathway ultimately participates in the multistep pathway leading to gastric carcinogenesis requires further study.

ACKNOWLEDGMENTS

N. L. Jones is the recipient of an American Digestive Health Foundation Research Scholar Award and a Canadian Institute of Health Research Operating Grant. A. S. Day was the recipient of a Research Initiative Award from the Canadian Association of Gastroenterology/Astra Zeneca Pharmaceuticals/Medical Research Council of Canada. H. A. Jennings was the recipient of a Summer Studentship Award from the Canadian Association of Gastroenterology. P. M. Sherman is the recipient of a Canada Research Chair in Gastrointestinal Disease.

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