Regulation of parietal cell migration by gastrin in the mouse

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Kirton, C. M., T. Wang, and G. J. Dockray. Regulation of parietal cell migration by gastrin in the mouse. Am J Physiol Gastrointest Liver Physiol 283: G787–G793, 2002. First published April 24, 2002; 10.1152/ajpgi.00538.2001.—Recent studies suggest that gastrin regulates parietal cell maturation. We asked whether it also regulates parietal cell life span and migration along the gland. Dividing cells were labeled with 5′-bromo-2′-deoxyuridine (BrdU), and parietal cells were identified by staining with Dolichos biflorus lectin. Cells positive for D. biflorus lectin and BrdU were reliably identified 10–30 days after BrdU injection in mice in which the gastrin gene had been deleted by homologous recombination (Gas-KO) and wild-type (C57BL/6) mice. The time course of labeling was similar in the two groups. The distribution of BrdU-labeled parietal cells in wild-type mice was consistent with migration to the base of the gland, but in Gas-KO mice, a higher proportion of BrdU-labeled cells was found more superficially 20 and 30 days after BrdU injection. Conversely, in transgenic mice overexpressing gastrin, BrdU-labeled parietal cells accounted for a higher proportion of the labeled pool in the base of the gland 10 days after BrdU injection. Gastrin, therefore, stimulates movement of parietal cells along the gland axis but does not influence their life span.

terochromaffin-like cells (1, 12, 14). Mice in which the gastrin gene has been deleted by homologous recombination (Gas-KO) are achlorhydric and do not respond to acute administration of the acid secretagogues gastrin, histamine, and carbachol (6). They do, however, secrete acid after treatment with gastrin for ≥24 h (2, 6). These observations, therefore, suggest that gastrin plays a role in parietal cell maturation. Interestingly, in transgenic mice with moderate hypergastrinemia (Ins-Gas mice), there is an initial increase in parietal cell numbers, although this is followed (>4 mo) by a progressive loss of glandular cells and foveolar hyperplasia (11, 18). Interpretation of the observations in these genetically modified animals is complicated by the fact that it is not yet clear whether gastrin also regulates the life span of parietal cells or influences their migration or that of their precursors along the gland axis. In the present study, we labeled parietal cell precursors at their last cell division using 5′-bromo-2′-deoxyuridine (BrdU) and tracked the distribution of labeled cells in Gas-KO and Ins-Gas mice. We found that the life span of parietal cells is similar in Gas-KO and wild-type mice but that the rate of progression of new parietal cells along the gland axis is slowed, suggesting a role for gastrin in cell migration.

METHODS

Animals. Gas-KO mice on a C57BL/6 background and Ins-Gas mice on a background of FVB/N have been described previously (10, 19). Animals were 11–13 wk of age at the start of experiments. Mice were housed in polycarbonate-bottomed cages with a strict light cycle (lights on at 0600 and lights off at 1800) and fed a commercial pellet diet and water ad libitum.

BrdU labeling in vivo. Cells in the S phase of the cell cycle were labeled with biotinylated BrdU (10 mg/ml, 100 μl ip; Sigma, Poole, Dorset, UK). Animals were killed at intervals up to 40 days later by a rising concentration of CO2. A blood sample was taken by cardiac puncture, and plasma was removed for radioimmunoassay. The stomach was removed, the contents were gently flushed with 0.1 M phosphate-buffered saline (PBS), and the tissue was fixed in 4% paraformaldehyde (1 h) and then immersed in 20% sucrose (24 h)

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Immunohistochemistry. Tissue was mounted in OCT mounting medium (BDH, Poole, UK) in dry ice-cooled isopropanol, and cryostat sections (5 μm) were processed for localization of BrdU using Texas red-conjugated avidin (10 μg/ml; Oncogene Research Products, Boston, MA). Parietal cells were then identified by staining with FITC-conjugated lectin from Dolichos biflorus (DBL, 10 ng/ml, 22°C for 1 h), washed in PBS, and mounted under Vectashield (Vector Laboratories, Peterborough, UK). Sections were examined using an Axioplan-2 microscope (Zeiss Vision, Welwyn Garden City, UK), and images were captured using a JVC-3 charge-coupled device camera at ×100 magnification and KS300 software (Zeiss Vision); images were imported into Scion Image version 4.0.2. The number of cells expressing BrdU and the number of these cells that were also positive for DBL were counted in well-oriented glands and expressed per millimeter of horizontal length across the mucosa. The position of all cells positive for BrdU and DBL was determined relative to the base of the gland and assigned to one of five gland segments defined as 0–19, 20–39, 40–59, 60–79, and 80–100% of gland length (from base (0%) to luminal surface (100%)). At least 300 glands per section were observed for five sections per mouse.

Identification of H^+K^-ATPase α-subunit in parietal cells. Previous work indicates that DBL is a specific marker for parietal cells (5). To validate this for identification of parietal cells in Gas-KO and C57BL/6 mice, the staining of cells with DBL and antibody to the H^+K^-ATPase α-subunit was examined. Frozen sections were rapidly thawed by immersion in water, dehydrated in 50% and then 75% ethanol (10 min), and incubated in 10% goat serum in PBS (30 min, 22°C), and the excess solution was removed. Sections were incubated with primary antibody to the COOH terminus of the H^+K^-ATPase α-subunit (Calbiochem) diluted to 1:400 (18 h, 4°C), washed (3 times in PBS), incubated with Texas red-conjugated secondary antibody (TI5000, Vector Laboratories; 2 h, 22°C), washed (3 times in PBS), mounted under Vectashield, and examined as described above.

Radioimmunoassay. Mouse blood was collected in heparin (1 U/ml) and centrifuged, and the plasma was stored at −20°C. The concentration of amidated gastrins in plasma was determined by radioimmunoassay using antibody L2, as described elsewhere (3).

Antibiotic treatment. C57BL/6 and Gas-KO mice were given daily injections of cefoperazone (100 mg/kg body wt sc; Sigma) and provided drinking water containing 5 mg/ml streptomycin (Sigma). After 10 days of treatment, the mice were injected with BrdU, and antibiotics were administered for up to 20 days later (20). Mice were then killed, and tissue was collected as described previously.

Statistics and counting. Comparisons were carried out by Student’s t-test or ANOVA as appropriate using the program PRISM (Graphpad Software, San Diego, CA).

RESULTS

Time course of BrdU labeling. Cells incorporating BrdU were observed in the gastric corpus 1 h after injection. Labeled cells were located mainly in the isthmus region of the gland (Fig. 1A). After 10 days, only a small proportion of the BrdU-labeled cells remained, and these were distributed throughout the gland (Fig. 1B). Cells stained for BrdU and DBL (Fig. 1C) were clearly identified 10 days after injection of BrdU, but up to 5 days after injection, only very rarely did cells appear to be costained for BrdU and DBL (data not shown). The very low level of apparent colocalization in these cases is presumably due to overlay in the section of a parietal cell and a BrdU-labeled cell and reflects the level of false-positive assignments. In
all subsequent experiments, day 10 was used as the first time point for identification of BrdU-labeled parietal cells.

Costaining of DBL and \(H^+\)-K\(^+\)-ATPase in parietal cells. To validate the use of DBL as a parietal cell marker in the present strains of mice, we examined the localization of DBL and the \(H^+\)-K\(^+\)-ATPase in parietal cells. In the corpus of C57BL/6 mice, 99\% of DBL-positive cells also exhibited \(H^+\)-K\(^+\)-ATPase immunoreactivity (Fig. 1D). Similarly, 99\% of DBL-positive cells in the corpus of the Gas-KO mouse also exhibited \(H^+\)-K\(^+\)-ATPase immunoreactivity (Fig. 1E). Conversely, we did not identify a population of cells in either mouse strain that exhibited \(H^+\)-K\(^+\)-ATPase immunoreactivity and were not stained for DBL. The data, therefore, suggest that in the Gas-KO mice the expression of DBL by parietal cells is maintained and DBL is a useful parietal cell marker.

Parietal cell life span. Cells labeled with BrdU and DBL were detected in C57BL/6 and Gas-KO animals 10–30 days after BrdU injection. In both strains, BrdU-labeled cells were virtually absent 40 days after BrdU injection. In both groups 10 and 20 days after BrdU injection and was reduced in both groups at day 30 (Fig. 2A). As expected, the plasma gastrin concentrations in Gas-KO mice were below the limit of detection (<5 pM) compared with 72 ± 14 pM in wild-type C57BL/6 mice. The data suggest that the life span of parietal cells does not depend on the presence of gastrin. Interestingly, the total number of BrdU-labeled cells was slightly greater in Gas-KO than in wild-type mice at days 10 and 20. The identity of the other labeled cells has not been systematically examined, but it is worth noting that labeled parietal cells account for a relatively high proportion of the remaining cells at day 30.

Parietal cell migration. To determine whether there were differences between Gas-KO and wild-type mice in the progression of parietal cells along the gastrin gland, we assigned every BrdU/DBL-labeled cell to a position in one of five segments of the gland (see METHODS). At 10 days after injection, BrdU-labeled parietal cells were found predominantly in regions corresponding to the isthmus, neck, and midregion of the gland, and there were no marked differences between Gas-KO and wild-type mice (Fig. 3A). The population of BrdU-labeled parietal cells in the more superficial regions (isthmus and neck) was largely absent after 20 days in C57BL/6 and Gas-KO mice.

Compatible with the idea that parietal cells migrate from the proliferative region in the isthmus to the base of the gland, the percentage of labeled cells in the isthmus was reduced from 63\% at day 10 to 35\% at day 20 in C57BL/6 mice, and from 54\% at day 10 to 32\% at day 20 in Gas-KO mice (Fig. 3B). The population of labeled cells in the other segments of the gland was gradually reduced from 10\% to 5\% at day 20 in both groups. The data suggest that the migration of parietal cells in C57BL/6 and Gas-KO mice is not dependent on the presence of gastrin.
of the gland, we found that, 20 days after administration of BrdU to C57BL/6 wild-type mice, most labeled cells were in the midregion of the gland. Moreover, by 30 days after BrdU administration, most labeled cells were in the base of the gland (Fig. 3, B and C). Interestingly, however, there were significant differences in the position of BrdU-labeled parietal cells between the Gas-KO and the wild-type mice at these time points. Thus, at 20 and 30 days after administration of BrdU, the portion of the gland containing the greatest number of BrdU-labeled parietal cells was closer to the base in the C57BL/6 mouse than in the Gas-KO mouse, and the differences between strains were significant ($P < 0.05$, ANOVA).

**Antibiotic treatment.** Because Gas-KO mice are achlorhydric, we considered the possibility that bacterial overgrowth in these animals might influence BrdU labeling or parietal cell migration; therefore, we studied mice after antibiotic treatment known to reduce bacterial overgrowth (20, 21). Antibiotic treatment of C57BL/6 mice did not influence the total number of BrdU-labeled cells, but there was a significant decrease in the total number of BrdU-labeled cells in the Gas-KO mice compared with untreated Gas-KO mice ($P < 0.05$, t-test; Fig. 4A). Interestingly, the number of labeled parietal cells was not influenced by antibiotic treatment in Gas-KO or C57BL/6 mice (Fig. 4B). Moreover, the position of the BrdU-labeled cells along the gland axis in the Gas-KO mice was not influenced by antibiotic treatment (Fig. 4, C and D).

**Effect of elevated plasma gastrin on parietal cell migration.** To determine whether elevated plasma gastrin in vivo might increase the rate of migration of parietal cells, we examined the distribution of BrdU-labeled cells after 10 days in mice with moderate hypergastrinemia due to ectopic expression of a human gastrin transgene in pancreatic β-cells (Ins-Gas mice). Plasma gastrin concentration in the Ins-Gas mice was 222 ± 30 pM and was significantly higher ($P < 0.05$) than in the corresponding wild-type strain, FVB/N (60 ± 2 pM). As expected, BrdU-labeled cells at 1 h after injection in wild-type and Ins-Gas mice were localized in the band corresponding to the isthmus region. As with C57BL/6 mice, it was not possible to identify BrdU-labeled parietal cells up to 5 days after administration of BrdU. However, after 10 days, labeled cells were distributed between the isthmus and the base of the gland (Fig. 5A). In the Ins-Gas mice,
BrdU-labeled parietal cells were significantly depleted from the isthmus region, whereas the population of cells in the most distal 20% of the gland was significantly higher than in FVB/N mice (Fig. 5B); a similar tendency was also found in animals 15 days after BrdU injection. Unexpectedly, there was a marked difference in the time course over which BrdU-labeled parietal cells were lost in FVB/N and Ins-Gas mice compared with C57BL/6 and Gas-KO mice. Thus, in FVB/N and Ins-Gas mice, there were virtually no BrdU-labeled parietal cells after 20 days, and even 15 days after BrdU the number of labeled parietal cells was depleted to ~30% of that at 10 days (not shown). In contrast, as noted above, labeled parietal cells in C57BL/6 and Gas-KO mice persist to day 30. The data suggest strain differences in parietal cell life span that are independent of gastrin.

DISCUSSION

The main finding of the present study is that gastrin determines the position of parietal cells along the axis of gastric glands. It has long been recognized that gastrin regulates acid secretion (17), and recent studies in mice in which the gastrin gene has been deleted indicate that gastrin also regulates parietal cell maturation (2, 6, 10). However, the extent to which gastrin might influence parietal cell life span, or migration along the gland, is unknown. The function of parietal cells is thought to depend in part on cell position along the gland (8), so whether gastrin regulates cell migration is potentially linked to mechanisms of cell maturation. The present observations suggest that gastrin does not influence the longevity of parietal cells but does influence parietal cell position, compatible with stimulation of migration along the gland axis.

Proliferating cells in gastric glands are located in the isthmus region, and newly formed parietal cells identified by incorporation of BrdU tend to be found in this part of the gland 10 days after administration of BrdU. A subpopulation of parietal cells is known to occur here and in the neck region of the gland (7). This population of labeled cells was virtually lost by 20 days after BrdU injection, and there was little difference in this respect between Gas-KO and wild-type C57BL/6 mice. Because at this time there are labeled cells in the midregion and base of the gastric glands, it seems possible that the isthmus/neck population slowly migrates toward the base of the gland or that this population of parietal cells has a shorter life span than the glandular cells. Nevertheless, gastrin appears not to influence the turnover of this population.

In contrast, there were clear differences between C57BL/6 and Gas-KO mice in parietal cell distribution in the midregion and base of gastric glands. The abundance of BrdU-labeled parietal cells in the base of the gland increased progressively up to day 30, when the bottom 20% of the gland accounted for >50% of labeled parietal cells in C57BL/6 mice. By 40 days after BrdU injection, labeled cells were no longer identifiable in either group. Interestingly, in Gas-KO mice, the bottom 20% of the gland seldom contained labeled parietal cells, and the major labeled population of cells after 30 days was higher in the gland, occupying a region 20–40% of the gland length from the base. The data suggest that parietal cell life span is 30–40 days in wild-type and Gas-KO mice and that the presence of gastrin is required for the colonization of the most basal part of the gland by parietal cells.

Stimulation of parietal cell migration by gastrin, such as that reported here, might be expected to influence the overall distribution of these cells in Gas-KO mice compared with wild-type mice. Interestingly, therefore, close inspection of the photomicrographs in previous descriptions of the stomach in mice in which the gastrin or the gastrin-cholecystokinin-B receptor gene has been deleted indicates that parietal cells are generally scarcer in the base of the gland than they are in wild-type mice (10, 13).

Studies in the rabbit indicate that distinct subpopulations of parietal cell can be identified that differ in acid-secretory capacity (8). In particular, parietal cells at the base of the gland appear to be less active than those in the middle or upper regions. The relevant mechanisms are not clear. It would seem, however, that impairment of acid secretion in Gas-KO mice is not attributable to mislocation of parietal cells along the gland axis. The achlorhydria of Gas-KO mice is
thought to contribute to the development of bacterial overgrowth in the stomach (20, 21), which might indirectly influence parietal cell migration. We found that prolonged administration of antibiotics decreased incorporation of BrdU in Gas-KO (but not C57BL/6) mice, compatible with the idea of a moderate hyperproliferative state in the Gas-KO mice due to inflammation attributed to bacterial overgrowth. Importantly, however, there was no evidence that similar considerations might influence the distribution of BrdU-labeled parietal cells.

To test whether elevated plasma gastrin concentrations might enhance migration, we studied Ins-Gas mice, which are known to exhibit moderate, chronic hypergastrinemia (18). These transgenic mice are on a background of the FVB/N strain; this is relevant, since we encountered an unexpected strain difference in parietal cell life span. Thus, at 20 days after BrdU administration to FVB/N mice, labeled parietal cells had virtually disappeared, whereas in C57BL/6 mice, labeled cells were similar to those at 10 days. Nevertheless, the distribution of labeled parietal cells in FVB/N mice after 10 days was distinct from that in Ins-Gas mice, compatible with increased migration in the latter toward the base of the gland. Together, these observations support the conclusions from Gas-KO mice that gastrin regulates parietal cell movement along the gland axis and is not only required for migration into the most distal part of the gland but, in elevated concentrations, can stimulate it.

Cell migration is an essential feature of the mechanisms that maintain gastric gland organization. Although there is a substantial body of work on cell migration involved in wound healing (9), little attention has been given to the control of gastric epithelial cell migration in normal circumstances. The present data suggest that gastrin is a factor that influences the movement of parietal cells toward the base of gastric glands. The rates of migration are relatively slow. On the basis of the present data, we calculate that gastrin may increase migration of parietal cells from \( \sim 0.1 \mu m/h \) (Gas-KO mice) to \( 0.25 \mu m/h \) (wild-type C57BL/6 mice). This rate of movement would seem to be distinct from the rapid epithelial cell migration that is involved in wound healing. However, recent studies suggest that there is expression of the gastrin-cholecystokinin-B receptor by surface epithelial cells after gastric mucosal wounding (16). Whether this is associated with functional control of cell migration in the wounded area is unclear. Nevertheless, this process is likely to be functionally distinct from the processes described in the present study. The cellular mechanisms by which gastrin controls migration remain to be established. Several of the major biological effects of gastrin are now recognized to be indirect and mediated by paracrine agents, including histamine, epidermal growth factor family members, and Reg (4). Further work is needed to determine whether gastrin influences migration in vivo directly or indirectly by these or other paracrine mediators.

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REFERENCES

