Overexpression of Glycine-Extended Gastrin Inhibits Parietal Cell Loss and Atrophy in the Mouse Stomach

Guanglin Cui,1 Theodore J. Koh,1 Duan Chen,2 Chun-Mei Zhao,2 Shigeo Takaishi,1 Graham J. Dockray,3 Andrea Varro,3 Arlin B. Rogers,4 James G. Fox,4 and Timothy C. Wang1

1Division of Gastroenterology, University of Massachusetts Medical School, Worcester, Massachusetts; 2Department of Cancer Research and Molecular Medicine and Laboratory Medicine, Norwegian University of Science and Technology, Trondheim, Norway; 3The Physiological Laboratory, University of Liverpool, Liverpool, United Kingdom; and 4Division of Comparative Medicine, Massachusetts Institute of Technology, Boston, Massachusetts.

ABSTRACT

Recently we have reported synergistic effects between glycine-extended gastrin (G-gly) and amidated gastrin-17 on acid secretion in short-term infusion studies. In the present study, we examined the long-term effect of G-gly on the atrophy-promoting effects of amidated gastrin in the mouse stomach with or without Helicobacter infection. Transgenic mice overexpressing amidated gastrin (INS-GAS mice), G-gly (MTI/G-gly mice), and both peptides (INS-GAS/G-gly mice) were used for assessment of acid secretion and ulcer susceptibility and histologic examination and scoring of preneoplastic lesions in response to the 3 and 6 months Helicobacter felis infection. We found that MTI/G-gly mice had normal gastric histology and acid secretion. Double transgenic (INS-GAS/G-gly) mice showed 2-fold increases in acid secretion compared with INS-GAS mice. Acute peptic ulcers after pyloric ligation were noted in 50% of the INS-GAS/G-gly mice but in none of the INS-GAS mice at 6 months of age. Whereas male INS-GAS mice had a >50% decrease in the numbers of parietal cell and enterochromaffin-like cell at 6 months of age, the male double transgenic mice had no such decrease. Overexpression of G-gly reduced the scores of preneoplasia in the stomach; however, it did not prevent the development of amidated gastrin-dependent gastric cancer in both H. felis-infected mice and uninfected mice. We conclude that G-gly synergizes with amidated gastrin to stimulate acid secretion and inhibits parietal cell loss in INS-GAS/G-gly mice. The overexpression of G-gly seems to increase the susceptibility to peptic ulcer disease and delay the development of Helicobacter-mediated gastric preneoplasia in this model.

INTRODUCTION

Gastric cancer develops through a series of discrete steps now known as the atrophy-metaplasia-dysplasia-carcinoma sequence (1). One critical stage in this multistep process is the induction of parietal cell loss, which results in gastric glandular atrophy in the stomach (1). It is now well established that infection with Helicobacter pylori (H. pylori) is the major cause of both gastric atrophy and gastric cancer (2, 3), but interestingly H. pylori is also the major pathogenetic factor for the development of duodenal ulcer disease (4). The mechanism by which a single bacterial agent leads to two distinct clinical outcomes has been a question of great interest, particularly because patients with H. pylori infection was associated with elevation in the levels of amidated gastrins, including gastrin-17 (G-17). This elevation provided a possible explanation for the increase in gastric acid secretion observed in ulcer patients and was labeled as the “gastrin link” (11). In addition, amidated gastrin concentrations are often elevated in patients who later progress to gastric cancer. Recent work from our laboratory (12) has indicated that in a transgenic mouse model of hypergastrinemia (i.e., INS-GAS mice), increased circulating concentrations of amidated gastrin leads over time to gastric glandular atrophy (characterized by loss of parietal and chief cells) and invasive gastric cancer.

A potential explanation for the diverse roles of gastrin in these diseases is related to gastrin processing and the role of incompletely processed gastrins. Gastrin is initially synthesized in the stomach as gastrin-34, which is then processed to glycine-extended gastrin (G-gly) and finally to amidated gastrin [gastrin-17 (G-17) and G-34; ref. 13]. In the majority of earlier studies, only the amidated gastrins were measured whereas the incompletely processed gastrins were largely ignored. However, studies from our laboratory as well as others have shown that incompletely processed gastrins are biologically active and play a role in both the growth and differentiation of the gastrointestinal mucosa (14–18). Finally, in our previous study in gastrin-deficient mice, infusions of G-17 alone led to an initial increase (at 1 and 6 days) in acid secretion followed by a diminution of acid secretion at 14 days. Although G-gly alone had no effect on acid secretion, the combination of G-17 and G-gly not only led to a higher level of acid secretion than G-17 alone at early time points but also prevented the later diminution (19).

Given these observations, we hypothesized that G-gly may be one of the factors influencing the host to be prone to either peptic ulcer or gastric cancer in the setting of Helicobacter infection. To address the long-term consequences of elevated G-gly levels with or without an increased circulating amidated gastrin, we compared transgenic MTI/G-gly mice that have increased circulating concentrations of G-gly levels that have increased circulating concentrations of G-gly (17) either with transgenic INS-GAS mice that produce elevated amidated gastrin or with double transgenic mice by crossing the two lines of mutant mice. Our findings suggest that the increased G-gly increases susceptibility to peptic ulcer disease and inhibits progression to gastric atrophy.

MATERIALS AND METHODS

Animals. Wild-type (WT) FVB/N mice (Charles River Lab., Wilmington, MA), INS-GAS transgenic mice (which have increased only amidated gastrin up to 500 pmol/L; approximately; ref. 12), MTI/G-gly transgenic mice (increased only G-gly up to 85 pmol/L; ref. 17), and INS-GAS/G-gly double transgenic mice (increased both amidated gastrin and G-gly up to 459 and 115 pmol/L respectively) were included in this study. INS-GAS and MTI/G-gly mice have been described previously in detail (12, 17) and were produced in the same way. The INS-GAS/G-gly mice were generated by first crossing MTI/G-gly mice to INS-GAS mice for three generations to homozygous for both transgenes. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. Requests for reprints: Timothy C. Wang, Division of Digestive and Liver Diseases, Department of Medicine, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, Box 83, New York, NY 10032. Phone: 212-342-3412 or 305-8156; Fax: 212-305-6443; E-mail: tww21@columbia.edu.
approved by the Animal Welfare Committees of University of Massachusetts Medical School or Massachusetts Institute of Technology.

Stomach Histology and Immunohistochemistry in INS-GAS and INS-GAS/G-gly Mice. The gastric histology of MTI/G-gly mice at different ages has been examined previously and reported by our laboratory to be grossly normal (17). Male INS-GAS and INS-GAS/G-gly mice were examined in this study. Midline strips along the lesser curvature of the stomach were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin. Sections were cut at 4 μm and then stained with H&E. Immunohistochemistry was done with avidin-biotin-peroxidase complex kits (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. The following primary antibodies were used: against chromogranin A to stain enterochromaffin-like (ECL) cells (working dilution 1:500, rabbit antiporcine, Immunostar, Hudson, WI), H⁺K⁺-ATPase β subunit to parietal cells (1:2,000, mouse antihist. Affinity Bioreagents, Golden, CO), somatostatin to D cells (1:600, rabbit anti-human, Dako Corp., Carpinteria, CA), and rabbit anti-human gastrin/cholecystokinin B (CCKB) receptor to show its distribution in the fundus (1:50, Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibodies were incubated at 4°C overnight in humidified chamber. 3-Amino-9-ethylcarbazole (Vector Laboratories) was used as a chromogen, and slides were counterstained with Mayer’s hematoxylin.

To measure gastric cell proliferation and apoptosis, male WT, INS-GAS, and INS-GAS/G-gly mice received injection intraperitoneally with 5’-bromo-2’-deoxyuridine (BrdUrd) at a single dose of 50 mg/kg, 1 hour before euthanasia. The BrdUrd incorporation was done with a monoclonal rat anti-BrdUrd antibody (1:70, Accurate Chemical and Scientific, Westbury, NY). Apoptosis in gastric cells was evaluated with a rabbit monoclonal antibody specific for cleaved (activated) caspase-3 antibody (1:100, Cell Signaling Technology, Beverly, MA) and terminal dUTP nick-end labeling (TUNEL) staining (Merek Bioscience Ltd., Nottingham, United Kingdom). In addition, double staining for antibodies against caspase-3 with H⁺K⁺-ATPase β subunit or fluorescence (FITC)-labeled Ulex europaeus agglutinin I lectin (1:400, Sigma, St. Louis, MO) to show apoptotic parietal cells and pit cells (also called mucus cells), respectively, according to our method published previously (20).

Cell Counting. Only immunoreactive epithelial cells with appropriate morphology and location in well-oriented sections were counted in at least five randomly selected visual fields under microscope (objective, ×20; eyepiece, ×10; visual field diameter, 1.85 mm, CX31, Olympus Optical Co., Ltd., Manila, Philippines). The immunoreactive epithelial cell densities were expressed as number per gland for parietal cells (at least 10 glands were selected per slide) and per field for ECL cells, fundic D cells, BrdUrd-labeling cells, and caspase-3-immunoreactive cells.

Determination of Gastric Acid Secretion and Susceptibility to Gastric Mucosal Damage in Transgenic Mice. Seven to 15 mice of each genotype (INS-GAS, INS-GAS/G-gly, and MTI/G-gly) at ages of 2 to 3 months and 11 to 12 months were selected for determination of acid secretion and at the age of 6 months for assessment of mucosal damage via pyloric ligation technique (21). Gastric juice was collected and measured with a pH meter (AR 25, Fisher Scientific, Houston, TX) by 0.01 N NaOH titration, and results were expressed as micro-equivalents. Grossly visible damage to the mucosa was assessed.

Heliobacter felis (H. felis) Infection in Transgenic Mice. Three to five mice of each genotype at 4 to 5 weeks of age were inoculated by mouth with H. felis (49179, American Type Culture Collection, Manassas, VA) as described previously (22). Male MTI/G-gly mice, INS-GAS mice, and WT mice were infected with H. felis for 3 months to examine the possible protective effects of G-gly alone, and gastric histologic score was evaluated. For long-term studies, both male and female INS-GAS, INS-GAS/G-gly, and WT FVB/N mice were infected with H. felis and euthanized at 6 months postinfection for gastric histologic evaluation. Epithelial hyperplasia (both antrum and fundus), gastric atrophy (loss of fundic glands), and inflammation were graded semiquantitatively as 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked; ref. 23).

Data Analysis. One way ANOVA (among three groups) and Mann-Whitney test (between two groups) were used; a P < 0.05 value was considered significant.

RESULTS

Overexpression of G-gly Maintains the Parietal Cell Population and Delays the Development of Gastric Atrophy in INS-GAS/G-gly Mice. Previous studies from our group showed that INS-GAS mice had a gradual loss of parietal and ECL cells over time, with eventual progression to gastric cancer (12), but MTI/G-gly mice maintained a normal gastric histology (17). To determine whether overexpression of G-gly expression influences parietal cell and ECL cell densities, we examined mucosal histology along with parietal cell density in WT, INS-GAS, and INS-GAS/G-gly mice at 2 to 3 months, 5 to 6 months, and 11 to 12 months of age (Fig. 1A). Parietal cell density was increased slightly at 2 to 3 months of age in both INS-GAS and INS-GAS/G-gly mice compared with WT mice. However, parietal cell number was decreased in INS-GAS mice at 5 to 6 and 11 to 12 months of age but not in the INS-GAS/G-gly double transgenic mice (P < 0.01; Fig. 1A). In addition to the changes in parietal cell number, INS-GAS mice showed similar changes in ECL cell and fundic D cell numbers, with an initial increase in ECL cell number at 2 to 3 months and a marked declined in ECL and D cells at 5 to 6 and 11 to 12 months relative to WT mice (Fig. 1B and C). INS-GAS/G-gly mice showed no decrease in ECL or fundic D cell number at these later time points. Nevertheless, both the INS-GAS and the INS-GAS/G-gly showed progressive foveolar hyperplasia, which was most notable in the older mice (data not shown). Previously we had reported that the gastrin/CCKB receptor was expressed mainly in parietal cells in male INS-GAS mice (20). In the current study, immunohistochemical analysis further showed that in both lines of transgenic mice, the gastrin/CCKB receptor was predominately expressed in the middle and lower third of the fundic glands (Fig. 1D and E), the primary location of parietal cells, ECL cells, and chief cells. The parietal cell seemed to be the main gastric cell type that was positive for gastrin/CCKB receptor immunoreactivity in both lines of transgenic mice. In association with the decrease in parietal cell density with age in the INS-GAS mice, the number of cells immunoreactive for the gastrin/CCKB receptor was markedly reduced, whereas these changes were delayed in INS-GAS/G-gly mice. In both lines of transgenic mice with gastric cancer, only a few cells could be detected with gastrin/CCKB receptor immunoreactivity.

Double Transgenic INS-GAS-G-gly Mice Have Hypersecretion of Gastric Acid and Increased Susceptibility to Peptic Uleration. MTI/G-gly mice examined at different ages had a normal gastric acid secretion level (Fig. 2A). INS-GAS mice at 2-months of age had a ~2-fold increase in acid secretion, whereas the MTI/G-gly mice showed no difference compared with WT controls. The double transgenic INS-GAS/G-gly mice at two-months of age had a ~2-fold higher rate of acid secretion compared with the INS-GAS mice (Fig. 2A). By 12 months of age, the rate of acid secretion had declined in both INS-GAS and INS-GAS/G-gly mice compared with their respective rates at 2 months, but it remained higher in INS-GAS/G-gly mice compared with INS-GAS, MTI/G-gly, and WT mice (Fig. 2A). It was noted that the INS-GAS/G-gly mice (particularly those <6 months of age) frequently had hemorrhagic gastric mucosal defects after pyloric ligation, whereas these lesions were not observed in MTI/G-gly mice. To explore this observation further, pyloric ligation was done on 15 male INS-GAS-G-gly and 15 male INS-GAS mice that were 5 to 6 months of age. The stomachs of INS-GAS mice were grossly normal (Fig. 2B), whereas the stomachs of ~50% (8 of 15) of the INS-GAS-G-gly double transgenic mice exhibited loss of mucosal surface integrity with bleeding, primarily in the fundus (Fig. 2C). Histologic analysis of affected stomachs confirmed the presence of acute erosions characterized by superficial epithelial cell necrosis, hemorrhage, and edema.
GLYCINE-EXTENDED GASTRIN AND ATROPHY IN MOUSE STOMACH

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Overexpression of G-gly Does Not Alter Gastric Cell Proliferation, but Does Modulate Apoptosis on Parietal Cells of Hypergastrinemic Mice. BrdUrd labeling was done in WT, INS-GAS, and INS-GAS/G-gly mice of different ages (Fig. 3). These studies revealed that most gastric cells with BrdUrd labeling were located in the neck cell region of the gastric glands. The proliferation-labeling index was higher in both the INS-GAS and INS-GAS/G-gly mice relative to WT mice at all ages (P < 0.05 and P < 0.01, respectively), with no statistical difference noted between INS-GAS and INS-GAS/G-gly mice (P > 0.05).

Apoptosis or programmed cell death plays an important role in maintaining gastric cell homeostasis and also seems to be an important trigger for progression to gastric atrophy. Thus, immunostaining for cleaved (activated) caspase-3 showed that apoptotic cells were increased in number in young (2–3 months) male INS-GAS and INS-GAS/G-gly mice. Generally, young male INS-GAS mice had a significantly higher apoptosis index in the gastric mucosa compared with male INS-GAS/G-gly mice of the same age (Fig. 4). Caspase-3–positive cells in young transgenic mice could be detected in both the gastric pit cells (nuclear positive) and gastric glands (mainly parietal cells, both cytoplasm and nuclear positive). In addition, increased staining of inflammatory cells could also be seen. However, double staining for antibodies against caspase-3 and H^+K^-ATPase β subunit or FITC-lectin showed that 2–3-month-old male INS-GAS mice clearly had increased glandular staining, particularly of parietal cells (Fig. 5A), whereas age matched INS-GAS/G-gly mice showed less glandular apoptosis, but a higher surface mucous (pit) cell apoptosis (Fig. 5B). These results were further confirmed by TUNEL staining (Fig. 5, C and D). In 5- to 6-month-old transgenic mice, the level of apoptosis was still elevated. The ratio of apoptotic cells/proliferation labeling index was higher in INS-GAS mice compared with INS-GAS/G-gly mice at both early time points (2 months, 0.87 versus 0.25; 5–6 months, 1.08 versus 0.49) but was decreased in both lines of transgenic mice at the age of 11 to 12 months (0.24 versus 0.23). The apoptotic gastric cell number was not statistically different between the two sets of transgenic mice at the older age (Fig. 4).

G-gly Inhibits Progression to Atrophy, but Does Not Prevent the Development of Gastric Cancer in INS-GAS/G-gly Mice with or without H. felis Infection. MTUG-gly mice examined at different ages had grossly normal stomach histology as described previously (17). Whereas INS-GAS/G-gly mice had lesser degrees of atrophy at all time points examined up until 1 year of age, they nevertheless had a progressive increase in mucosal thickness similar to that observed in INS-GAS mice. At 12 months of age, a significant degree of hyperplasia and metaplasia with occasional dysplasia was evident in both

Fig. 1. Fundic gastric cell densities in gastrin transgenic mice. A, at 2 to 3 months of age, parietal cell density was slightly increased in both INS-GAS mice (II) and INSGAS/G-gly mice (III; P > 0.05, significant from each other). At 5 to 6 months and 11 to 13 months of age, parietal cell density was decreased in INS-GAS mice compared with INS-GAS/G-gly mice of age (P < 0.05, significant in INS-GAS compared with INS-GAS/G-gly mice). B, at 2 to 3 months of age, the density of ECL cells was initially increased in both INS-GAS mice (II) and INS-GAS/G-gly (III) male transgenic mice compared with WT mice (II, P < 0.05). At later time points (5–6 months and 11–12 months old), ECL cell density is decreased in INS-GAS mice (P < 0.01, significant from WT mice and INS-GAS/G-gly mice). The density of ECL cells in male INS-GAS/G-gly mice was similar to WT controls at these later time points (P > 0.05). C, at 2 to 3 months of age, the density of fundic D cells was similar in all experimental groups, but at 5 to 6 months and 11 to 12 months of age, fundic D cells were decreased in male INS-GAS mice compared with WT controls and INS-GAS/G-gly mice (P < 0.05; P < 0.01; significant from WT mice and INS-GAS/G-gly mice). D and E, gastrin/CKCB receptor immunohistochemistry in male INS-GAS mice and INS-GAS/G-gly mice at the age of 3 months. The gastrin/CKCB receptor was mainly expressed in the middle and basal parts of fundic glands in both lines of transgenic mice. The parietal cell is the main cell type immuno-reactive for gastrin/CKCB receptor in both male INS-GAS mice (D) and INS-GAS/G-gly mice (E). 3-Amino-9-ethylcarbazole chromogen, counterstained by Hematoxylsin, original magnification 400X.
INS-GAS and INS-GAS/G-gly mice. At 18 months of age, all male INS-GAS and INS-GAS/G-gly mice had evidence of intramucosal or invasive gastric cancer (Fig. 6A and B). Interestingly, the cancer lesions in the INS-GAS/G-gly mice seemed to be somewhat more advanced and tended to show more submucosal invasion compared with those observed in the INS-GAS mice.

The inhibitory effect of G-gly on *H. felis*-induced gastric atrophy was examined in transgenic mice overexpressing G-gly. Three-month *H. felis* infection in male MTI/G-gly mice resulted in a decreased histologic score compared with INS-GAS mice and WT mice with *H. felis* infection (*P < 0.05*, Table 1). Six-month *H. felis* infection studies of WT, INS-GAS, and INS-GAS/G-gly mice of both genders were then done. In general, males of both transgenic genotypes showed much greater histologic scores compared with WT male FVB/N mice in response to 6 months of *H. felis* infection; furthermore, male transgenic genotypes showed more severe lesions than females (Table 2). In male INS-GAS/G-gly mice, the scores for glandular atrophy and corpus and antral metaplasia were decreased compared with male INS-GAS mice (*P < 0.05*, Table 2). At the time of euthanasia (7 months old), gastric cancer was histologically evident in 100% of male INS-GAS and 86% of male INS-GAS/G-gly mice, a difference that was not statistically significant (*P > 0.05*).

**DISCUSSION**

Our current results show the biological activity of G-gly and the importance of G-gly as a key modulator of parietal cell function in the
mouse. We show here that overexpression of G-gly is able to synergize with G-17 to maintain acid hypersecretion, inhibit progression to atrophy, and increase ulcer susceptibility.

Given that the effect of G-gly on the gastric mucosa seems to be observed primarily in the setting of G-17 stimulation (19), one can postulate that the mechanism may involve modulation or antagonism of pathways downstream of CCK-B receptor signaling. Todisco et al. (24) have shown that amidated gastrin (G-17) stimulates rat pancreatic AR42J cell proliferation by activation of the early genes c-fos and c-jun through a mitogen-activated protein kinase signaling pathway, whereas G-gly stimulates cell growth by post-translational activation of c-jun through c-Jun NH2-terminal kinase activation. In the INS-GAS/G-gly model, the effect of G-gly does not seem to involve any change in the rate of cellular proliferation, because BrdUrd-labeling rates in double transgenic mice were generally the same as those in INS-GAS single transgenic mice. In addition, the effect of G-gly was not specific for parietal cells, because similar protective effects were observed with ECL cell and somatostatin (D) cell. Although we cannot exclude that amidated and glycine-extended gastrin are acting directly on the gastric stem cell, we suspect that a major target of the effects of G-gly may be the parietal cell. Immunohistochemical studies confirmed that parietal cell is the main source of gastrin/CCKB receptor expression in the gastric fundus of both INS-GAS mice and INS-GAS/G-gly mice. Recently, a cytoskeletal protein, ezrin, which is expressed predominantly in parietal cells, was shown to be decreased in gastrin-deficient mice and up-regulated by administration of G-17 and G-gly (25) and may be involved in parietal cell maturation.

The balance between cell proliferation and apoptosis is important for gastric mucosal homeostasis. Increased apoptosis in the gastric mucosa has been observed with increased cell turnover and is a predisposing factor in gastric carcinogenesis (26). Gastrin is a critical trophic factor for the oxyntic mucosa both in humans and animals (27). An association between apoptosis and hypergastrinemia has been reported previously in a rodent (Mastomyx) model using an H2 receptor antagonist (28). Moreover, given the finding of accelerated parietal cell loss in INS-GAS mice, we considered the possibility that this was attributable to increased apoptosis, and we showed that the expression of G-gly led to significant protection against G-17–stimulated apoptosis. The apoptotic cell/proliferation labeling index ratio measured in 2- to 6-month INS-GAS/G-gly mice was lower than that in age-matched INS-GAS mice. The majority of apoptotic cells in young INS-GAS mice could be found in the middle and lower third of the gastric glands, where parietal cells and chief cells are common, whereas many apoptotic cells in young INS-GAS/G-gly mice were located in the gastric pit region. The difference in apoptotic cell distribution between younger INS-GAS mice and INS/GAS/G-gly mice provides a partial explanation for the increased parietal cell loss and earlier progression to atrophy in male INS-GAS mice. In contrast, an antiapoptotic effect of amidated gastrin was reported recently in the rat pancreatic AR42J cell line, the malignant esophageal epithelial cell lines (OE19 and OE21), and the Barrett’s esophagus cell line OE33, via the activation of protein kinase B/Akt signaling pathway (29, 30).

Protein kinase B/Akt is a serine-threonine protein kinase activated in response to growth factors (such as insulin, platelet-derived growth factor, and epidermal growth factor) that inhibits apoptotic pathways.

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Table 1 Gastric histologic scores in male MTI/G-gly mice and INS-GAS mice with 3-month H. felis infection

<table>
<thead>
<tr>
<th></th>
<th>WT (FVB) H. felis (+) male</th>
<th>INS-GAS H. felis (+) male</th>
<th>MTI/G-gly H. felis (+) male</th>
<th>MTI/G-gly H. felis (−) male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>2.167 ± 0.33†</td>
<td>1.88 ± 0.48†</td>
<td>1.30 ± 0.20†‡</td>
<td>0.50 ± 0.29†‡</td>
</tr>
<tr>
<td>Atrophy</td>
<td>2.17 ± 0.17</td>
<td>2.13 ± 0.25†</td>
<td>1.70 ± 0.13†‡</td>
<td>0.50 ± 0.29†‡</td>
</tr>
<tr>
<td>Glandular Metaplasia</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Intestinal</td>
<td>1.83 ± 0.44</td>
<td>*</td>
<td>1.30 ± 0.30†‡</td>
<td>0.17 ± 0.17†‡</td>
</tr>
<tr>
<td>Metaplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>1.83 ± 0.44</td>
<td>2.25 ± 0.65†</td>
<td>1.10 ± 0.24†‡</td>
<td>0.50 ± 0.00‡</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>1.67 ± 0.33</td>
<td>1.63 ± 0.63†</td>
<td>0.50 ± 0.16†‡</td>
<td>0.00‡</td>
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* Data not available.
† P < 0.05; MTI/G-gly mice with or without H. felis infection compared with H. felis infected WT mice or INS-GAS mice.
‡ P < 0.05; MTI/G-gly mice with or without H. felis infection compared with each other.
The divergent effects of gastrin on cellular apoptosis observed in these studies may be resulted from the different model system used (malignant cell lines versus intact whole animal) and the different duration of gastrin stimulation. For example, whereas gastrin can clearly stimulate ECL cell growth, Kimura et al. (32) have found that over-stimulation by amidated gastrin can in fact lead to ECL cell damage and death.

It is now well established in several model systems that loss of parietal and chief cells represents an important biological trigger for the progression to gastric metaplasia and cancer (12, 33–35). In addition, parietal cells play an important role in the modulation of gastric stem cell proliferation and differentiation (36, 37). Thus, factors such as G-gly that maintain parietal cell mass and acid secretory capacity might influence the overall response to infection with gastric Helicobacter. The INS-GAS/G-gly double transgenic mice were highly susceptible to peptic ulcer disease after pyloric ligation (a procedure that induces maximal acid secretion) whereas INS-GAS and WT animals showed no such susceptibility. The effects of G-gly on slowing the rate of G17-mediated parietal cell loss seem to be of some clinical relevance. Although the mice did not develop chronic duodenal ulcer disease (either with or without H. felis infection), the atrophy-resistant and ulcer-susceptible phenotype shows some similarity to the duodenal ulcer model in humans.

An inhibitory effect of G-gly on gastric preneoplastic lesions was found in transgenic mice overexpressing G-gly. Reduced histologic scores of gastric preneoplastic changes were found in MTI/G-gly mice with 3 months of H. felis infection or INS-GAS/G-gly mice with 6 month of H. felis infection. The development of gastric atrophy was clearly inhibited or delayed compared with age-matched INS-GAS mice and WT mice. Overexpression of G-gly in transgenic mice provided relative protection against the development of atrophy, possibly attributable in part to the suppression of inflammation. Interestingly, gut hormones have been postulated to act as local modulators of gastric inflammation. Somatostatin has been noted to have an anti-inflammatory function, and gastrin has been shown to suppress somatostatin and D cell numbers in mice (38, 39). In this study, we showed a decreased fundic D cell number in INS-GAS/G-gly mice, an effect that was abrogated by overexpression of G-gly. Additional studies are needed to determine whether D cells contribute to the prevention of the development of atrophy and to establish the mechanism of action of G-gly on somatostatin in H. felis infection.

In both lines of H. felis-infected transgenic mice (INS-GAS and INS-GAS/G-gly), gastric cancer was found mainly in male animals rather than females. This gender specificity of tumor occurrence seems consistent with our previous findings (40) and is also concordant with the greater incidence of gastric carcinoma in men. Importantly, overexpression of G-gly did not prevent the eventual progression to gastric cancer either in the uninfected or the H. felis-infected INS-GAS/G-gly mice. This was somewhat surprising, given that the development of gastric atrophy was clearly inhibited or delayed at all earlier time points. Given this discrepancy, one possible conclusion is that the G-gly–expressing mice showed a more rapid progression to cancer once severe atrophy had developed. In fact, histologic evaluation of the gastric cancer lesions suggests that those from the double transgenic mice are somewhat more aggressive in appearance. This likely reflects the dual nature of the G-gly effect (41), and the unique nature of the MTI/G-gly–transgenic mouse model. The MTI/G-gly mice exhibit not only increased circulating levels of G-gly but also broad expression of G-gly in most epithelial tissues. In tissues such as the colon, G-gly clearly has proliferative effects (17, 42). Thus, our current interpretation of the data would be consistent with a model in which circulating G-gly inhibits progression to atrophy (and cancer), but G-gly expression (by proliferating progenitor cells) can enhance later progression of the tumor. Future studies will be required to explore these diverse effects of G-gly on the gastric epithelium.

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