Gastrin stimulates the growth of gastric pit with less-differentiated features

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Konda, Yoshitaka, Hitoshi Kamimura, Hiromi Yokota, Naoki Hayashi, Kentaro Sugano, and Toshiyuki Takeuchi. Gastrin stimulates the growth of gastric pit with less-differentiated features. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G773–G784, 1999.—Gastrin stimulates the growth of gastric mucosa by increasing mostly its glandular region but is not known to induce the growth of a pit region where its major constituent cells, gastric surface mucous (GSM) cells, turn over rapidly. To investigate the effect of gastrin on GSM cells, we generated hypergastrinemic mice by expressing a human gastrin transgene. We obtained a hypergastrinemic mouse line whose average serum gastrin level is 671 ± 252 pg/ml (normal level <150 pg/ml). Gastrin-positive cells were found in the fundic mucosa. The gastric mucosa exhibited hypertrophic growth, which was characterized by an elongated pit with an active proliferative zone, but the glandular region containing parietal cells was normal or reduced in size. The GSM cells contained fewer mucous granules than those of control littermates and lost reactivity to the GSM cell-specific cholera toxin β-subunit lectin. GSM cells along the foveolar region and many mucous neck cells became Alcian blue positive, suggesting the appearance of a mucus in these cells. We suggest that gastrin stimulates the growth of the proliferative zone of gastric glands, which results in the elongation of the pit region whose GSM cells exhibit less-differentiated features.

gastri and the initiation of a gastric cancer (22, 44). This hypothesis is favored by the fact that the GSM cells of atrophic gastritis exhibit more mitotic activity than those in healthy individuals (34).

Gastrin is synthesized in G cells as the precursor progastrin and then is processed by proteolysis and amidation reactions to amidated gastrin (17 amino acids long) (50). In the amidation reaction, the glycine residue at the carboxy-terminal end serves as the substrate for the amidation enzymes. Gastrin thus formed exhibits gastric acid-secreting activity three orders of magnitude higher than does glycine-extended gastrin (G-Gly) (36). In contrast, both gastrin and gastric mucosal growth. Such transgenic mice were produced by Wang et al. (53). The mice in their model expressed gastrin under the control of an insulin promoter, which
resulted in gastrin production in the pancreatic β-cells, and expressed gastrin under the control of a human gastrin promoter, which resulted in the production of a noncleaved gastrin precursor in the liver. Wang et al. (53) demonstrated that gastrin is more potent for gastric mucosal growth, whereas progastrin is more potent for colonic mucosal growth. In their gastrin-producing transgenic model, the serum gastrin level increased twofold compared with the level of littermate controls (~130 vs. 70 pg/ml). In hypergastrinemia, however, due to either ZE tumor or atrophic gastritis, serum gastrin levels are often elevated 10-fold or more (9). For producing such hypergastrinemic mice, gastrin expression is desirable not only in endocrine cells but also in nonendocrine cells. We were successful in making such a gastrin expression vector by utilizing the consensus cleavage site of the proprotein-processing endoprotease furin, Arg-X-(Lys/Arg)-Arg (18). Amidation enzyme is distributed widely in almost every tissue including nonneuroendocrine cells, which are able to produce amidated peptides when their genes were expressed (11, 18, 25). We expressed a gastrin cDNA under the control of a β-actin promoter, which exhibits strong expression in a variety of tissues (1, 21). Thus gastrin should be highly produced in mice expressing this mutated gastrin precursor.

The present study analyzed the hypertrophic gastric mucosa of hypergastrinemic mice. The hypertrophic mucosa was comprised of an elongated pit region with an active proliferative zone. The GSM cells consisting of the elongated pit exhibited less differentiated features by immunocytochemical and electron microscopy analyses.

**MATERIALS AND METHODS**

Generation of gastrin-expressing transgenic mice. We used a human gastrin cDNA, for which the peptide product contains two mutations (18). One mutation is a processing site at the amino terminus of gastrin: Asp-Pro-Ser-Lys-Lys (native) was changed to Asp-Arg-Ser-Lys-Lys (mutant). This tetrabasic site was efficiently cleaved by furin, which is distributed in many cell types (55). The other mutation is at the carboxy terminus of gastrin after glycine: the progastrin sequence was terminated after the glycine position by inserting a stop codon. With this modification, the mutated progastrin was efficiently cleaved and amidated by matured progastrin and was efficiently cleaved and amidated in nonneuroendocrine cells (18). The mutated progastrin cDNA was inserted into the XhoI site of the pCXN2 vector (39), whose expression is based on the chicken β-actin promoter. We excised the gastrin expression unit with SalI from the vector and microinjected the excised DNA into oocytes from ICR mice (Nippon Clea, Osaka, J. Japan). The oocytes were transferred to pseudopregnant ICR female mice according to standard procedures (19). Neonatal mice were screened for the presence of the human gastrin transgene and the endogenous mouse gastrin gene by a PCR method using oligonucleotides that bracket the 190-bp DNA on the human gastrin cDNA (5'-AACAGGGACCTGGAGCTACC-3' and 5'-GTTTCTCATCTCAGCAGCTGC-3') and the 300-bp mouse gastrin genomic DNA including the 110-bp intron II (5'-AATGAGGACCTGGACACGCC-3' and 5'-CTGCTTCTCTTCCACACCGC-3'), respectively (16). We obtained five mice with the gastrin transgene. Transgenic lines were mated and propagated to obtain hypergastrinemic mice.

Morphological studies. For periodic acid-Schiff (PAS) staining, stomach tissue sections were fixed in 10% formaldehyde for 3 h at 4°C and stained with PAS, using the standard method after diastase digestion (31). Proliferation of the mouse gastric mucosa was examined by two methods, staining of proliferating cell nuclear antigen (PCNA) and incorporation of the thymidine analog bromodeoxyuridine (BrdU) (31). Brdu (80 mg/kg body wt; Sigma Chemical, St. Louis, MO) was injected intraperitoneally into 20-wk-old mice 2 h before killing. Stomach tissues were removed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Small pieces of the sample underwent saccharose replacement and then were frozen for microtome sectioning. The following antibodies were used as a first antibody for immunostaining: rabbit anti-gastrin polyclonal antibody (Zymed Laboratory, South San Francisco, CA), mouse monoclonal anti-PCNA antibody (PC10, DAKO, Glostrup, Denmark), mouse anti-BrdU monoclonal antibody (BioMeda, Foster City, CA), rabbit anti-histamine polyclonal antibody (Chemicon International, Temecula, CA), and rabbit anti-somatostatin polyclonal antibody (Peninsula Laboratory, Belmont, CA). Monoclonal antibody to H-K-ATPase was prepared by injecting purified rabbit gastric microsomal fraction with endogenous H-K-ATPase activity into mice; this antibody recognizes the tertiary structure of H-K-ATPase made of α- and β-subunits. An LSAB2/horseradish peroxidase staining kit (DAKO) was used as the secondary antibody reaction system.

For lectin binding studies, FITC-labeled cholera toxin β-subunit (CTB) and Dolichos biflorus (DBA) (Sigma) were used to identify a gastric epithelial cell lineage (13, 14). Characteristics of mucous cells were examined by Alcian blue staining (at pH 2.5 for acidic mucin including sialomucin and sulfomucin and at pH 1.0 for sulfomucin) and paradoxical concanavalin A staining (PCS) for mucous neck cells (23, 46). For examination via electron microscopy, gastric tissues were fixed in 2.5% glutaraldehyde-2.0% formaldehyde in 0.1% sodium cacodylate buffer. They were postfixed in 1% OsO4, treated with 0.5% uranyl acetate, and embedded in Epon. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with an H-800 electron microscope (Hitachi, Tokyo, J. Japan).

RIA.s. RIA for amidated gastrin was performed using a gastrin assay kit (gastrin RIA kit II, Dainabot, Tokyo, J. Japan). This antibody is specific for gastrin with an amide moiety. The assay for G-Gly was performed as described previously, using the antibody 8237 (18). This antibody does not cross-react with amidated forms of gastrin but cross-reacts 100% with CCK-Gly (8).

Measurement of acid secretion. Gastric acid secretion was measured according to the method described previously (38). Briefly, control and transgenic mice (~20 wk old) were fasted for 3 h and then anesthetized with ether. After the abdominal wall was incised, the pylorus was ligated, and the incision was sutured. The gastric fluid in the stomach was collected for 4 h after the pylorus ligation. For maximal acid output, acid secretion was stimulated by injecting pentagastrin (250 µg/kg body wt) at the pylorus ligation. The gastric fluid was titrated with 0.1 N NaOH to pH 7.0 using a microtitrator.

RNA analysis. Isolated total RNA was treated with DNase I (GIBCO BRL) for RT-PCR. Expression was assessed by RT-PCR using 5'-AACAGGGACCTGGAGCTACC-3' and 5'-GTTTCTCATCAGCAGCTGC-3' for human gastrin mRNA (134 bp) and 5'-AATGAGGACCTGGACACGCC-3' and 5'-AGAAGGAGGATGGACC-3' for mouse gastrin mRNA (135 bp).
RESULTS

Generation of transgenic mice. We selected transgenic mice with the 190-bp human gastrin DNA fragment using PCR and then mated them to propagate a transgenic line. We deduced the genotype of human gastrin DNA again using PCR and then classified the mice into one of three genotypes: those with 300-bp bands and without 190-bp bands, nontransgenic (genotype $+/-$); those with 300-bp and 190-bp bands (genotype $+/+$); and those with 300-bp bands and roughly two times thicker 190-bp bands (genotype $+/-$) (Fig. 1A). After classification, plasma gastrin levels of mice fasted overnight were measured by RIA (Fig. 1B). The values from the $+/-$ mice averaged $113 \pm 46$ pg/ml with a maximum of 204 pg/ml, those from the $+/-$ mice averaged $278 \pm 62$ pg/ml, and those from $+/+$ mice were distributed from 317 pg/ml to 1,207 pg/ml with an average of $671 \pm 252$ pg/ml (Fig. 1B). Although the classification of genotype, depending on the thickness of the 190-bp bands, is not absolute, we were able to select a hypergastrinemic mouse group. We used mice from the $+/-$ group whose gastrin levels were over the average for the following experiments. We also measured G-Gly in several mice of each genotype group. Although the antibody to G-Gly (antibody 8237) recognizes G-Gly as well as CCK-Gly (8), plasma G-Gly levels were not elevated in both the $+/-$ and $+/-$ hypergastrinemic mouse groups and remained in the same range as those in the $+/+$ control mouse group (Fig. 1C), suggesting that the mutated gastrin expressed from the transgene was efficiently processed to amidated gastrin.

Expression of gastrin. Gastrin content was evaluated in a variety of tissues. Compared with gastrin levels of control mice, those of the transgenic mice were strikingly high in the corpus of the stomach. The levels were noticeably high in the small intestine and detectable in the lung, heart, foregut, liver, and kidney (Fig. 2A). The gastrin content in the corpus was comparable to that in the antrum where gastrin is originally produced. This marked expression of the human gastrin transgene was also confirmed by RT-PCR (Fig. 2B). Gastrin was immunostained in many epithelial cells in the fundic mucosa of HG mice (Fig. 2C, b and c) but not at all in the control fundic mucosa (Fig. 2Ca). Gastrin-positive cells looked smaller than parietal cells. In the pit region, they appeared to be GSM cells (Fig. 2Cc). In the glandular region, they may be mucous neck cells by their small size. GSM cells and mucous neck cells are exocrine cells and secrete mucus into gastric lumen. Because gastrin is a secretory peptide, it may be stored in mucous granules. Exocrine cells release secretory proteins into an exocrine duct as well as into a blood stream, as exemplified by serum amylase and pepsinogen. We think that gastrin is produced in GSM-type small cells in the fundic mucosa.

We then analyzed a molecular form of gastrin by Sephadex G-50 gel filtration. Gastrin from the antrum of both control and transgenic mice exhibited a major peak at the gastrin-17 (Gly-17) position with a minor peak at the Gly-34 position (Fig. 2D, b and d). In contrast, gastrin from the corpus of transgenic mice was eluted as a single peak at the G-17 position without a distinct peak at the Gly-34 position (Fig. 2Dc), indicating that the gastrin contained in the corpus is derived from the gastrin transgene.
Overgrowth of gastric pit relative to a reduced parietal cell mass. The stomachs from the +/- group hypergastrinemic mice at 7–8 mo of age were ~30–50% heavier in weight, and their mucosa was markedly thicker than that of controls, although the mucosa was higher in some parts and lower in other parts (Table 1, higher part = 1.03 ± 0.32 mm, lower part = 0.48 ± 0.15 mm). The gastric pits with PAS-positive staining were highly elongated and displayed an orderly structure (Fig. 3, A and B). This finding is in contrast to the gastric pit of TGF-α-overexpressing transgenic mice, which display disorderly growth with cystic distensions (10, 46). Both the higher and lower parts of the pit were longer than the control pit (Table 1). The gastric mucosa of normal mice was full of H-K-ATPase-positive cells, with a relatively short pit region (Fig. 3C). In contrast, in the mucosa of transgenic mice, the H-K-ATPase-positive glandular region was normal to
reduced in height. Overgrowth of gastric pit relative to a parietal cell mass in the transgenic mouse mucosa was also confirmed by using parietal cell-specific lectin DBA (Fig. 3, E and F). To examine the actual decrease in the absolute number of parietal cells, we counted the number of parietal cells together with ECL cells per gastric gland unit (Table 2). However, we could not obtain the absolute number of parietal cells because the section of the transgenic mouse gastric mucosa was thicker in some parts and thinner in other parts. We presume that total parietal cell mass may not be increased because the transgenic mouse parietal cell region was normal to reduced in height.

We then measured acid secretion capacity in control and transgenic mice. Basal acid output levels were not elevated in transgenic mice, although their basal acid output was presumed to be stimulated by high levels of gastrin (Fig. 4). Maximal acid output levels were also similar between the two groups by gastrin stimulation. Likewise, maximal acid output levels by carbachol (60 µg/kg body wt) were also similar between the two groups. Because maximal acid output reflects total parietal cell mass, the mass may be similar between the control and transgenic mouse gastric mucosae. Acid secretion in the transgenic mice may be well balanced to a normal range between high levels of gastrin and somatostatin because somatostatin cells were increased as described in Endocrine-type cells.

Increase of proliferative zone. The isthmus at the base of the pit region is known as the proliferative zone (17, 27, 29), which was confirmed by PCNA staining and the incorporation of BrdU. In normal mucosa, PCNA-positive cells were scattered from the middle pit region to the upper glandular region (Fig. 5A). In the mucosa of transgenic mice, PCNA-positive cells were clustered heavily at the isthmus region and also scattered to the upper pit region (Fig. 5B). The distribution of BrdU-positive cells is consistent with that of the PCNA data, but the BrdU-positive cell number is limited. In the normal mucosa, only a few positive cells were scattered at the upper third zone of the gastric mucosa (Fig. 5C), as in the rat gastric mucosa (31). In the transgenic mouse mucosa, a higher number of BrdU-positive cells were distributed in the same zone (Fig. 5D). The labeling index of the cells with BrdU in the whole gastric gland unit was 0.71 ± 0.24% in the normal fundic gland; in contrast, it was 5.5 ± 1.0% in the transgenic mouse gland. PCNA facilitates DNA replication by polymerase δ and remains in the nucleus for a few days after cell division (43, 54) so that many more

Table 1. Gastric mucosal thickness in control and transgenic mice

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<th>Control Mice</th>
<th>Transgenic Mice</th>
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<td>Higher part</td>
<td>Lower part</td>
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<tr>
<td>Gastric mucosa, mm</td>
<td>0.57 ± 0.22</td>
<td>1.03 ± 0.32*</td>
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<td>Pit region, mm</td>
<td>0.17 ± 0.04</td>
<td>0.62 ± 0.08t</td>
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<td>Submucosa to serosa, mm</td>
<td>0.30 ± 0.07</td>
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Values are means ± SE of 6 mice. *P < 0.05, †P < 0.01 vs. control values.

Fig. 3. Overgrowth of gastric mucosa in HG mice. A, C, and E show gastric mucosa of normal mice; B, D, and F show gastric mucosa of transgenic mice. A and B: diastase-resistant periodic acid-Schiff staining. Pit region was elongated in the gastric mucosa of transgenic mice. C and D: horseradish peroxidase (HRP) reaction of H-K-ATPase. Brown-colored H-K-ATPase-positive parietal cells were distributed in the whole mucosa, except in the upper fourth of gastric glands in normal mice. In contrast, H-K-ATPase-positive cells were confined in the lower third of the mucosa in transgenic mice. E and F: staining with Dolichos biflorus (DBA) lectin. DBA is specific for parietal cells. DBA staining confirms H-K-ATPase staining in C and D. Scale for A, B, C, and D = 100 µm; scale for E and F = 100 µm.
PCNA-positive cells are observed than BrdU-positive cells.

Less-differentiated features of GSM cells. We assessed differentiated features of GSM cells using GSM cell-specific lectin CTB, electron microscopy, and mucus staining. CTB was positively stained along the foveola-facing membranes of GSM cells in the pit region of control mice (Fig. 6A). In contrast, CTB was not positive in the pit region of transgenic mice (Fig. 6B). Thus surface mucous cells in the transgenic mice did not express CTB-specific carbohydrate moieties. In electron microscopy, mucous granules were rich in luminal-side GSM cells of control mucosa (Fig. 7, A and C). Granules are composed of at least two types: small, dense-cored ones and large gray ones (Fig. 7C). Mucous granules in the transgenic mouse mucosa were reduced in number (Fig. 7, B and D). The cytoplasm of GSM cells was full of enlarged rough endoplasmic reticulum (ERs) (Fig. 7D). In the middle portion of transgenic mouse pits, GSM cells were aligned in an orderly fashion (Fig. 7E) and contained various sizes of granules from small to much larger ones (Fig. 7F), which were larger than those in control GSM cells. Large, gray granules were also surrounded by enlarged ERs, such as those in Fig. 7D. ER is often enlarged in cells with actively producing secretory proteins. Gastrin is reported to stimulate mucin biosynthesis in the rat gastric corpus mucosa (20). These types of GSM cells were observed along the elongated pit of transgenic mice.

We then stained the mucosa with Alcian blue, at pH 2.5 for acidic mucin including sialomucin and sulfomucin (45) and at pH 1.0 for sulfomucin (23). No Alcian blue staining at pH 2.5 was observed in the control mucosa (Fig. 8A). In the mucosa of transgenic mice, Alcian blue at pH 2.5 stained positively along the luminal and foveolar surfaces of GSM cells in the upper pit region and staining was also positive in many cells inside the glandular mucosa (Fig. 8B). Alcian blue-staining at pH 2.5 in Fig. 8A and B is a demonstration of Alcian blue-staining at pH 2.5 in the transgenic mouse mucosa. Alcian blue-staining at pH 2.5 was not observed in the control mucosa (Fig. 8A). In the mucosa of transgenic mice, Alcian blue at pH 2.5 stained positively along the luminal and foveolar surfaces of GSM cells in the upper pit region and staining was also positive in many cells inside the glandular mucosa (Fig. 8B). Alcian blue-staining at pH 2.5 was not observed in the control mucosa (Fig. 8A). In the mucosa of transgenic mice, Alcian blue at pH 2.5 stained positively along the luminal and foveolar surfaces of GSM cells in the upper pit region and staining was also positive in many cells inside the glandular mucosa (Fig. 8B). Alcian blue-staining at pH 2.5 was not observed in the control mucosa (Fig. 8A). In the mucosa of transgenic mice, Alcian blue at pH 2.5 stained positively along the luminal and foveolar surfaces of GSM cells in the upper pit region and staining was also positive in many cells inside the glandular mucosa (Fig. 8B).

Table 2. Parietal cell and ECL cell counts in the gastric gland unit

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<th>Control Mice</th>
<th>Transgenic Mice</th>
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<td></td>
<td>Higher part</td>
<td>Lower part</td>
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<tr>
<td>Parietal cells</td>
<td>21.3 ± 0.7</td>
<td>33.8 ± 2.0</td>
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<tr>
<td>ECL cells</td>
<td>2.8 ± 0.2</td>
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Values are means ± SE of 3 mice. Cells in 40 gastric glands were counted and averaged. ECL cells, enterochromaffin-like cells.

Fig. 4. Basal and maximal acid output. Open columns, basal acid output. Solid columns, maximal acid output. Control, acid output in control mice. TG, acid output in transgenic mice. Maximal acid output was measured after stimulation with 250 µg/kg pentagastrin. Values are means ± SE of at least 4 mice.

Fig. 5. Increase of proliferative zone in transgenic mice. A and C show gastric mucosa of normal mice. B and D show gastric mucosa of transgenic mice. A and B: HRP reaction of proliferating cell nuclear antigen (PCNA). Brown-colored nuclei were localized in the upper fourth of the glands, except in the luminal epithelial cell area in normal mucosa. In the mucosa of transgenic mice, PCNA-positive cells were thickly localized at the base of the pit region. C and D: HRP reaction of bromodeoxyuridine. Distribution pattern was similar but less dense than that of PCNA in both normal and HG mice. Scale for A–D = 100 µm.

Fig. 6. Staining with cholera toxin β-subunit (CTB) lectin. A: gastric pit of control mice. B: gastric pit of transgenic mice. CTB is specific for gastric surface mucous (GSM) cells located in the upper gastric pit. Scale for A and B = 100 µm.
Fig. 7. Electron micrograph of GSM cells. A and C: upper gastric pit of normal mice. B and D: upper gastric pit of transgenic mice. E and F: middle region of gastric pit of transgenic mice. Arrowheads in A indicate rich amounts of mucous granules. PC, parietal cell. Arrowhead in B indicates the fewer number of mucous granules. Mitochondria (Mt) are shown in C. Arrowhead in F indicates a large gray granule. Bottom arrow and top arrow indicate a small gray granule and a small, dense-cored granule, respectively. Scale for A, B, and E = 10 µm; scale for C, D, and F = 2 µm.
positive cells in the glandular mucosa were smaller in size than parietal cells (Fig. 8C). To identify this Alcian blue-positive cell type, we then stained the mucosa with PCS, specific for mucous neck cells (45), and with anti-PCNA antibody as shown in Fig. 5, A and B. PCS-positive neck cells were scattered among parietal cells in the lower two-thirds of the control mucosa (Fig. 8E). In contrast, they were localized in the lower one-fourth to one-third of the transgenic mouse mucosa (Fig. 8F). By size and distribution, Alcian blue-positive cells appeared to be mucous neck cells that were surrounded by parietal cells (Fig. 8, C and G). The Alcian blue-positive cell layer was lower than the PCNA-positive proliferative zone (Fig. 8, F and H). In contrast to Alcian blue staining at pH 2.5, Alcian blue staining at pH 1.0 was not positive in the transgenic mouse mucosa (Fig. 8D).

Endocrine-type cells. Histamine-producing ECL cells, another gastrin-target cell type, were stained for histamine. They were almost similar in number between the control and transgenic mice (Fig. 9, A and B). This finding is different from the report by Wang et al. (53), who demonstrated an increased number of ECL cells by argyrophil staining. In contrast, somatostatin-producing D cells were increased in the mucosa of transgenic mice (Fig. 10, A and B). Only a few somatostatin-positive cells were present in the normal fundic mucosa, whereas a number of small D cells were scattered in the transgenic mouse mucosa [Fig. 10, B and C (enlargement)]. Thus, although gastrin receptors are present in parietal cells, ECL cells, and D cells (7, 35), only somatostatin-producing D cells increased in number in the transgenic mice.

**DISCUSSION**

In this study, we generated hypergastrinemic mice, whose gastric mucosa was overgrown by the elongation...
of its pit region. Although we used a chicken β-actin promoter that has been utilized for transgene expression (1, 21), we unexpectedly found that high expression of the gastrin transgene was limited to the mucosa of the gastric corpus (Fig. 2). The expression of TGF-α under the control of a ubiquitously active metallothionein promoter was observed in the gastric mucosa in some mouse lines and in the liver in other lines (24, 46). Thus, even if we use a ubiquitously active promoter, the site of gene expression appears to be affected by many factors, such as gene products and chromosomal integrated sites. Although the gastrin gene was not widely expressed in our transgenic mouse line, we obtained a transgenic mouse line with an average of 671 ± 252 pg/ml plasma gastrin levels.

The thickened mucosa of our hypergastrinemic mice resulted from the elongation of its pit region, unlike the hypertrophic gastric mucosa observed in ZE syndrome individuals, which is caused by the expansion of the glandular region (15), and that observed in a rat model infused with gastrin for 28 days (40). In ZE patients and gastrin-infused rat models, hypergastrinemia induces hypertrophy of the glandular mucosa and an increase in ECL cells. Our hypergastrinemic mouse model presented an increase in somatostatin-producing D cells; thus it might not exhibit an increase in parietal cells and ECL cells. Another possibility is that the gastrin transgenic mice were exposed to high levels of gastrin for prolonged periods of time, thus the sensitivity to gastrin may be decreased and the parietal and ECL cell number may be also decreased to normal levels. In the gastrin-expressing models by Wang et al. (53), the glandular region appeared thick in the mice that produced a noncleaved gastrin precursor from the liver, whereas the pit region appeared elongated in those mice that produced gastrin from the pancreatic β-cells. The thickened mucosa of our model appeared similar to that of their gastrin-producing model but not to their progastrin-producing model. It remains unclear, however, whether gastrin induces the growth of the pit region and whether progastrin induces the growth of the glandular region.

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Fig. 9. HRP reaction of histamine. A: gastric mucosa of normal mice. B: gastric mucosa of transgenic mice. Brown-colored histamine-positive enterochromaffin-like cells were scattered in the glandular mucosa in both normal and transgenic mice (arrowheads). Scale for A and B = 50 µm.

Fig. 10. HRP reaction of somatostatin. A: gastric mucosa of normal mice. B: gastric mucosa of transgenic mice. C: enlargement of squared area in B. Brown-colored somatostatin-positive D cells were not seen in the control glandular mucosa but were seen scattered in the glandular mucosa of transgenic mice (arrowheads). Scale for A and B = 50 µm; scale for C = 10 µm.
The elongated gastric pit of the transgenic mouse model exhibited less-differentiated features, determined by the following observations. First, cell proliferation was highly active, as shown by BrdU incorporation and PCNA-positive staining. Second, there were virtually no parietal cells over the proliferative zone, which were limited to the glandular region. Third, GSM cell-specific staining by CTB lectin was not observed in the transgenic mouse mucosa. Fourth, mucous granules in the GSM cells of the top pit region were decreased in number and larger in size, and those in the GSM cells of the middle pit region appeared large and gray and were surrounded by enlarged ERs. Finally, Alcian blue-stained cells appeared along the luminal and foveolar surface of the pit. Furthermore, some of mucous neck cells were transformed to Alcian blue-positive cells. Because Alcian blue-positive cells were overexpressed in the gastric mucosa of the TGF-α-overexpressing mouse, resembling that of Menetrier’s disease, appearance of Alcian blue-positive cells suggests a premalignant change of gastric mucosa (23). Thus the elongated pit of the transgenic mouse model is composed of less-differentiated GSM cells, which are generated from the extensively active proliferative zone.

Gastrin induces extensive cell mitosis in atrophic gastritis (34). In type A gastritis, a loss of parietal cells occurs due to autoimmune mechanisms. Recently, a mouse model lacking parietal cells was made by using herpes simplex virus thymidine kinase DNA or diphtheria toxin fragment A DNA as a transgene (4, 32). This concerted signaling of gastrin and TGF-α might lead to the formation of an orderly arrayed gastric gland unit. These concerted signals might lead to the formation of an orderly arrayed gastric gland unit. This concerted signaling of gastrin and TGF-α was demonstrated in the neogenesis of islet β-cells from pancreatic duct cells (52). Overexpression or underexpression of one of these factors, however, may lead to the abnormal development of gastric mucosa. Recently, Koh et al. (30) demonstrated an atrophic change of gastric mucosa in gastrin gene-disrupted mice, characterized by a decrease of parietal cells and ECL cells and an increase of mucous neck cells. By overexpressing gastrin, we showed abnormal elongation of gastric pits composed of less-differentiated GSM cells in this study. The thickened gastric mucosa with an elongated GSM/pit cell region will serve as an important model for studying the role of gastrin in the growth and maturation of a GSM/pit cell lineage.

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