IT HAS BEEN ESTABLISHED THAT Helicobacter pylori induces mucosal inflammation or gastritis and subsequently major cellular and physiological changes in the stomach (10). In particular, H. pylori colonization of the stomach correlates with an increase in gastric G cells, gastrin expression, and secretion (10). Basal levels of acid secretion rise, presumably due to the elevated levels of circulating gastrin. It is assumed that the high gastric acid levels stimulate the production of somatostatin through putative chemoreceptors on the D cell. Once the D cell is activated, secreted somatostatin should inhibit the G cell by paracrine mechanisms (23).

Rather, what are observed in H. pylori-infected patients are reduced numbers of D cells and somatostatin in the face of elevated gastrin production and acid secretion (12, 24). To explain these findings, a direct inhibitory effect by inflammatory cytokines on D cells has been proposed (2, 3, 5). Evidence for the immunoregulation of somatostatin originates from in vitro studies demonstrating that cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-8 inhibit somatostatin release from isolated rabbit and canine fundic D cells (2).

Gastrin null mice created by homologous recombination are hypochlorhydric (11, 13). We have recently studied these mice in greater detail and have found that they also exhibit significant gastric mucosal inflammation caused by bacterial overgrowth as a consequence of reduced gastric acidity (30). The present study extends these findings by measuring changes in tissue somatostatin and D cell numbers in gastrin null mice. As reviewed above, the levels of tissue somatostatin and the D cell numbers are quite relevant to understanding the regulation of gastric acid secretion. Thus it is important to evaluate the reason for suppressed tissue somatostatin levels during gastritis. Our studies in the gastrin-deficient and omeprazole-treated mice (OM) indicate that in the presence of gastritis, the stomach is stimulated to maximize acid output (30). Thus it is logical to propose that, at the same time, the stomach will act to suppress the inhibitors of gastric acid secretion. Because somatostatin is a major inhibitor of gastrin secretion and therefore acid secretion, we hypothesize that during inflammation, somatostatin production is actively inhibited.

Analyzing the D cell population in the gastrin-deficient mice, we observed and report here that both D cells and somatostatin levels remain elevated despite significant gastritis. This raised the interesting possibility that gastrin may be the regulator of the D cell. We tested this hypothesis by infusing gastrin into the gastrin-deficient mice in the presence of antibiotics to resolve the inflammation. We found that the gastrin
infusion suppressed both fundic and antral D cell populations and tissue somatostatin levels. We also examined changes in D cells in mice made hypergastrinemic by omeprazole.

**MATERIAL AND METHODS**

**Animals.** Gastrin-deficient (G−/−) mice and strain-matched (C57Bl/6 × 129/Sm) wild-type controls (G+/+) were bred by homozygous mating. G+/+ (n = 10) and G−/− (n = 12) mice were maintained in individual sterile microisolator cages in nonbarrier mouse rooms for 16 wk. All mice were fasted overnight with access to water ad libitum before analysis. The study was performed with the approval of the University of Michigan Animal Care and Use Committee, which maintains an American Association for Accreditation of Laboratory Animal Care facility.

**Antibiotic treatment.** At 13 wk of age, G+/+ and G−/− were treated with antibiotics by adding 5 mg/kg of streptomycin to their drinking water for 3 wk. At the same time, mice were given a subcutaneous injection of 100 mg/kg kg−1day−1 of colopectazone (Sigma) (26). Each day, their feces were collected, weighed, suspended in 1 ml PBS by vortexing, and then diluted 1:100. One hundred microliters of the suspension were cultured on Luria broth plates and incubated at 37°C for 24 h. The colonies on each plate were counted, and the results were expressed as colony-forming units (CFU) per gram of feces. After 5 days of antibiotic treatment, the CFU per gram feces decreased significantly from 300 × 105 to 85 × 105 CFU/g feces. The bacterial count continued to decrease over the next 15 days of treatment (data not shown), and bacterial counts were undetected in mouse feces by day 20. Therefore, we chose this duration for treatment. Mice were killed after 20 days of antibiotic treatment and analyzed.

**G-17 infusion.** G−/− mice were anesthetized with xylazine (20 mg/ml) and ketamine (100 mg/ml) at 8 wk of age. A midline incision was made in the skin below the ribcage. Another incision was then made in the abdominal muscle directly under the cutaneous incision. The microsomatic pump (model 1002; Alzet), delivering 5 μg/kg h−1 of rat nonsulfated gastrin (G-17; Bachem) was inserted into the peritoneal cavity. The muscle and skin incisions were then closed by silk sutures. Mice were continuously infused with G-17 for 14 days before death and analysis.

**Omeprazole treatment.** Eight-week-old G+/+ mice were made hypochlorhydric by treating with a single intraperitoneal injection of omeprazole per day (400 μmol/kg) for 8 wk. The stock solution of omeprazole (80 μmol/ml) was dissolved in DMSO/PEG (4.5:0.5 vol/vol) and stored at −20°C until use. Eight-week-old control mice were treated in the same manner with the vehicle alone. All mice were killed at 16 wk of age and analyzed. In a separate group, 8-wk-old G+/+ animals were also treated with omeprazole for 8 wk but were treated with the same antibiotic regimen as described above for 20 days. These animals were also killed at 16 wk of age.

**Quantitative and qualitative microbiology.** The wet weight of the antral and fundic tissue was determined. Tissue was homogenized briefly with a polytron PT-2000 (Kinematica, Cincinnati, OH) in 1 ml saline, and appropriate dilutions were spread on blood agar plates containing Campylobacter-base agar (Difco, MI) supplemented with 5% horse blood (Colorado Serum, Denver, CO) and 2 μg/ml nystatin (Sigma, St. Louis, MO). Plates were incubated under aerobic, microaerophilic, and anaerobic conditions (CampyPak Plus, GasPak Plus; Anaerobic Systems) at 37°C for 3–5 days. The number of colonies was counted, and the CFU per gram of tissue were calculated. Comparisons between groups were based on the log concentrations of bacteria. For statistical analysis, the total viable count was determined by a total colony count of all bacterial types on aerobic, facultative anaerobic, and anaerobic cultures. Single colonies were used to identify major bacterial species by the Medical Microbiology Department at the University of Michigan Hospital using specific nutrient culture conditions, biochemical assays, and the API System for fermenting and nonfermenting gram-negative bacteria.

To exclude the presence of *H. pylori*, an aliquot of the homogenate was spread on selective blood plates supplemented with 5 μg/ml vancomycin and 10 μg/ml trimethoprim lacate (Sigma, MO), and then incubated for 5 days at 37°C under microaerophilic conditions. Single colonies from these plates were tested for urease (using a drop of urea broth: 10 g urea, 0.5% wt/vol phenol red, 0.22 g Na2HPO4·H2O, 0.51 g Na2HPO4, 100 mg NaN3, and 500 ml distilled water at pH 6.2), catalase (using 3% H2O2 solution), and oxidase (DrySlide) activity. None of the tested colonies were positive for any of the three enzyme tests. The quantitative urease activity was performed on the remaining homogenate to exclude other urease-positive *Helicobacter* species. Briefly, homogenate was centrifuged for 10 min at 7,500 rpm, and the pellet was resuspended in 200 μl of urea broth containing phenol red. After the reaction mixture was incubated at 37°C overnight, the reaction mixture was centrifuged and the supernatants were placed in microtiter plates. The extent of the phenol red color change was recorded in an automated ELISA reader at 550 nm against a *H. pylori* standard curve. All colonies tested were below the standard curve for *H. pylori*, suggesting an absence of urease-positive *Helicobacter* spp. In addition, DNA was extracted from stomach tissue of G−/− and G+/+ mice and a pure culture of *H. pylori* using a DNeasy tissue kit (Qiagen) according to the manufacturers protocol. *Helicobacter* primer pairs C97 and C98 and C97 and C05 were used to amplify 16S rRNA amplicons of ~400 and 1200 bp, respectively. The primer sequences were generated according to Fox et al. (10) and were as follows: C97, 5′-GCT ATG ACG GGT ATC C-3′ (276–291 forward); C98, 5′-GAT TTT ACC CCT ACA CCC-3′ (681–698 reverse); and C05, 5′-ACT TCA CCC CAG TCG CTG-3′ (1478–1494 reverse). PCR conditions are as previously published (10). Analysis of the *H. pylori* DNA samples by PCR with the *Helicobacter* genus-specific primers produced the expected 400- and 1200-bp amplicons with *H. pylori* DNA extracted from the stomachs of G+/+ mice infected with the SS1 strain (16). Neither of the 400- and 1200-bp amplicons was amplified from DNA extracted from uninfected G+/+ or G−/− mice, confirming the culture results demonstrating there were no *Helicobacter* spp. present in these mice.

*Immunohistochemistry.* A longitudinal section of the stomach (spanning both the fundic and antral regions) was fixed in 4% paraformaldehyde/PBS, paraffin-embedded, and 5 μm sections were prepared. Sections were deparaffinized, then permeabilized in 3% H2O2 and 100% ethanol. Nonspecific antigenic sites were blocked with 20% normal goat serum/ PBS and 0.1% Triton X-100 for 30 min before a 2-h incubation with a 1:200 dilution of rabbit anti-somatostatin (Zymed) antibody. A 1:500 dilution of the secondary anti-rabbit IgG antibody was added for 30 min and visualized with avidin-biotin complex using the Vectorstain Elite ABC Kit and diaminobenzidine for substrate (Vector Laboratories, Burlingame, CA). Sections were also stained with hematoxylin and eosin (H&E). The morphometric results were expressed as the average number of cells counted per gland. A total of 10 oriented glands in random fields was counted for each mouse.
The results were expressed as the average number of cells counted per gland.

Sections stained by H&E were graded on the intensity of inflammation and metaplasia by a pathologist blinded to the treatment and mouse genotypes according to Eaton et al. (9), a grading system developed for the histological quantification of gastritis in mice. A score of 0 or 1 was given to those sections showing no inflammation, 2 for gastritis (inflammatory infiltrate sufficient to displace glands), and 3 for marked inflammation with metaplasia, where metaplasia is defined as the loss of normal fundic morphology with replacement of mucous-secreting glands (9). The presence of metaplasia was confirmed by a periodic acid Schiff (PAS)/alcian blue stain.

**Blood collection.** After death, ~1 ml of blood was collected by cardiac puncture, aliquoted into tubes that were lithium heparinized, and centrifuged at 15,000 rpm for 15 min at 4°C. Plasma was collected immediately and stored at −20°C until assayed.

**Fig. 1.** Representative hematoxylin and eosin stained sections of inflamed stomach from a 16-wk-old wild-type (G⁺/⁺; A), gastrin-deficient (G⁻/⁻; B), G⁻/⁻ treated with antibiotics (C), or G⁻/⁻ mouse infused with G-17 (D). The arrows indicate areas of inflammation. Histological scores (means ± SE) are shown. n = 10 for the G⁺/⁺ group, n = 12 for the G⁻/⁻ groups.

**Fig. 2.** Analysis of T and B lymphocytes by flow cytometry. T and B lymphocytes isolated from the stomachs of G⁺/⁺ mice treated with antibiotics, G⁻/⁻ mice treated with antibiotics, and G⁻/⁻ mice infused with G-17 were analyzed by flow cytometry and expressed as the total cell numbers in preparation. *P < 0.05 vs. G⁺/⁺ mice, n = 10 for G⁺/⁺ group and n = 12 for G⁻/⁻ group.

**Fig. 3.** Bacterial counts from the stomachs of G⁺/⁺ and G⁻/⁻ mice treated with either antibiotics or G-17 infusion. Total bacterial counts in the mouse fundus (A) and antrum (B) represented as the means ± SE of colony-forming units (CFU) per gram of tissue. Note numbers for G⁺/⁺ and G⁻/⁻ mice treated with antibiotics previously reported (30).

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Peptide extraction. Gastric sections were weighed and added to 500 µl of boiling double-distilled water. After the biopsies were boiled for 5 min, they were compressed using a glass rod, and the tissue was boiled for an additional 5 min, and then microfuged at 10,000 rpm for 15 min. The supernatant collected was designated as the water extract containing gastrin. The pellet was resuspended in 500 µl of boiling 3% acetic acid, boiled for 10 min, and microfuged as above. The supernatant collected was the acid extract containing somatostatin. Extracts were stored at −20°C until assayed by radioimmunoassay.

Somatostatin radioimmunoassay. Somatostatin concentrations in water and acid biopsy extracts were measured using appropriate volumes and dilutions. Antiserum 1001 [CURE, Univ. of California Los Angeles (UCLA)] was used, which detects both somatostatin-14 and somatostatin-28 (18). [125I]Tyr-somatostatin-14 was used as the label, and somatostatin-14 (50 pmol/l) was used to generate the standard curve. The ID₅₀ was 8 fmol/ml, and the inter- and intra-assay coefficients of variation were <5 and 12%, respectively.

Gastrin radioimmunoassay. Plasma and the water and acid tissue extracts were assayed for gastrin amide (G-17) using appropriate volumes and dilutions (empirically determined). Samples were incubated in duplicate at 4°C with [¹²⁵I]¹⁵Met human G-17 label and antiserum 1296 (CURE, UCLA). Antiserum 1296 recognizes all COOH-terminal fragments larger than the pentapeptide and measures G-17 sulfated and nonsulfated identically. G-17 (50 pmol/l) was used to generate the standard curve. The ID₅₀ was 1 fmol/ml, and inter- and intra-assay coefficients of variation were <2 and 11%, respectively.

Gastric acid concentrations. The stomachs from G⁻/⁻ mice infused with G-17 were opened along the greater curvature and washed with 2 ml normal saline (pH 7.0). All mice were fasted overnight before gastric samples were collected. The contents were centrifuged at 3,000 rpm for 5 min, and the supernatant was collected. The supernatant was titrated using 0.005 N NaOH, and gastric acidity was expressed in microequivalents.

Cell preparation and flow cytometry. Lymphocytes and epithelial cells were isolated from the gastric mucosa according to a previous method (7). Briefly, the stomach was dissected into 2-mm-size pieces. The pieces were first incubated in 20 ml Hank’s balanced salt solution containing 5% BSA, 1 mM 1,4-dithiothreitol, and 1 mM EDTA for 1 h with vigorous shaking at 37°C to release the epithelial cell population. This first cell suspension was passed through a filter (50-µm Filcon filter, DAKO), collected, and washed twice with RPMI 1640 mammalian cell culture medium containing 5% fetal...
calf serum. The stripped mucosa was then subjected to enzymatic digestion in 20 ml of RPMI medium containing 1 mg/ml dispase II (Roche Molecular Biochemicals) for two 30-min incubations at 37°C with vigorous shaking to remove mucosal lymphocytes. The lymphocytes were collected, washed, and surface labeled for sal lymphocytes. The lymphocytes were collected, washed, with vigorous shaking to remove mucosal digestion in 20 ml of RPMI medium containing 1 mg/ml calf serum. The stripped mucosa was then subjected to enzymatic digestion in 20 ml of RPMI medium containing 1 mg/ml dispase II (Roche Molecular Biochemicals) for two 30-min incubations at 37°C with vigorous shaking to remove mucosal lymphocytes. The lymphocytes were collected, washed, and surface labeled for flow cytometry. Isolated T cells, B cells, and leukocytes were labeled with FITC-conjugated anti-mouse CD3 (Pharmingen), CD19 (Pharmingen), and phycoerythrin-conjugated anti-mouse CD45 (Pharmingen), respectively. D cells were labeled with a rabbit antisomatostatin (Vector Laboratories) antibody after permeabilization (29). The total epithelial cell population was quantified using a monoclonal anti-human cytokeratin-18 (keratin RCK106) antibody (11416; Cappel ICN Pharmaceuticals). FITC-conjugated anti-mouse and anti-rabbit immunoglobulins (Cappel ICN Pharmaceuticals) were used to detect the cytokeratin-18 or somatostatin primary antibodies, respectively. Labeled cells were then analyzed by flow cytometry using a Coulter Elite ESP Cell Sorter (Bechman-Coulter Electronics). A total of 10,000 cells was analyzed for all cell types. Changes in T and B cells were calculated as follows: cell number in total cell prep × %CD45 + cells × %CD3 + CD19 + cells = number of T or B cells in the mucosa (1). D cells were expressed as a percentage of the number of cytokeratin-18-positive cells.

Statistical analysis. The results were statistically tested by unpaired t-test or one-way ANOVA as appropriate using commercially available software (GraphPad Prism, GraphPad Software, San Diego, CA). A P value < 0.05 was considered significant.

RESULTS

Mucosal inflammation in gastrin-deficient G−/− mice. We examined mucosal changes by histology and by flow cytometry to quantify T and B cell populations. Significant inflammation was observed in G−/− (Fig. 1B) compared with the G+/+ mice (Fig. 1A). The histological grades for all mice were scored by a pathologist blinded to the experiment. The mean histological score for G−/− mice was 3.58 ± 0.38, which was significantly higher than in G+/+ mice (2.71 ± 0.32).

Table 1. Tissue somatostatin concentrations in the gastric mucosa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissue</th>
<th>Somatostatin, pmol/g</th>
<th>Circulating Gastrin, pmol/l</th>
<th>Gastric Acid, µeq</th>
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<tr>
<td></td>
<td>Fundus</td>
<td>Antrum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+/+</td>
<td>339 ± 114</td>
<td>1,272 ± 301</td>
<td>54 ± 8#</td>
<td>2.71 ± 0.31#</td>
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<tr>
<td>AB</td>
<td>538 ± 143</td>
<td>1,608 ± 348</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G−/−</td>
<td>368 ± 51</td>
<td>1,156 ± 180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>280 ± 91</td>
<td>1,225 ± 237</td>
<td>558 ± 114 #</td>
<td>1.86 ± 0.36#</td>
</tr>
<tr>
<td>G-17</td>
<td>106 ± 10*</td>
<td>497 ± 98*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. wild-type (G+/+) group. #Previously reported in Ref. 30; G−/−, gastrin-deficient mice; AB, antibiotics.

Table 2. Tissue somatostatin concentrations in G+/+ OM mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissue</th>
<th>Somatostatin, pmol/g</th>
<th>Circulating Gastrin, pmol/l</th>
<th>Gastric Acid, µeq</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fundus</td>
<td>Antrum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+/+ V</td>
<td>212 ± 30</td>
<td>1,783 ± 133</td>
<td>34 ± 4#</td>
<td>5.62 ± 0.70#</td>
</tr>
<tr>
<td>G+/+ V + AB</td>
<td>235 ± 42</td>
<td>1,621 ± 119</td>
<td>44 ± 5#</td>
<td>5.03 ± 0.66#</td>
</tr>
<tr>
<td>G+/+ OM</td>
<td>186 ± 22*</td>
<td>507 ± 139*</td>
<td>159 ± 11#</td>
<td>1.62 ± 0.59#</td>
</tr>
<tr>
<td>G+/+ OM + AB</td>
<td>268 ± 76</td>
<td>1,496 ± 65*</td>
<td>52 ± 16#</td>
<td>1.90 ± 0.86#</td>
</tr>
</tbody>
</table>

Values are means ± SE. V, vehicle; OM, omeprazole. *P < 0.05 vs. G+/+ control group. #Previously reported in Ref. 30.
SUPPRESSION OF SOMATOSTATIN BY HYPERGASTRINEMIA.

Fig. 6. Quantitation of D cells. A: %anti-somatostatin-positive cells per cytokeratin-18-positive cells was determined by flow cytometry and is shown for controls (open bars), controls + antibiotics (filled bars), omeprazole (stippled bars), and omeprazole + antibiotic (hatched bars)-treated G+/+ mice. B: number of D cells per gland in controls (open bars), controls + antibiotics (closed bars), omeprazole (stippled bars), and omeprazole + antibiotic (hatched bars)-treated G+/+ mice were counted. *P < 0.05 vs. controls, n = 8.

significantly greater than that of the G+/+ mice (0.75 ± 0.13). To determine whether the presence of bacteria was the cause of the inflammation in the G−/− mice, a group was treated with antibiotics for 20 days. After antibiotic treatment, the histological grade scored in the G−/− mice decreased to normal (0.50 ± 0.15). Although the histological scores decreased after G−/− mice were infused with G-17 (1.42 ± 0.19), this grade was significantly greater than that for the G+/+ animals. To support the qualitative analysis of mucosal inflammation, flow cytometry was used to quantify changes in the number of T and B lymphocytes. Consistent with the histological grading, there was a significant increase in T and B cells in G−/− mice that resolved with antibiotics. As observed in the histological analysis, G-17 infusion reduced the T and B cell populations (Fig. 2). However, the lymphocyte population after G-17 infusion did not return completely to baseline levels.

G-17 infusion reduced bacterial overgrowth in the G−/− mice. Figure 3 illustrates the total number of bacterial counts in the G+/+ vs. G−/− mice in both the fundus (Fig. 3A) and antrum (Fig. 3B). After antibiotic treatment and G-17 infusion, there was a significant reduction in the total number of bacteria in both the fundus and antrum. However, these numbers were significantly greater than the bacterial counts for the G+/+ mice treated with antibiotics. Overall, the total number of bacteria in the antrum was significantly greater in the G−/− mice compared with the G+/+ animals.

D cells and tissue somatostatin were suppressed after G-17 infusion. To evaluate the response to inflammation, changes in D cells and tissue somatostatin concentrations were measured in G−/− mice. Immunohistochemical staining revealed positive anti-somatostatin antibodies in both the fundus and antrum of the G−/− mice (Fig. 4, A and B). These cells were quantified by morphometric analysis and showed that after antibiotic treatment, there was no change in the number of D cells in either the fundus or antrum (Fig. 5B). In contrast to the antibiotic treatment after G-17 infusion, there was a reduction in the number of D cell staining in both the fundus and antrum (Fig. 4, C and D). After G-17 infusion, there was a significant decrease in D cell numbers in both fundic and antral regions of the stomach (Fig. 5B). In
addition, there was a significant reduction in both fundic and antral tissue somatostatin concentrations after G-17 infusion (Table 1). Thus hypergastrinemia had a greater suppressive effect than inflammation on the D-cell numbers and somatostatin levels. The decrease in somatostatin was consistent with an increase in circulating gastrin (558 ± 114 pmol/l) in the G−/− mice infused with G-17 compared with resting levels in the G+/+ mice (54 ± 8 pmol/l; Table 1). The reduction in somatostatin during G-17 infusion also correlated with an increase in gastric acidity. Gastric acidity increased from 0.48 ± 0.10 μeq in the untreated G−/− mice to 1.86 ± 0.36 μeq in the G-17-infused mice. However, gastric acidity did not reach acid levels measured in the G+/+ mice (2.71 ± 0.31 μeq; Table 1). This result was similar to the incomplete recovery of acid secretion observed by Friis-Hansen et al. (11). There was no change in gastric acidity in G−/− mice after antibiotic treatment. Flow cytometry, which evaluated the total number of D cells in the epithelium, correlated with the morphometric analysis (Fig. 5A). Both morphometric and flow cytometric analyses revealed that there was a trend toward lower D cell numbers in the G−/− mice. This may reflect the modest inhibitory effect of inflammation on the D cell. Slightly fewer D cells were counted in the antrum compared with the fundus, although there were threefold higher tissue somatostatin levels in the antrum compared with the fundus. Therefore, it appears that the concentration of somatostatin per cell is highest in the antrum. In addition, there was no significant difference in somatostatin concentrations between G+/+ and G−/− mice (Table 1).  

Hypergastrinemia in G+/+ OM suppressed D cells and tissue somatostatin. Collectively, our results suggest that somatostatin is suppressed by increasing circulating gastrin concentrations. Furthermore, hypergastrinemia induced by omeprazole treatment is also associated with decreased somatostatin. Therefore, to further evaluate the role of hypergastrinemia in the downregulation of somatostatin, G+/+ mice were made hypochlorhydric and hypergastrinemic with omeprazole treatment for 2 mo. The circulating gastrin concentrations in these mice were 159 ± 11 pmol/l (Table 2), as reported in other animal models and human subjects (7). Histological and flow cytometric evaluation revealed that G+/+ OM also showed significant inflammation (30). After 2 mo of omeprazole treatment, D cell numbers were significantly decreased in both the fundus and antrum compared with untreated animals (Fig. 6B). This correlated with a significant reduction in tissue somatostatin concentrations in both the fundus and antrum in the OM compared with controls (Table 2). After antibiotic treatment, the elevated circulating gastrin concentrations returned to resting levels (34 ± 4 pmol/l). Subsequently, we found that resolution of the inflammation correlated with a return of tissue somatostatin levels (Table 2) and D cell numbers to baseline (Fig. 6B). The flow cytometry was consistent with the morphometry (Fig. 6A). The return of the D cells to baseline levels occurred in the presence of hypochlorhydria documented by acid concentrations of 1.62 ± 0.59 μeq in OM compared with 5.62 ± 0.70 μeq in untreated animals (Table 2).  

Figure 7 illustrates the differences between the G−/− and OM models. G+/+ mice treated with omeprazole become hypochlorhydric and thus are susceptible to bacterial overgrowth and inflammation. A consequence of the inflammation is the increase in plasma gastrin (Fig. 7A) (30). When these mice were treated with antibiotics and the inflammation was resolved, plasma gastrin concentrations normalized despite the stomach remaining hypochlorhydric (Fig. 7B and Table 2). Examining the fate of the D cell population, we found that D cell numbers were depressed in the hypergastrinemic state induced by omeprazole (G+/+; Fig. 6). Certainly, this is as expected from prior studies suggesting that low acid levels inhibit the D cell (23). However, we challenged this dogma by administering antibiotics to the OM to eliminate the inflammation-induced hypergastrinemia. With the reduction in plasma gastrin levels, the D cells returned to baseline levels despite persistent hypochlorhydria (Fig. 7B and Table 2). Therefore, we concluded that D cells are regulated by fluctuations in gastrin and not gastric acid, which did not change.  

The results in the omeprazole model are supported by studies using G−/− mice. G−/− mice are hypochlorhydric due to a genetic deficiency of gastrin. These mice
exhibit significant inflammation and increased G cell density (30) despite a lack of gastrin. Because D cell numbers and somatostatin concentrations were unchanged even after antibiotic treatment, this led us to propose that gastrin may be the critical factor required to suppress somatostatin production (Fig. 7C). Confirming our suspicions, it was only after the infusion of G-17 into the gastrin null mice that we observed a reduction in somatostatin and D cell density (Fig. 7D). Therefore, these results clearly show that hypergastrinemia is the major factor modulating the D cell during bacterial infection.

DISCUSSION

We have shown that hypochlorhydria, induced either genetically by deletion of the gastrin gene or chemically by treatment with omeprazole, causes bacterial overgrowth that results in gastric inflammation (30). There were slightly lower D cell numbers in G−/− mice, which may reflect an effect of chronic inflammation on somatostatin in the G−/− mice. Whereas antibiotic treatment of G−/− mice resolved the inflammation and normalized cell populations such as parietal and G cells (30), we showed that there was no significant effect on D cells or tissue somatostatin content. When G−/− mice were infused with G-17, raising circulating gastrin concentrations to levels observed in hypergastrinemic animals, D cell numbers and tissue somatostatin levels were suppressed. Similarly, hypergastrinemic G+/+ OM also exhibited reduced D cell numbers and tissue somatostatin concentrations. After omeprazole mice were treated with antibiotics, plasma gastrin concentrations normalized, correlating with resolution of the inflammation. Consistent with lower circulating gastrin levels, D cell numbers returned to baseline. This was also observed in the OM despite persistent hypochlorhydria. Therefore, we conclude from these studies that the suppression of somatostatin during bacterial infection is due to the hypergastrinemia induced in response to inflammation and not the hydrogen ion concentration as previously proposed.

In wild-type mice and human subjects, omeprazole induces significant hypergastrinemia, presumably due to the decrease in gastric acid that in turn inhibits D cell secretion of somatostatin (14, 15). Moreover, in the absence of somatostatin, inhibition of the G cell is removed, allowing serum gastrin levels to rise (6, 21, 22). Because we have shown that omeprazole treatment induces a gastritis and that the inflammation is a major activator of the G and parietal cell, our model demonstrates that G cell numbers should increase and serum gastrin levels should rise (30). Indeed, this is what we observed, and this is also what has been observed in other models of chemical achlorhydria in rodents and in human subjects (4, 17, 28). In these prior reports, modulation of the G and D cell population was postulated to be due to changes in gastric acid. Our model would support the notion that the regulation of gastrin and somatostatin by gastric acid is indirect. Rather, the absence of acid permits bacterial overgrowth or microbial metabolites to induce inflammation. Consistent with this hypothesis, we found that in normal mice treated with omeprazole and antibiotics to resolve the inflammation, gastrin levels decreased and D cells returned to their normal resting numbers despite low gastric acidity. Thus, in the face of low gastric acid due to omeprazole, gastrin levels were suppressed simply by treating the animals with antibiotics. This in turn released the inhibition on the D cell. If hypoacidity were the major direct regulator, as previously proposed, then the D cell numbers and tissue somatostatin should have remained low. Although prior studies have indicated that gastrin stimulates somatostatin release, these studies were performed on isolated cell populations or in whole animals in the absence of inflammation, hypochlorhydria, or bacterial overgrowth (8, 31). In addition, these studies examined the effect of acutely administering gastrin. Thus we emphasize that the results shown here document an inhibitory effect of gastrin on somatostatin secretion in vivo when hypergastrinemia is induced by chronic inflammation. Because the studies were performed in vivo, it is not known under these conditions whether gastrin stimulates the release of other ligands, e.g., heparin-binding epidermal growth factor, TGF-α, or trefoil proteins, or acts directly on the D cell (19, 20, 25, 27). Certainly both possibilities may also occur in concert.

In summary, the findings reported here clearly show that hypergastrinemia, induced by inflammation, results in decreased D cell numbers. The stomach responds to the presence of inflammation by increasing gastric acidity, elevating G cell numbers, and gastrin secretion that, in turn, stimulates parietal cells (30). Coincidently with the changes to increase acid secretion, hypergastrinemia also blocks the somatostatin inhibitory pathway (Fig. 8). As a result, the stomach is able to maximize its gastric acid output in an attempt to clear bacterial infection.

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