Helicobacter pylori Eradication Prevents Progression of Gastric Cancer in Hypergastrinemic INS-GAS Mice

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Abstract
Helicobacter pylori infection results in chronic gastritis, which may progress to gastric cancer. In this study, we investigated the efficacy of H. pylori eradication in preventing the progression of gastritis to gastric cancer in H. pylori–infected transgenic INS-GAS mice. H. pylori infection induced severe dysplasia and gastric cancer classified as high-grade and low-grade gastrointestinal intraepithelial neoplasia (GIN) in INS-GAS mice at 28 weeks postinfection (WPI). H. pylori eradication therapy using omeprazole, metronidazole, and clarithromycin was administered p.o. at 8, 12, or 22 WPI. Compared with untreated infected mice, H. pylori eradication at 8, 12, and 22 WPI significantly reduced the severity of dysplasia (P < 0.01). Moreover, H. pylori eradication at 8 WPI completely prevented the development of GIN (P < 0.001). Although not as effective as early antimicrobial treatment, prevention of progression to high-grade GIN was achieved by H. pylori eradication at 12 and 22 WPI (P < 0.05). Consistent with reduced gastric pathology, H. pylori eradication at all time points significantly down-regulated gastric Interferon-γ, tumor necrosis factor-α, inducible nitric oxide synthase, and Reg 1 mRNA levels (P < 0.05) and reduced epithelial proliferation in the corpus (P < 0.01) compared with untreated infected mice. We concluded that H. pylori eradication prevented gastric cancer to the greatest extent when antibiotics are given at an early point of infection, but that eradication therapy given at a later time point delayed the development of severe dysplastic lesions. [Cancer Res 2008;68(9):3540–8]

Introduction
Helicobacter pylori was first identified in the antrum of patients with active chronic gastritis and peptic ulcers (1). H. pylori is now recognized as the major cause of gastric cancer and has been classified as a group I carcinogen by WHO (2, 3). H. pylori infection causes persistent chronic gastritis, which in susceptible individuals may progress to atrophy, intestinal metaplasia, dysplasia, and finally, intestinal-type gastric cancer (3, 4). Therefore, H. pylori eradication should, in theory, prevent the development of H. pylori–associated gastric diseases. Eradication of H. pylori in humans has been associated with prevention or regression of preneoplastic lesions including atrophic gastritis and intestinal metaplasia (5–9). The effectiveness of H. pylori eradication in preventing the development of gastric cancer remains controversial. Antibiotic treatment during knee or hip replacement surgery reduced the incidence of gastric cancer in these patients during the postoperative period, probably due to eradication of H. pylori (10, 11). The optimal effect of antibiotic eradication therapy in preventing gastric cancer has been observed in H. pylori–infected patients who did not have precancerous gastric lesions before H. pylori eradication therapy (12). However, in other studies, H. pylori eradication did not reduce the overall prevalence of dysplasia or gastric cancer (6, 9, 12, 13). Because it typically requires several decades for gastric cancer to develop in susceptible hosts acquiring H. pylori infection at an early age (4), H. pylori eradication trials continue to raise key questions about which patients would benefit from H. pylori eradication and at what stage of gastric disease would H. pylori antimicrobial eradication prevent the progression to gastric cancer.

Several animal models have been used to examine whether H. pylori eradication is effective in the reversal of preneoplastic gastric lesions and in preventing the progression of these preneoplastic lesions to gastric cancer. Antibiotic eradication therapy reversed the histologic progression of dysplasia in H. pylori–infected Mongolian gerbils (14). In Helicobacter felis–infected C57BL/6 mice that developed gastric cancer within 16 months postinfection, gastric cancer was completely prevented by H. felis antimicrobial eradication therapy given within the first 6 months of infection. In contrast, antibiotics given to H. felis–infected mice at 12 months postinfection did not arrest progression of dysplasia but reduced the incidence of invasive gastric cancer (15).

Recent studies suggest an association between hypergastrinemia, Helicobacter infection, and gastric cancer in humans and mice (16–19). In the absence of Helicobacter infection, transgenic INS-GAS mice that overexpress amidated gastrin have elevated gastric acid secretion and an increased parietal cell number at 1 to 3 months of age. With increasing age, these INS-GAS mice lose parietal cell mass and develop hypochlorhydria, gastric atrophy, metaplasia, and dysplasia. At 20 months of age, INS-GAS mice spontaneously develop invasive gastric cancer (18, 20). The development of gastric cancer is accelerated by gastric Helicobacter spp. infection, and lesion severity is more profound in male INS-GAS mice (16, 18, 19, 21). The purpose of this study was to examine the effect of H. pylori eradication at different stages of progression from gastritis to gastric cancer in INS-GAS male mice.

Materials and Methods

Mice. The animal protocol was reviewed and approved by the Massachusetts Institute of Technology Committee on Animal Care. Specific pathogen-free (including Helicobacter spp.) male INS-GAS mice on a FVB/N background were used in this study (20). Mice were maintained in a facility accredited by the Association for Assessment and Accreditation of
Laboratory Animal Care International and housed on hard wood bedding in microisolator, solid-bottomed polycarbonate cages, and given a commercial rodent diet (Probol RMH 3000, PMI Nutrition International) and water ad libitum.

**Experimental design.** Fifty-four 6- to 8-wk-old male mice were infected by oral gavage with 0.2 mL of *H. pylori* SS1 on alternate days for a total of three doses (22, 23). The *H. pylori* inoculum for oral gavage was adjusted with PBS to an absorbance of 1.0 at 600 nm (approximate dose of 10^7 colony-forming units/mL; ref. 23). Helicobacter-uninfected mice were sham dosed with 0.2 mL of PBS. Infected mice were dosed p.o. with omeprazole (400 μmol/kg/d; Sigma-Aldrich), metronidazole (14.2 mg/kg/d; Sigma-Aldrich), and clarithromycin (7.15 mg/kg/d; gift from Chugai Pharmaceuti-
cal Co.) in a 0.2-mL volume twice a day for 7 d (24). This antimicrobial regimen previously showed 100% eradication of *H. pylori* in infected C57BL/6 mice (24). Treatment was administered at 8, 12, or 22 weeks post *H. pylori* infection (WPI). Mice were euthanized at 28 WPI.

**Tissue collection and histologic analysis.** Following CO2 asphyxiation, blood was immediately collected by cardiac puncture. The stomach and proximal duodenum were removed and the stomach incised along the greater curvature. Linear gastric strips from the lesser curvature were fixed overnight in 10% neutral-buffered formalin, embedded, cut to 4-
 thickness, and stained with H&E. Tissue sections were scored for gastric lesions including atrophy, epithelial defects, and dysplasia, and characterized by loss of parietal and chief cells (Fig. 1; Table 1). Corpus hypertrophy was quantified manually for the K67 labeling index (LI), and results were averaged from three to four mice in each group. The remainder of the gastric tissue was snap-frozen in liquid nitrogen and stored at −70°C for DNA and RNA analyses.

**Confirmation of *H. pylori* eradication by quantitative PCR.** A longitudinal strip of gastric tissue from the greater curvature was digested with proteinase K at 55°C overnight, followed by DNA extraction with phenol/chloroform/isooamyl alcohol (25:24:1) and ethanol precipitation. *H. pylori* colonization levels in gastric tissue were quantified by a fluorogenic quantitative PCR assay with a dual-labeled TaqMan probe and FAM reporter fluorescence (Applied Biosystems). DNA was amplified using Power SYBR Green PCR reagent (QIAGEN) using primers for *H. pylori* (F: TATCCATTCTATTTTGTCTCTAGCT; R: CATATGTCACGTCGTGACATGC) (25). The standard curve included a 10^3- to 10^7-copy dilution series of a *H. pylori* isolate (SS1 strain) as previously described (28). In brief, 96-well flat-bottomed plates were coated with 100 μL of antigen (10 μg/mL) overnight at 4°C. sera were diluted to a ratio of 1:100 and added to the wells. Biotinylated secondary goat anti-mouse antibodies, clones A85-1 and 5.7 (BD Pharmingen, San Jose, CA), were used for detecting IgG1 and IgG2a, respectively. Incubation with extravidin peroxidase (Sigma-Aldrich) was followed by treatment with 2,2′-azino-bis(3-ethylbenzthiazolesulfonic acid) substrate (Kirkegaard & Perry Laboratories) for color development. The absorbance was recorded at A405 and A595 with a plate reader per manufacturer’s protocol (Power WaveX Select, Bio-Tek Instruments).

**Statistical analysis.** Gastric lesion scores and Ki67 LI for proliferation indices were compared by the Mann-Whitney U test. Expression levels of cytokines, iNOS, Reg 1, and IgG titers were compared using Student’s t-test. Incidences of low-grade and high-grade GIN in the treatment groups were compared with controls by Fisher’s exact t test. Statistical analysis was done using a commercial software (GraphPad Prism 4.0, GraphPad Software, Inc.) with significance at *P < 0.05.*

**Results**

**Confirmation of *H. pylori* eradication in INS-GAS mice that received antimicrobial therapy.** To assess the effect of *H. pylori* eradication on progression of gastric cancer in INS-GAS mice, antimicrobial therapy using the combination of omeprazole, metronidazole, and clarithromycin was administered p.o. to mice at 8, 12, or 22 WPI. Quantitative PCR indicated that *H. pylori* was successfully eradicated in all mice (100%) treated at 8 or 12 weeks post infection (WPI). In 12 of 14 (85.7%) animals treated at 22 WPI, *H. pylori* was successfully eradicated (Fig. 1). Only mice in which *H. pylori* eradication was successful were used for further analysis.

**H. pylori** infection promoted the development of premalignant lesions and gastric cancer in INS-GAS mice. Helicobacter infection promotes gastric carcinogenesis in INS-GAS mice, particularly in males (18, 19). As previously noted (19), uninfected INS-GAS mice at 28 to 34 weeks of age developed progressive gastric lesions including atrophy, epithelial defects, and dysplasia, accompanied with minimal inflammation, severe hyperplasia, and intestinal metaplasia (Fig. 1; Table 1). Corpus hypertrophy was observed at necropsy as thickened mucosal folds in *H. pylori*–infected INS-GAS mice at 22 and 28 WPI. *H. pylori*–infected INS-GAS mice developed chronic atrophic gastritis with profound changes in mucosal architecture, restricted mainly to the corpus and characterized by loss of parietal and chief cells (Fig. 1). Compared with age-matched uninfected mice, infected INS-GAS mice had more severe inflammation (*P < 0.001*), oxyntic atrophy (*P < 0.05*), hyperplasia (*P < 0.05*), epithelial defects (*P < 0.001*), intestinal metaplasia (*P < 0.05*), and dysplasia (*P < 0.001*) at 22 WPI, and higher degrees of inflammation (*P < 0.001*), dysplasia (*P < 0.001*), and hyperplasia (*P < 0.01*) at 28 WPI (Fig. 2; Table 1). Compared with infected mice at 22 WPI, infected mice at 28 WPI had more severe dysplasia and atrophy (*P < 0.05*).

**H. pylori** eradication at 8 WPI significantly reduced gastritis and premalignant lesions. *H. pylori*–infected INS-GAS mice received antimicrobial eradication therapy at 8, 12, or 22 WPI and were euthanized at 28 WPI. Infected mice that received *H. pylori* antimicrobial eradication therapy at 8 WPI had gastric architecture indistinguishable from that of uninfected age-matched mice (Fig. 1). Compared with untreated *H. pylori*–infected INS-GAS mice, *H. pylori* antimicrobial eradication therapy at 8 WPI inhibited the development of corpus dysplasia, inflammation, atrophy, hyperplasia, epithelial defects (all *P < 0.001*), and intestinal metaplasia (*P < 0.05; Fig. 2A). Additionally, these mice exhibited less severe corpus inflammation, atrophy, and epithelial defects...
H. pylori antimicrobial eradication therapy at 12 WPI and were untreated H. pylori dilated glands and glandular dysplasia (Fig. 1). Compared with euthanized 16 weeks later had distorted mucosal architecture with intestinal metaplasia. but comparable atrophy, hyperplasia, epithelial defects, and metaplasia (H. pylori had statistically less severe dysplasia, inflammation, and intestinal atrophy, and moderate hyperplasia on H&E stain. Tissue from a treated INS-GAS mouse 22 WPI, exhibiting moderate dysplasia, moderate inflammation, severe atrophy, and moderate hyperplasia on H&E stain. Tissue from a treated INS-GAS mouse 22 WPI, exhibiting severe dysplasia, mild inflammation, severe atrophy, and moderate to severe hyperplasia on H&E stain. Tissue from an untreated INS-GAS mouse 22 WPI, exhibiting severe dysplasia, moderate inflammation, severe atrophy, and moderate to severe hyperplasia on H&E stain. n, numbers of mice with successful H. pylori eradication per group size.

than did the uninfected mice (P < 0.05, P < 0.01, and P < 0.01, respectively; Fig. 2A).

Microscopically, H. pylori–infected INS-GAS mice that received H. pylori antimicrobial eradication therapy at 12 WPI and were euthanized 16 weeks later had distorted mucosal architecture with dilated glands and glandular dysplasia (Fig. 1). Compared with untreated H. pylori–infected INS-GAS mice, infected INS-GAS mice that received H. pylori antimicrobial eradication therapy at 12 WPI had statistically less severe dysplasia, inflammation, and intestinal metaplasia (P < 0.01, P < 0.05, and P < 0.05, respectively), but both groups had similar degrees of atrophy, hyperplasia, and epithelial defects (Fig. 2A). Compared with uninfected mice, infected INS-GAS mice that received H. pylori antimicrobial eradication therapy at 12 WPI had more severe dysplasia and inflammation (P < 0.001) but comparable atrophy, hyperplasia, epithelial defects, and intestinal metaplasia.

H. pylori–infected INS-GAS mice that received H. pylori antimicrobial eradication at 22 WPI and were euthanized 6 weeks later had thickened mucosal folds and developed corpus hyperplasia and dysplasia (Fig. 1). Microscopically, these mice developed statistically less severe dysplasia, inflammation, atrophy, and epithelial defects compared with untreated H. pylori–infected INS-GAS mice (P < 0.01; Fig. 2A). Compared with uninfected mice, infected INS-GAS mice that received H. pylori antimicrobial eradication therapy at 22 WPI had more severe dysplasia, hyperplasia, and epithelial defects (P < 0.05). Additionally, it is important to note that the two mice in which H. pylori was not eradicated developed low-grade or high-grade GIN (data not shown).

Among the infected INS-GAS mice that received H. pylori antimicrobial eradication therapy, mice receiving antimicrobial therapy at 8 WPI had significantly lower scores of dysplasia, inflammation, atrophy, and hyperplasia compared with mice receiving antimicrobial therapy at 12 or 22 WPI (P ≤ 0.05), and had less severe epithelial defects compared with mice receiving antimicrobial therapy at 12 WPI (P < 0.001). Most gastric lesions

**Table 1. Gastric lesions at 22 and 28 WPI**

<table>
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<tr>
<th>Weeks*</th>
<th>H. pylori</th>
<th>Lesion indices, median (range)</th>
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Abbreviations: D, dysplasia; I, inflammation; A, atrophy; H, hyperplasia; ED, epithelial defects; IM, intestinal metaplasia.

*Equivalent to WPI.
†Significant difference between 22 and 28 WPI in uninfected mice, P < 0.05.
‡Significant difference between uninfected and infected mice at 22 WPI, P < 0.001.
§Significant difference between uninfected and infected mice at 22 WPI, P < 0.05.
¶Significant difference between 22 and 28 WPI in infected mice, P < 0.05.
**Significant difference between uninfected and infected mice at 28 WPI, P < 0.001.
were comparable in infected mice that received eradication therapy at 12 or 22 WPI, except for epithelial defects that were more severe in the 12 WPI group ($P < 0.01$).

**$H. pylori$ eradication at 8 WPI prevented progression to low-grade and high-grade gastrointestinal intraepithelial neoplasia.** None of the uninfected INS-GAS mice between 34 to 36 weeks of age developed GIN. In contrast, all untreated $H. pylori$–infected INS-GAS mice ($n = 10$) at 28 WPI (34–36 weeks old) developed gastric cancer: 2 (20%) with low-grade GIN and 8 (80%) with high-grade GIN (Fig. 2B). In the $H. pylori$–infected mice that received antimicrobial eradication therapy at 8 WPI ($n = 11$), 10 (91%) of them did not have GIN and 1 (9%) had low-grade GIN. In the infected mice that received antimicrobial eradication therapy at 12 WPI ($n = 9$), 1 (11%) did not have GIN, 7 (78%) had low-grade GIN, and 1 (11%) had high-grade GIN. Six of the 12 (50%) infected mice that received antimicrobial eradication therapy at 22 WPI did not have GIN, and the remainder (50%) had low-grade GIN.

Compared with uninfected mice, $H. pylori$–infected mice that received antimicrobial eradication therapy at 8 WPI had a similar incidence of GIN ($P = 0.38$). In contrast, infected mice that received antimicrobial eradication therapy at 12 or 22 WPI and untreated $H. pylori$–infected mice had a higher incidence of low-grade and high-grade GIN ($P < 0.05$) when compared with uninfected mice. Compared with $H. pylori$–infected INS-GAS mice that did not receive eradication therapy, the incidences of low-grade and high-grade GIN were statistically lower in infected mice receiving antimicrobial eradication therapy at 8, 12, or 22 WPI ($P < 0.05$). Among the infected mice receiving antimicrobial eradication therapy, therapy at 8 WPI resulted in the lowest incidences of GIN compared with eradication therapy at 12 or 22 WPI ($P < 0.05$). The incidences of low-grade and high-grade GIN were statistically

**Figure 2.** A, histologic scores of dysplasia, inflammation, atrophy, hyperplasia, epithelial defects, and intestinal metaplasia. B, incidences of non-GIN, low-grade GIN (a dysplasia score of 3.0), and high-grade GIN (dysplasia scores ≥3.5). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, compared with uninfected mice. $^\sharp$, $P < 0.05$; $^\sharp\sharp$, $P < 0.01$; $^\sharp\sharp\sharp$, $P < 0.001$, compared with $H. pylori$–infected mice that did not receive antimicrobial therapy. $\dagger$, $P < 0.05$; $\dagger\dagger$, $P < 0.01$; $\dagger\dagger\dagger$, $P < 0.001$, comparison as indicated.
Interestingly, gastric IFN-γ mRNA levels were lower in infected mice that received antimicrobial therapy at 8 WPI than in uninfected mice and in mice receiving eradication therapy at 12 or 22 WPI ($P < 0.05$). Compared with untreated H. pylori–infected mice, mRNA levels of IFN-γ were significantly reduced in infected mice receiving H. pylori antimicrobial eradication therapy at 8, 12, and 22 WPI ($P < 0.05$).

Compared with uninfected mice, gastric TNF-α mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice ($P < 0.001$) and in infected mice that received H. pylori antimicrobial therapy at 12 WPI ($P < 0.01$; Fig. 3B). Infected mice that received H. pylori eradication therapy at 8 WPI had reduced gastric TNF-α mRNA levels ($P < 0.001$), whereas infected mice that received H. pylori eradication therapy at 22 WPI had similar gastric TNF-α mRNA levels ($P = 0.71$), relative to uninfected mice. Compared with untreated H. pylori–infected mice, gastric TNF-α mRNA levels were significantly reduced in all mice that received eradication therapy ($P < 0.001$). Among the infected mice receiving eradication therapy, infected mice that received antimicrobial eradication therapy at 8 WPI had significantly lower TNF-α mRNA levels compared with those mice that received antimicrobial eradication therapy at 12 or 22 WPI ($P < 0.001$ and $P < 0.05$). Infected mice that received antimicrobial eradication therapy at 12 WPI had higher TNF-α mRNA levels compared with mice that received antimicrobial eradication therapy at 22 WPI ($P < 0.05$).

Compared with uninfected mice, gastric iNOS mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice ($P < 0.001$; Fig. 3C). Compared with untreated H. pylori–infected mice, gastric iNOS mRNA levels were significantly down-regulated by H. pylori antimicrobial eradication therapy at all time points ($P < 0.05$). Among the infected mice that received H. pylori antimicrobial eradication therapy, those receiving antimicrobial eradication therapy at 8 WPI had the lowest iNOS mRNA levels compared with mice receiving antimicrobial eradication therapy at 12 or 22 WPI ($P < 0.001$).

The magnitude of H. pylori–specific antibody responses was affected by H. pylori eradication. Compared with uninfected mice, H. pylori–specific, Th1-associated IgG2a levels were significantly elevated in all infected mice with or without H. pylori antimicrobial eradication therapy ($P < 0.01$; Fig. 4A). H. pylori antimicrobial eradication therapy at all time points had no significant effect on H. pylori–specific IgG2a levels compared with untreated H. pylori–infected mice. However, infected mice that received antimicrobial eradication therapy at 22 WPI had statistically higher H. pylori–specific IgG2a levels compared with those receiving antimicrobial eradication therapy at 8 or 12 WPI ($P < 0.05$ and $P < 0.01$, respectively). Compared with uninfected mice, H. pylori–specific, Th2-associated IgG1 responses were also elevated in infected mice receiving H. pylori antimicrobial eradication therapy at 12 and 22 WPI ($P < 0.05$ and $P = 0.06$, respectively) and in untreated infected mice ($P = 0.08$). Compared with untreated H. pylori–infected mice, H. pylori–specific IgG1 levels were reduced in mice that received antimicrobial eradication therapy at 8 WPI ($P < 0.01$), but were not affected by antimicrobial eradication therapy at 12 or 22 WPI ($P = 0.11$). H. pylori–specific IgG1 levels in mice that received antimicrobial eradication therapy at 8 WPI were also lower than those in mice that received antimicrobial eradication therapy at 12 or 22 WPI ($P < 0.01$ and $P < 0.05$, respectively).

**Antimicrobial eradication therapy was associated with decreased gastric IFN-γ, TNF-α, and iNOS mRNA levels compared with untreated infected mice.** Given the importance of the inflammatory response in the pathogenesis of H. pylori gastritis, we analyzed selected proinflammatory cytokines and iNOS mRNA levels in the gastric tissue at 28 WPI. Compared with uninfected mice, gastric IFN-γ mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice ($P < 0.001$; Fig. 3B). H. pylori–infected mice that received H. pylori antimicrobial therapy at 12 and 22 WPI also had up-regulated gastric IFN-γ mRNA levels compared with uninfected mice ($P < 0.001$ and $P < 0.05$, respectively).

Similar to infected mice receiving eradication therapy at 12 and 22 WPI ($P = 0.12$), gastric IFN-γ mRNA levels were lower in infected mice that received antimicrobial therapy at 8 WPI than in uninfected mice and in mice receiving eradication therapy at 12 or 22 WPI ($P < 0.05$). Compared with untreated H. pylori–infected mice, mRNA levels of IFN-γ were significantly reduced in infected mice receiving H. pylori antimicrobial eradication therapy at 8, 12, and 22 WPI ($P < 0.05$).

Compared with uninfected mice, gastric TNF-α mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice ($P < 0.001$) and in infected mice that received H. pylori antimicrobial therapy at 12 WPI ($P < 0.01$; Fig. 3B). Infected mice that received H. pylori eradication therapy at 8 WPI had reduced gastric TNF-α mRNA levels ($P < 0.001$), whereas infected mice that received H. pylori eradication therapy at 22 WPI had similar gastric TNF-α mRNA levels ($P = 0.71$), relative to uninfected mice. Compared with untreated H. pylori–infected mice, gastric TNF-α mRNA levels were significantly reduced in all mice that received eradication therapy ($P < 0.001$). Among the infected mice receiving eradication therapy, infected mice that received antimicrobial eradication therapy at 8 WPI had significantly lower TNF-α mRNA levels compared with those mice that received antimicrobial eradication therapy at 12 or 22 WPI ($P < 0.001$ and $P < 0.05$). Infected mice that received antimicrobial eradication therapy at 12 WPI had higher TNF-α mRNA levels compared with mice that received antimicrobial eradication therapy at 22 WPI ($P < 0.05$).

Compared with uninfected mice, gastric iNOS mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice ($P < 0.001$; Fig. 3C). Compared with untreated H. pylori–infected mice, gastric iNOS mRNA levels were significantly down-regulated by H. pylori antimicrobial eradication therapy at all time points ($P < 0.05$). Among the infected mice that received H. pylori antimicrobial eradication therapy, those receiving antimicrobial eradication therapy at 8 WPI had the lowest iNOS mRNA levels compared with mice receiving antimicrobial eradication therapy at 12 or 22 WPI ($P < 0.001$).

The magnitude of H. pylori–specific antibody responses was affected by H. pylori eradication. Compared with uninfected mice, H. pylori–specific, Th1-associated IgG2a levels were significantly elevated in all infected mice with or without H. pylori antimicrobial eradication therapy ($P < 0.01$; Fig. 4A). H. pylori antimicrobial eradication therapy at all time points had no significant effect on H. pylori–specific IgG2a levels compared with untreated H. pylori–infected mice. However, infected mice that received antimicrobial eradication therapy at 22 WPI had statistically higher H. pylori–specific IgG2a levels compared with those receiving antimicrobial eradication therapy at 8 or 12 WPI ($P < 0.05$ and $P < 0.01$, respectively). Compared with uninfected mice, H. pylori–specific, Th2-associated IgG1 responses were also elevated in infected mice receiving H. pylori antimicrobial eradication therapy at 12 and 22 WPI ($P < 0.05$ and $P = 0.06$, respectively) and in untreated infected mice ($P = 0.08$). Compared with untreated H. pylori–infected mice, H. pylori–specific IgG1 levels were reduced in mice that received antimicrobial eradication therapy at 8 WPI ($P < 0.01$), but were not affected by antimicrobial eradication therapy at 12 or 22 WPI ($P = 0.11$). H. pylori–specific IgG1 levels in mice that received antimicrobial eradication therapy at 8 WPI were also lower than those in mice that received antimicrobial eradication therapy at 12 or 22 WPI ($P < 0.01$ and $P < 0.05$, respectively).

**Antimicrobial eradication therapy was associated with decreased gastric IFN-γ, TNF-α, and iNOS mRNA levels compared with untreated infected mice.** Given the importance of the inflammatory response in the pathogenesis of H. pylori gastritis, we analyzed selected proinflammatory cytokines and iNOS mRNA levels in the gastric tissue at 28 WPI. Compared with uninfected mice, gastric IFN-γ mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice ($P < 0.001$; Fig. 3B). H. pylori–infected mice that received H. pylori antimicrobial therapy at 12 and 22 WPI also had up-regulated gastric IFN-γ mRNA levels compared with uninfected mice ($P < 0.001$ and $P < 0.05$, respectively).
of gastric mucosa (29) and is up-regulated in H. felis–infected INS-GAS mice (30). To further investigate the possible mechanisms for the inhibitory effect of H. pylori antimicrobial eradication therapy on progression of gastric lesions, we analyzed gastric Reg 1 mRNA levels. Compared with those in uninfected mice, gastric Reg 1 mRNA levels were up-regulated in untreated H. pylori–infected INS-GAS mice (P < 0.001; Fig. 5A). Infected mice receiving antimicrobial eradication therapy at 8, 12, or 22 WPI had gastric Reg 1 mRNA levels that were comparable with each other and with those of uninfected mice. Additionally, antimicrobial eradication therapy at all time points significantly reduced Reg 1 expression compared with untreated, infected mice (P < 0.01).

Gastric mucosal cell proliferation was also reduced in all mice that received H. pylori antimicrobial eradication therapy. Epithelial proliferating cells detected by Ki67 immunohistologic staining were mainly in the isthmus regions of corpus mucosa in uninfected INS-GAS mice and H. pylori–infected mice that received eradication therapy at 12 WPI (Fig. 5B). Proliferating cells in the corpus expanded from isthmus regions to hypertrophic foveolar regions in untreated H. pylori–infected INS-GAS mice. Compared with uninfected mice, corpus epithelial proliferation, measured by Ki67 LI, was comparable in infected mice that received H. pylori eradication at 8 WPI (P = 0.093), but was higher in infected mice that received H. pylori eradication at 12 or 22 WPI in untreated H. pylori–infected mice (P < 0.05; Fig. 5C). H. pylori eradication at 8, 12, and 22 WPI significantly reduced Ki67 LI in the corpus compared with untreated H. pylori–infected mice (P < 0.01). Among the infected mice receiving H. pylori eradication therapy, mice receiving eradication therapy at 8 WPI had the lowest corpus epithelial LI when compared with mice receiving eradication therapy at 12 or 22 WPI (P < 0.05 and P = 0.058, respectively).

**Discussion**

In this study, we used the well-characterized INS-GAS male mouse model to elucidate the effect of H. pylori eradication therapy conducted at different stages of H. pylori–associated gastric pathology. H. pylori–infected INS-GAS mice developed GIN or gastric cancer at 28 WPI, accompanied by inflammation, loss of parietal and chief cells, and hypertrophy of foveolar glands. When H. pylori antimicrobial eradication therapy was instituted at 8 WPI, the risk of GIN was reduced to a comparable level of uninfected mice. Decreases in gastric inflammation, mRNA levels of proinflammatory cytokines and Reg 1, and epithelial cell proliferation in infected mice successfully treated with antimicrobials arguably contributed to lower dysplasia and reduced gastric cancer risk, particularly in those mice treated at 8 WPI. Eradication therapy at 12 and 22 WPI also resulted in a statistically lower degree of gastric inflammation, dysplasia, mRNA levels of proinflammatory cytokines and Reg 1, and epithelial cell proliferation and prevented progression to high-grade GIN, when compared with the data recorded in untreated H. pylori–infected mice. However, eradication therapy at 12 and 22 WPI did not reverse selected histopathologic changes, including inflammation, hyperplasia, and dysplasia, to the levels of uninfected mice. Nonetheless, it is also possible that the protective effect of H. pylori eradication at 12 or 22 WPI in INS-GAS mice may not be seen at 28 WPI. A longer period of time after H. pylori eradication therapy may be required in INS-GAS mice to observe the benefit of treatment during the late stages of H. pylori infection. These data in aggregate indicate that antimicrobial eradication therapy attenuated progression of H. pylori–induced gastric cancer, with early intervention providing the maximum benefit.

In comparable rodent studies, antimicrobial eradication therapy at 50 or 75 WPI prevented dysplasia in H. pylori–infected Mongolian gerbils (14). H. felis–infected C57BL/6 mice developed gastritis and invasive gastric cancer at 16 months postinfection (15). These lesions could be prevented and mucosal architecture restored if H. felis eradication therapy was instituted within 6 months of infection (15). Although H. felis eradication therapy at 12 months postinfection reduced the incidence of gastric cancer, it had little effect on the reversal of dysplasia in the C57BL/6 model (15). Consistent with the H. felis C57BL/6 model, our data show that H. pylori eradication administered at the early stages of H. pylori infection attenuated gastric inflammation, restored gastric mucosal architecture, and prevented the development of gastric cancer in INS-GAS mice. Moreover, because mature parietal cells are necessary to maintain the integrity of gastric mucosa, parietal cell loss may have resulted in dysregulation of gastric stem cell homeostasis and migration-associated differentiation of pit and zymogenic cells (31). Independent studies from our laboratory confirmed parietal cell loss in uninfected INS-GAS mice after 5 months of age (approximately equivalent to 12–14 WPI in our current study; ref. 18). Parietal cell mass and epithelial cell...
differentiation in *H. pylori*-infected INS-GAS mice may be partially restored and onset of precancerous lesions may be delayed by *H. pylori* eradication therapy given at 8 WPI, but not by antimicrobial therapy given at 12 WPI or later.

Gastric inflammation in INS-GAS mice positively correlated with epithelial proliferation. Previous studies suggest that IFN-γ induces proliferation of gastric epithelial cells (32, 33). Increased cell proliferation is a biomarker of gastric cancer risk, and reversal to a normal epithelial proliferation has been associated with a reduced gastric cancer risk (34, 35). In the current study, gastric epithelial proliferation rates and *Reg 1* mRNA levels in *H. pylori*-infected INS-GAS mice receiving *H. pylori* eradication therapy at 8 WPI returned to levels similar to those of uninfected mice, but not those in mice receiving eradication treatment at 12 or 22 WPI. With accumulated genetic damage in gastric mucosa as a result of long-standing *H. pylori* infection, *H. pylori* infection per se may no longer be necessary for sustained dysplasia and progression to gastric cancer.

Unexpectedly, *H. pylori*-infected mice receiving eradication therapy at 8 WPI had significantly lower degrees of gastric inflammation as evidenced by less severe atrophy and down-regulated IFN-γ, TNF-α, and iNOS mRNA levels relative to the *H. pylori*-unrelated gastric inflammation in uninfected INS-GAS mice. The mechanism by which antimicrobial eradication therapy exerted a protective effect on spontaneous gastric inflammation in INS-GAS mice is unknown. One possible explanation is that antimicrobial therapy eradicates not only *H. pylori* but also other microorganisms that could cause gastric inflammation, particularly when there is an elevated gastric pH (36, 37). Our laboratory observed that six of the eight bacterial species of altered Schaedler flora (37) were present in the stomach of *H. pylori*-infected dyspeptic patients who received a triple antimicrobial therapy regimen with omeprazole, metronidazole, and clarithromycin. Certain species of bacteria were eradicated by the antimicrobial therapy regimen with omeprazole, metronidazole, and clarithromycin.
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Cancer Research

Helicobacter pylori Eradication Prevents Progression of Gastric Cancer in Hypergastrinemic INS-GAS Mice

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doi:10.1158/0008-5472.CAN-07-6786

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