

Diphtheria Toxin-mediated Ablation of Parietal Cells in the Stomach of Transgenic Mice*

(Received for publication, November 21, 1995)

Qitang Li, Sherif M. Karam‡, and Jeffrey I. Gordon§

From the Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110 and the ‡Department of Anatomy, Faculty of Medicine, Kuwait University, Safat 13110, Kuwait

The self-renewing epithelial populations present in the gastric units of the mouse stomach are descended from a multipotent stem cell and undergo an orderly migration-associated differentiation followed by apoptosis. The steady state census of the three principal cell types (acid-producing parietal cells, mucus-producing pit cells, and pepsinogen and intrinsic factor-producing zymogenic cells) is accurately controlled, despite marked differences in the rates of migration of each lineage. A transgenic mouse model has been created to define functional interrelationships between the proliferation, differentiation, and death programs of these lineages. Nucleotides -1035 to $+24$ of the noncatalytic β subunit gene of mouse H^+/K^+ -ATPase were used to direct expression of an attenuated diphtheria toxin A subunit in the parietal cell lineage. These transcriptional regulatory elements are not active in members of the pit and zymogenic lineages. Stomachs, prepared from postnatal day 28–80 transgenic mice and their normal littermates, were subjected to single- and multilabel immunohistochemical studies as well as qualitative and quantitative light and electron microscopic morphologic analyses. The toxin produced complete ablation of differentiated parietal cells. Loss of parietal cells was accompanied by a 5-fold increase in the number of undifferentiated granule-free cells located in the proliferative compartment of gastric units. This amplified population of granule-free cells included the multipotent stem cell as well as committed precursors of the pit and zymogenic lineages. Loss of mature parietal cells was also associated with (i) a block in the differentiation program of the zymogenic lineage with an accumulation of pre-neck cells and a depletion of their neck and mature zymogenic cell descendants, and (ii) an ~ 2 -fold amplification of pit cells. These findings are consistent with the notion that epithelial homeostasis within gastric units is maintained by instructive interactions between their different cell lineages. Unlike pit and zymogenic cells, parietal cells complete their differentiation in the gastric unit's proliferative compartment before undergoing a bipolar migration along the unit. Thus, the mature parietal cell is in a strategic position to influence decision-making among gastric epithelial cell precursors and to modulate the migration-associated terminal differentiation programs of the pit and zymogenic lineages.

The glandular epithelium of the mouse stomach is self-renewing. Self-renewal occurs in tube-shaped mucosal invaginations known as gastric units (1). The gastric unit represents a model system for studying how the proliferation, differentiation, and death programs of several distinct lineages are coordinated to maintain a steady state population of cells and whether these programs are controlled in a cell autonomous or nonautonomous fashion.

Each gastric unit located in the corpus of the adult mouse stomach contains an average of 200 cells (1). The apical third of the gastric unit, known as the pit region, is populated with ~ 40 mucus-producing pit cells (2). A centrally positioned isthmus contains one or more active multipotent stem cells and their undifferentiated yet committed descendants (3). The neck region is located just below the isthmus and contains ~ 12 mucus-producing neck cells (4). The base of the gastric unit is inhabited by ~ 70 pepsinogen- and intrinsic factor-positive zymogenic cells (4). The gastric unit's ~ 25 acid-producing parietal cells are distributed in all four of its regions (5), as are members of the enteroendocrine lineage (6).

Tritiated thymidine labeling-electron microscopic (EM)¹ autoradiographic studies have identified the presumptive stem cell and its committed, undifferentiated descendants (3). These studies have also provided morphologic descriptions of the differentiation programs of each of the principal epithelial lineages (2–6). A pit cell precursor devoid of secretory granules gives rise to pre-pit cells within the isthmus. Pre-pit cells become pit cells when they enter the pit region. They migrate up the pit and upon reaching the surface epithelium degenerate and die. The entire sequence is completed in 3 days (2). Members of the zymogenic lineage differentiate during a downward migration from the isthmus. A granule-free pre-neck cell precursor produces pre-neck cells, which are transformed to neck cells as they migrate from the isthmus to the neck. Neck cells complete their journey through the neck region in 14 days (4). Upon arrival at the upper portion of the base of gastric units, they become pre-zymogenic cells. Terminal differentiation to zymogenic cells occurs during a continued downward descent to the lower portion of the base of gastric units. Zymogenic cells die by necrosis or apoptosis. The sequence is completed in ~ 190 days (4). Conversion of undifferentiated granule-free cells to pre-parietal cells occurs in the isthmus and takes ~ 1 day (5). Differentiation of pre-parietal to parietal cells also takes place in the isthmus. Parietal cells subsequently undergo a bipolar migration to both the pit and base. Death

* This work was supported by Grant DK33487 from the National Institutes of Health (to J. I. G.) and Grant MDA275 from Kuwait University (to S. M. K.). 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.

§ To whom correspondence should be addressed: Dept. of Molecular Biology and Pharmacology, Box 8103, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110. E-mail: jgordon@pharmdec.wustl.edu.

¹ The abbreviations used are: EM, electron microscopy; DT-A, fragment A of diphtheria toxin; hGH, human growth hormone; DBA, *D. biflorus* agglutinin; GSII, *Griffonia simplicifolia* II agglutinin; AAA, *Anguilla anguilla* agglutinin; BrdUrd, 5-bromo-2'-deoxyuridine; InF, intrinsic factor; P, postnatal day; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; G_{M1} , Gal β Nac β 4[Neu5Ac α 2,3]Gal β 4Glc β 2Cer.

ensues and cells are disposed of by extrusion or phagocytosis. The overall turnover time for parietal cells is 54 days (5).

A steady state cellular census is maintained among these different epithelial lineages (1) despite differences in their rates and direction of migration. How is homeostasis achieved? Are instructive interactions between cells critical for controlling decisions about proliferation, differentiation, and death, or is regulation entirely cell autonomous? In a self-renewing system, an entirely cell autonomous mechanism of control poses the risk that perturbations in the rate of proliferation, or migration, or death could result in unopposed changes in cellular census.

The parietal cell lineage represents an attractive starting point for examining the general issue of whether one lineage influences the proliferation, differentiation, or death programs of the gastric unit's other lineages. Parietal cells are derived from three precursors (3). Although the majority (~95%) of parietal cells originate from an undifferentiated granule-free cell, thymidine labeling-EM studies indicate that the daughters of pre-pit cell precursors and the daughters of pre-neck cell precursors can each adopt two fates in the adult mouse (3). Ninety-nine percent of the progeny of a pre-pit cell precursor become pre-pit cells, but 1% become pre-parietal cells. Likewise, a small fraction (~2%) of the daughters of the pre-neck cell precursor become pre-parietal cells, while the remainder adopt a pre-neck cell fate.

These observations raise a question about whether perturbations in parietal cell differentiation could modify decisions concerning the fate of daughters of the pre-neck and pre-pit cell precursors. One recent experiment addressed this issue (7). Functional mapping studies of transcriptional regulatory elements in the mouse H^+/K^+ -ATPase β subunit gene, performed in transgenic animals using light and EM immunohistochemical techniques, have shown that its nucleotides -1035 to +24 are only active in pre-parietal cells and their differentiated parietal cell descendants (7, 8). When H^+/K^+ -ATPase β subunit^{-1035 to +24} was used to direct expression of Simian virus 40 T antigen, a 50–70-fold amplification of pre-parietal cells occurred so that they became the predominant cell type in the gastric units of adult (postnatal day 28–80) transgenic animals. Differentiation to mature parietal cells was blocked. Remarkably, even though the transgene was not expressed in members of the pit or zymogenic lineages, there was a block in conversion of pre-zymogenic to zymogenic cells. The effect was quite specific; conversion of pre-neck to neck cells and neck to pre-zymogenic cells was not perturbed (7). There was also a 2–3-fold increase in mature pit cells.

Although this experiment revealed an apparent interrelationship between the differentiation programs of the parietal, zymogenic, and pit cell lineages, it was not possible to determine whether the phenotype reflected the consequences of amplifying the normally rare isthmal pre-parietal cell and distributing these cells to "ectopic sites" along the length of gastric units or whether the phenotype was due to depletion of mature parietal cells. To distinguish between these possibilities, we have used H^+/K^+ -ATPase β subunit^{-1035 to +24} to direct expression of an attenuated diphtheria toxin A fragment (DT-A) to pre-parietal and parietal cells so that they would be destroyed.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice—pIBI30-176 (9) contains a mutant fragment A of diphtheria toxin (*tox176*) with a Gly¹²⁸ → Asp substitution (10). *tox176* was excised from pIBI30-176 using *Pst*I and inserted into the *Pst*I site of pBluescript II SK⁺. *tox176* was released from the resulting recombinant plasmid by digestion with *Hinc*II and ligated to *Bam*HI-digested pHKATPhGH1 (8), yielding pBSHKATPDTAhGH. This placed *tox176* immediately downstream of mouse H^+/K^+ -ATPase β subunit^{-1035 to +24} (11, 12) and immediately upstream of nucleotides +3

to +2150 of the human growth hormone (hGH) gene. The 4.0-kilobase pair H^+/K^+ -ATPase β subunit^{-1035 to +24}/DT-A (*tox176*)/hGH insert in pBSHKATPDTAhGH was excised by digestion with *Not*I and *Xho*I, separated from vector sequences by agarose gel electrophoresis, purified by glass bead extraction (Geneclean II, BIO 101, Inc., Vista, CA), suspended in TE buffer (10 mM Tris, pH 7.4, 0.2 mM EDTA), sterilized by passage through a 0.22- μ m filter (Ultrafree-MC, Millipore) and injected into FVB/N oocytes. Injected eggs were transferred to pseudo-pregnant Swiss Webster females using standard methods (13).

Eighty-seven live-born mice were screened using the polymerase chain reaction, tail DNA, and two primers that span intron 2 of the hGH gene. Primer 1 (5'-AGGTGGCCTTTGACACCTACCAGG-3') anneals to the antisense strand in exon 1 of hGH. Primer 2 (5'-TCCTGTTGTGTTTCCTCCCTGTTGG-3') hybridizes to the sense strand in exon 3. The following thermocycling conditions were used to generate a 360-base pair polymerase chain reaction product: denaturation = 94 °C for 90 s, annealing = 55 °C for 90 s, and extension = 72 °C for 90 s for a total of 30 cycles, followed by 72 °C for 10 min. Six transgenic founders were identified. Pedigrees were established from 5 of these founders by crosses to normal FVB/N littermates. One founder was sterile.

Mice were maintained in a specific pathogen-free state in microisolator cages under a 12 h light cycle. Animals were given a standard irradiated chow diet (Pico Lab Rodent Diet 20; PMI Feeds) *ad libitum*.

Single and Multilabel Light Microscopic Immunohistochemical Studies—Postnatal day 28 (P28), P35, P42, and P80 transgenic mice and normal littermates ($n = 2-4$ /pedigree/time point) were given an intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdUrd; 120 mg/kg) and 5-fluoro-2'-deoxyuridine (12 mg/kg) 1.5 h before sacrifice by cervical dislocation. The stomach was removed, fixed in Bouin's solution (8), and then washed in 70% ethanol. Five-micron-thick sections were cut, deparaffinized, rehydrated in PBS, and incubated in PBS-blocking buffer (bovine serum albumin (1%, w/v), powdered skim milk (0.2%, w/v), Triton X-100 (0.3%, v/v)) for 15 min at room temperature. Primary antisera were diluted in PBS-blocking buffer and incubated overnight at 4 °C with the sections. Slides were then washed in PBS, and antigen-antibody complexes were detected using Cy3-labeled donkey anti-rabbit or anti-goat Ig (Jackson ImmunoResearch; final dilution = 1:500 in blocking buffer). After a final series of washes in PBS, slides were mounted in PBS/glycerol (1:1, v/v).

The following panel of antibodies and lectins were used: rabbit anti-rat intrinsic factor (InF; 1:1000; Ref. 8); rabbit anti-pepsinogen sera (1:500; Ref. 8); rabbit anti- β subunit of rat H^+/K^+ -ATPase (amino acids 2-23; dilution = 1:500; Ref. 8), goat anti-BrdUrd sera (1:1000; Ref. 14); *Dolichus biflorus* agglutinin (DBA; carbohydrate specificity = α GalNAc epitopes; cellular specificity = FVB/N parietal cells; Refs. 7, 8, and 15); *Griffonia simplicifolia* II (GSII, GlcNAc β 1, 4; FVB/N neck cells; Refs. 8, 15, and 16), cholera toxin B subunit (ganglioside G_{M1}; FVB/N pit and surface mucous cells; Refs. 15 and 17) and *Anguilla anguilla* agglutinin (AAA, α -L-fucose; FVB/N pit and surface mucous cells; Ref. 15). All lectins contained a fluorescein isothiocyanate (FITC) tag and were diluted to 5 μ g/ml with PBS-blocking buffer (minus the powdered skim milk).

Confocal Microscopy—A Molecular Dynamics Multiprobe 2001 inverted confocal microscope was used to scan sections of mouse stomach at a confocal optical plane of 0.6 μ m. Prior to scanning, slides were subjected to immunohistochemical staining as described above.

Morphologic Studies—P28, P35, and P42 transgenic mice from pedigree 6 and their normal littermates ($n = 2-3$ animals/time point) were anesthetized. Lactated Ringer's solution was infused into their left ventricle followed by fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.2% tannic acid in 0.1 M sodium cacodylate buffer, pH 7.4). The stomach was removed and portions of its zymogenic zone (7, 18) diced into 1–2-mm³ pieces. Tissue fragments were then placed in fixative for 2 h at 4 °C, incubated overnight in 0.1 M sodium cacodylate, pH 7.4, and post-fixed for 1 h at 4 °C in 2% osmium tetroxide (reduced with 3% sodium ferrocyanide). The samples were dehydrated in graded alcohols, infiltrated with Poly/Bed 812 (Electron Microscopy Sciences), and then embedded so that the base-to-pit axis of gastric units was parallel to the cutting surface. Sections (0.5 μ m thick) were stained with 0.1% toluidine blue for light microscopy. Adjacent sections were cut to 0.1 μ m thickness and examined with a JEOL model 1200 electron microscope.

Quantitation of Cell Number—Normal FVB/N mice were sacrificed at P1, P15, P28, P42, and P80. Transgenic mice from pedigree 6 were sacrificed at P28, P35, and P42. Serial 0.5- μ m-thick sections were stained with toluidine blue and examined under a 100 \times objective lens. Gastric units cut along the length of their central lumen were selected to quantitate cell types. Cells were identified according to criteria described in Refs. 1 and 7.

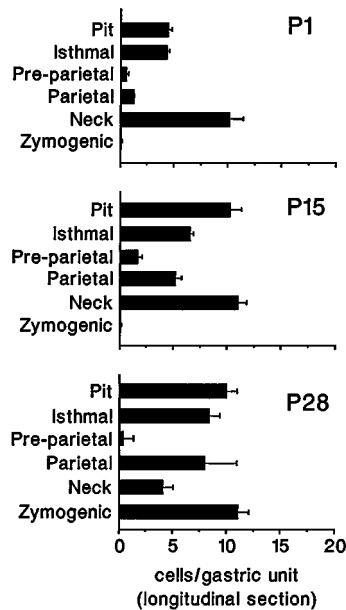


FIG. 1. Quantitation of epithelial lineages that populate gastric units in normal P1, P15, and P28 FVB/N mice. Cells were identified in toluidine blue-stained 0.5- μ m-thick sections of gastric units located middle third of the stomach (1). The mean \pm S.E. are plotted. Isthmal cells = pre-pit, pre-neck, and granule-free cells.

RESULTS AND DISCUSSION

Experimental Design

H^+/K^+ -ATPase β subunit^{-1035 to +24}/reporter transgenes are activated by embryonic day 18 (7). Expression in the parietal cell lineage is sustained at least through the first year of life.² Fig. 1 describes developmental changes in the number of parietal, zymogenic, and pit cells and their precursors in the gastric units of postnatal day 1, 15, and 28 FVB/N mice. Parietal and pit cell populations are present when H^+/K^+ -ATPase β subunit^{-1035 to +24} first becomes active. Their numbers increase through P15 and reach adult levels by the time weaning is completed. Although neck cells are also evident at P1, mature zymogenic cells are not apparent until after P15. The steady state levels of parietal, zymogenic, and pit cells noted at P28 remain constant through the next several months of life (7). Based on these findings, we elected to survey the gastric epithelium of pedigrees of FVB/N transgenic mice containing a H^+/K^+ -ATPase β subunit^{-1035 to +24}/diphtheria toxin-A fragment fusion gene from postnatal days P28 to P80.

Fragment A of diphtheria toxin (DT-A) functions as an ADP-ribosyl transferase. When introduced into cells, it ADP-ribosylates and inactivates elongation factor 2, causing inhibition of protein synthesis and cell death (10). Because of its extreme cytotoxicity, even very low basal levels of H^+/K^+ -ATPase β subunit^{-1035 to +24}/wild type DT-A expression could, in theory, result in the death of nontarget cell populations. Therefore, we used an attenuated DT-A, *tox176*, which is 15–30-fold less cytotoxic than wild type DT-A (10), to ensure that the ablation would be limited to members of the parietal cell lineage.

Finally, since previous EM immunohistochemical studies of H^+/K^+ -ATPase β subunit^{-1035 to +24} expression had been conducted using the hGH gene as a reporter (7), *tox176* was inserted into exon 1 of hGH, upstream of hGH's initiator Met codon. This served two purposes. It provided introns and a polyadenylation signal to enhance the chances of efficiently processing the transgene's DT-A containing mRNA transcript. It also reduced the chances of perturbing the pre-parietal cell-

specific pattern of H^+/K^+ -ATPase β subunit^{-1035 to +24} transcriptional activation.

H^+/K^+ -ATPase β Subunit^{-1035 to +24}/DT-A (*tox176*) Produces Ablation of Parietal Cells

The proximal third of the adult FVB/N mouse stomach (forestomach) contains a stratified squamous epithelium and lacks gastric units. The glandular epithelium that lines the distal two thirds of the stomach can be subdivided into a proximal half or zymogenic zone, and more distal mucoparietal and pure mucous zones (18). Gastric units in the zymogenic zone (corpus) contain the four epithelial lineages alluded to above. The zymogenic lineage is not represented in gastric units located within the mucoparietal zone while both the zymogenic and parietal lineages are absent from the pure mucous zone.

The forestomach, zymogenic, mucoparietal, and pure mucous zones of transgenic mice and their normal littermates were compared. Hematoxylin- and eosin-stained sections of the forestomach disclosed no histopathologic changes in transgenic animals. In normal littermates, differentiated parietal cells were readily detected in the zymogenic and mucoparietal zones based on their reaction with an antibody to the noncatalytic β subunit of H^+/K^+ -ATPase and with *D. biflorus* agglutinin (Fig. 2, A and B). In contrast, neither of these reagents reacted with cells present in the zymogenic or mucoparietal (or pure mucous) zones of transgenic mice (Fig. 2, C and D).

Comparisons of toluidine blue stained thin (0.5 μ m) sections prepared from the zymogenic and mucoparietal zones provided independent confirmation of the loss of parietal cells in transgenic animals (Fig. 3, A–D; Table I).

Previous [³H]thymidine labeling-EM autoradiographic studies had demonstrated that degeneration and apoptosis of parietal cells is normally confined to the pit and base regions of gastric units (5). Dead cells are either extruded into the lumen of gastric units, phagocytosed by neighboring cells, or engulfed by macrophages that invade from the surrounding mesenchyme (5). These processes were increased as early as P28–P35 in transgenic gastric units (e.g. Figs. 3D and 4A).

Pre-parietal cells were identified by viewing serial 0.5- and 0.1- μ m sections of gastric units under light and electron microscopes, respectively. Pre-parietal cells were largely confined to the isthmus region of gastric units in both transgenic and normal mice (Fig. 4A). Steady state levels of pre-parietal cells were 7-fold elevated in transgenic animals (from an average of 0.2–1.4 cells/longitudinally sectioned gastric unit; cf. Table I). Many of the pre-parietal cells present in transgenic gastric units were in various states of degeneration (Fig. 4, A and C). These results contrast with those obtained from identically aged FVB/N H^+/K^+ -ATPase β subunit^{-1035 to +24}/SV40 TAG transgenics raised in the same barrier facility. Their pre-parietal census ranged from 50 to 70 cells per longitudinally sectioned of gastric unit (i.e. a 250–350-fold amplification; cf. Ref. 7). Moreover, the pre-parietal cells were distributed in coherent vertical bands or clusters from the pit to the base rather than being limited to the isthmus.

Surveys of P28–P80 transgenic mice revealed a persistent absence of parietal cells as defined by immunohistochemical and morphologic criteria. Not surprisingly, H^+/K^+ -ATPase β subunit^{-1035 to +24}/DT-A (*tox176*) mice were unable to acidify their gastric contents. Luminal pH ranged from 6 to 7 in non-fasting transgenic animals and from 1 to 2 in normal littermates sacrificed at the same time of day ($n = 6$).

The Effects of Ablating Mature Parietal Cells on Other Gastric Epithelial Cell Lineages

Pit—Differentiated pit and surface mucous cells in normal 1–18-month-old FVB/N mice bind cholera toxin B subunit and

² Q. Li and J. I. Gordon, unpublished observations.

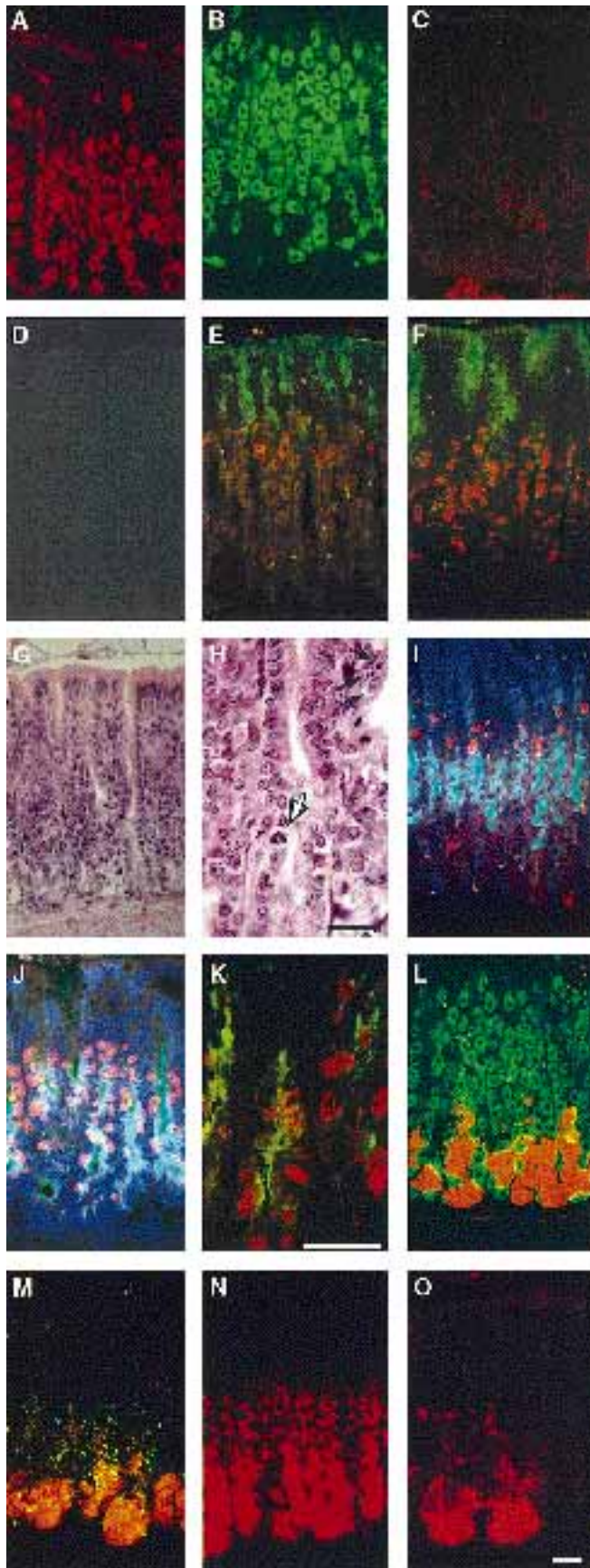


FIG. 2. Single- and multilabel immunohistochemical studies of $H^+/K^+-ATPase$ β subunit^{-1035 to +24/DT-A (tox176)} transgenic mice. Five- μm sections were prepared from the zymogenic zone of the stomachs of P35 transgenic mice and their age-matched normal FVB/N littermates. Panel A, section from a normal mouse incubated with

Anguilla anguilla agglutinin (15–17). Histochemical studies with these lectins suggested that there was an increase in pit cell number in the zymogenic gastric units of transgenic mice (e.g. Fig. 2, E and F). An ~2-fold amplification of pit cells was confirmed by surveying toluidine blue-stained thin sections (Fig. 3, B and E) and was statistically significant (Table I). A similar modest increase in pre-pit cell precursors was also noted (Table I).

Isthmus—Normal and transgenic mice were given an intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdUrd) 1.5 h prior to sacrifice to label gastric epithelial cells in S phase. Transgenic animals had a statistically significant 4-fold increase in BrdUrd-positive cells. These cells were confined to the central region of zymogenic gastric units (Fig. 2, E and F). A comparable increase in M phase cells was also documented (Fig. 2G and H; Table I).

Members of the enlarged proliferating cell population were identified using light and electron microscopy. Ablation of mature parietal cells was associated with a 4–5-fold increase in the number of (proliferating) granule-free cell precursors in the isthmus (Figs. 4, A–C; Table I). These isthmal precursors became the third most populous cell type in transgenic gastric units, in contrast to the situation in normal gastric units where their fractional representation was considerably lower (Table I).

Neck and Base—Loss of parietal cells was accompanied by a partial block in the differentiation program of the zymogenic cell lineage, resulting in accumulation of pre-neck cells and depletion of neck and zymogenic cells. Pre-neck cell number

rabbit anti-rat β subunit of $H^+/K^+-ATPase$ sera. Antigen-antibody complexes were visualized with Cy-3 labeled donkey anti-rabbit Ig. Parietal cells appear red. Panel B, section from a normal mouse incubated with FITC-labeled DBA. Parietal cells that react with the lectin appear green. Multilabel studies indicated that >98% of normal P35 FVB/N parietal cells coexpress the β subunit of $H^+/K^+-ATPase$ and fucosylated glycoconjugates recognized by DBA (data not shown). Panels C and D, sections prepared from a transgenic littermate and processed as in panels A and B, respectively. No specific cellular staining for the β subunit (C) or glycoconjugates recognized by DBA (D) are evident, indicating the absence of parietal cells. Panel E, section from a normal mouse incubated with FITC-labeled AAA and goat anti-BrdUrd. Antigen-antibody complexes were detected with Cy-3-labeled donkey anti-goat Ig. Pit and surface epithelial cells that produce glycoconjugates recognized by AAA appear green. S phase cells in the isthmus appear orange-red. Panel F, section from a transgenic littermate, processed as in E. The number of S phase (BrdUrd-positive orange) cells is greater when compared to the age- and gender-matched normal cagemate shown in E. Panels G and H, low and high power views of a hematoxylin- and eosin-stained section prepared from a transgenic mouse. The high power view provides evidence of increased proliferation (e.g. open arrow pointing to M phase cell) and increased apoptosis (solid arrows). Panel I, section of a normal FVB/N stomach incubated with FITC-labeled GSII, goat anti-BrdUrd, Cy3-labeled donkey anti-goat Ig, and bis-benzidine. GSII-positive neck (and pre-neck) cells appear aqua, while S phase cells appear red. Bis-benzidine stains nuclei purple. Panel J, section from a transgenic littermate processed as in I. Note the increased number of BrdUrd-positive cells. Panel K, section from a transgenic mouse incubated with FITC-labeled GSII, goat anti-BrdUrd, and Cy3-labeled donkey anti-goat Ig. This high power scanning confocal microscopic view of the isthmus shows BrdUrd-positive (red) pre-neck cells that produce glycoconjugates recognized by GSII (green). Panel L, double exposure of the section shown in panel B after incubation with FITC-labeled DBA, rabbit InF, and Cy3-labeled donkey anti-rabbit Ig. Differentiated, InF-positive zymogenic cells (orange) are located in the base of these normal mouse gastric units. Panel M, double exposure of the section shown in panel C after treatment with the reagents described in panel L. Panels N and O, sections from normal (N) and transgenic (O) littermates were incubated with rabbit anti-rat pepsinogen and Cy3-labeled donkey anti-rabbit Ig. Comparison of the two panels indicates that the number of pepsinogen-positive cells is reduced in transgenic gastric units and that they are confined to the base. Bars = 25 μm .

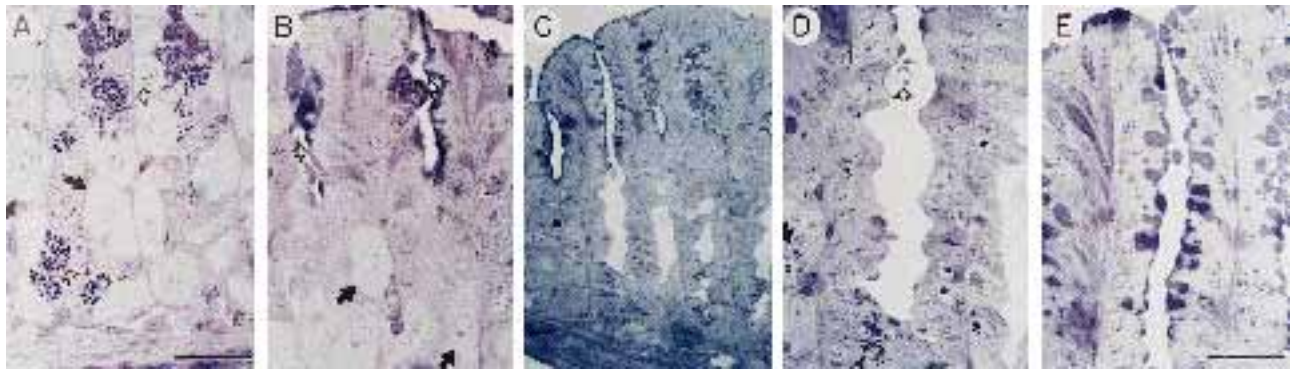


FIG. 3. Toluidine blue-stained sections of gastric units present in a postnatal day 35 $H^+/K^+-ATPase \beta$ subunit^{-1035 to +24/DT-A} (*tox176*) transgenic mouse and its normal littermate. Panel A, base of a normal FVB/N gastric unit. Differentiated parietal cells are evident (closed arrows). Zymogenic cells (e.g. open arrow) contain numerous dense secretory granules. Panel B, pit region of a normal gastric unit. Parietal cells are indicated by closed arrows. Pit cells with apical granules are denoted by open arrows. Panel C, lower power view of a section taken from the zymogenic zone (corpus) of a transgenic littermate's stomach. Panel D, high power view of the section in panel C, showing a dilated central lumen in the gastric unit with a degenerated, extruded cell (open arrow), apoptotic bodies (e.g. closed arrow), a pre-zymogenic cell (closed arrowhead), and the absence of parietal cells. Panel E, another high power view of panel C. Comparison with panel B emphasizes the increase in pit cell number associated with ablation of parietal cells. Bars = 25 μm .

TABLE I

Comparison of epithelial cell populations in the gastric units of P42 transgenic $H^+/K^+-ATPase \beta$ subunit^{-1035 to +24/DT-A} mice and their normal FVB/N littermates

Cell type	Normal	Transgenic
Pit	14 \pm 0.8 ^a	21.3 \pm 0.6
Pre-pit	3 \pm 0.2	5 \pm 0.3
Granule-free	2.1 \pm 0.3	9 \pm 0.8
Pre-parietal	0.2 \pm 0.1	1.4 \pm 0.2
Parietal	8.5 \pm 0.5	0 \pm 0
Pre-neck	2.4 \pm 0.2	10.1 \pm 1.2
Neck	4.5 \pm 0.3	2.2 \pm 0.5
Pre-zymogenic	1.1 \pm 0.2	1.3 \pm 0.2
Zymogenic	6.5 \pm 0.6	1 \pm 0.3
Enteroendocrine	0.5 \pm 0.3	1.9 \pm 0.2
Total	42.8 \pm 1.2	53.2 \pm 1.5
Mitosis	0.3 \pm 0.1	1.2 \pm 0.2
Apoptosis	0 \pm 0	4.8 \pm 0.6

^a The mean number of cells/longitudinal section \pm S.E. was determined by surveying toluidine blue-stained 0.5- μm -thick sections of gastric units ($n = 11-20$).

was increased 4–5-fold (Table I). This increase was due, at least in part, to increased cell division. Normal FVB/N pre-neck and neck cells bind the lectin *Griffonia simplicifolia* II (GSII; Refs. 15 and 16). *Proliferating BrdUrd*⁺-GSII⁺ pre-neck cells were identifiable using scanning confocal microscopy (Fig. 2, I–K). Differentiated pepsinogen- and intrinsic factor-positive zymogenic cells were detectable in P28-P80 transgenic mice although their numbers were reduced (Fig. 2, L–O). Quantitative analyses of serial toluidine blue-stained 0.5- μm -thick sections confirmed a statistically significant 2-fold decrease in neck cells and a 6-fold reduction in zymogenic cells (Table I). EM established that those neck and zymogenic cells that were present had normal morphology (Fig. 4D).

Apoptosis—Apoptosis was increased in transgenic gastric units (Fig. 2H; Table I). Light microscopic analysis of thin sections plus electron microscopy failed to show signs of apoptosis in the multipotent isthmal stem cell or its granule-free committed pre-pit and pre-neck cell daughters. These microscopic surveys confirmed that the augmentation in apoptosis was confined to members of the parietal cell lineage, consistent with the predicted consequence of expressing DT-A.

Prospectus

No cell culture system can recapitulate the spatial and temporal complexities of epithelial proliferation, differentiation,

and death in gastric units. Thus, genetically manipulatable *in vivo* models are needed to explore the functional interrelationships between these programs and to identify the underlying molecular mediators.

This report describes a transgenic mouse model where diphtheria toxin-mediated ablation of mature parietal cells is associated with a perturbation in the proliferation and differentiation programs of the pit and zymogenic lineages. The results provide direct evidence that loss of mature parietal cells reduces the efficiency of zymogenic cell differentiation from pre-neck cell precursors, while at the same time producing an amplification of pit cells. These findings are consistent with the notion that epithelial homeostasis within gastric units is maintained by instructive interactions that occur between different lineages.

The responses to the ablation of mature parietal cells suggest that both positive and negative regulatory cascades operating within gastric units derive from, and/or are dependent upon, this cell type. It is not yet clear whether instructive interactions involving parietal cells require direct cell-cell contacts or whether they involve short or long range paracrine loops. Our data underscore the fact that the mature parietal cell is in a strategic position to influence decision making. Parietal cells complete their differentiation in the isthmus, unlike pit and zymogenic cells. Thus, DT-A-induced degeneration of mature isthmal parietal cells could have marked effects on decisions concerning the proliferative status or fate of isthmal-based precursors of the pit and zymogenic lineages (e.g. through the release of positively and/or negatively acting factors from these dying cells or as a consequence of the loss of their normal instructive functions).

As noted in the Introduction, one fascinating feature of the self-renewing gastric unit is its ability to maintain an accurate steady state census of its component cell types even though these cells migrate at different rates along the base-pit axis. The bipolar migration of mature parietal cells through the pit and neck/base regions of gastric units could provide a series of extra-isthmal instructive interactions that serve to modulate progression through the pit and zymogenic cells' terminal differentiation programs.

The regulatory significance of cell-cell contacts involving parietal cells can now be assessed using a strategy analogous to that recently employed in the small intestine of chimeric-transgenic mice, namely disruption of endogenous cadherin-mediated cell adhesion by employing a lineage-specific promoter such as $H^+/K^+-ATPase \beta$ subunit^{-1035 to +24} to direct expres-

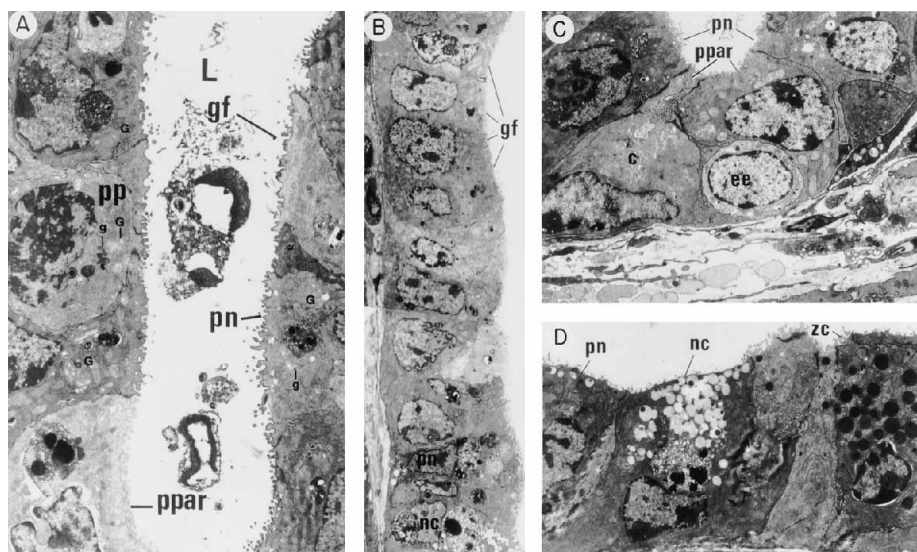


FIG. 4. Transmission electron microscopic view of gastric units from a postnatal day 42 $H^+/K^+-ATPase$ β subunit $^{-1035 \text{ to } +24}/DT-A$ (*tox176*) mouse. Panel A, longitudinal section of a gastric unit. The midportion of this unit contains a granule-free precursor (*gf*), a dividing pre-pit cell (*pp*), a pre-neck cell (*pn*), and a pre-parietal cell (*ppar*). The pre-parietal cell shows signs of degeneration with partial dissolution of cytosol, dense lysosomal bodies, and a dilated nuclear envelope. The remnants of two extruded cells are seen in the central lumen (*L*). *g*, secretory granules; *G*, Golgi apparatus. Magnification, $\times 5000$. Panel B, montage of the midportion of a gastric unit showing amplified population of granule-free (*gf*) precursors. A pre-neck (*pn*) and neck (*n*) cell are also evident. Magnification, $\times 3000$. Panel C, the pre-parietal cell (*ppar*) in the mid-left portion of the panel appears normal and contains apical microvilli, numerous mitochondria, and a characteristic secretory canaliculus (*c*). The pre-parietal cell in the mid-right portion of the panel contains mitochondria with an abnormal pale matrix, indicative of early degeneration. Two pre-neck cells (*pn*) are present as is an enteroendocrine cell (*ee*). Panel D, existing populations of pre-neck, neck, and zymogenic cells have normal morphology. The pre-neck cell (*pn*) contains scattered secretory granules with electron dense cores. The number of these secretory granules is markedly increased in a neck cell (*nc*). A fully differentiated zymogenic cell (*zc*) contains large (900–1300 nm) granules with uniform electron density. Magnification, $\times 6000$.

sion of a dominant negative cadherin (19, 20). The ability to readily identify the committed precursor cells of the gastric unit's principal epithelial lineages using morphologic criteria permits a more complete description of the effects of such perturbations on decision making than is achievable in other self-renewing systems, including the small intestine's crypt-villus unit.

Acknowledgments—We thank David O'Donnell, Maria Karlsson, Lisa Roberts, Anita Mathew, and Amal Wagdi for technical assistance; Ian Maxwell for supplying p130–176 DNA; David Alpers for providing antibodies to pepsinogen and intrinsic factor; and Michael Caplan for his gift of antisera to the β subunit of $H^+/K^+-ATPase$.

REFERENCES

- Karam, S. M., and Leblond, C. P. (1992) *Anat. Rec.* **232**, 231–246
- Karam, S. M., and Leblond, C. P. (1993) *Anat. Rec.* **236**, 280–296
- Karam, S. M., and Leblond, C. P. (1993) *Anat. Rec.* **236**, 259–279
- Karam, S. M., and Leblond, C. P. (1993) *Anat. Rec.* **236**, 297–313
- Karam, S. M. (1993) *Anat. Rec.* **236**, 314–332
- Karam, S. M., and Leblond, C. P. (1993) *Anat. Rec.* **236**, 333–340
- Li, Q., Karam, S. M., and Gordon, J. I. (1995) *J. Biol. Chem.* **270**, 15777–15788
- Lorenz, R. G., and Gordon, J. I. (1993) *J. Biol. Chem.* **268**, 26559–26570
- Breitman, M. L., Rombola, H., Maxwell, I. H., Klintworth, G. K., and Bernstein, A. (1990) *Mol. Cell. Biol.* **10**, 474–479
- Maxwell, F., Maxwell, I. H., and Glode, L. M. (1987) *Mol. Cell. Biol.* **7**, 1576–1579
- Canfield, V. A., and Levenson, R. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 8247–8251
- Morley, G. P., Callaghan, J. M., Rose, J. B., Toh, B. H., Gleeson, P. A., and van Driel, I. R. (1992) *J. Biol. Chem.* **267**, 1165–1174
- Hogan, B., Costantini, F., and Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Cohn, S. A., Simon, T. C., Roth, K. A., Birkenmeier, E. H., and Gordon, J. I. (1992) *J. Cell Biol.* **119**, 27–44
- Falk, P., Roth, K. A., and Gordon, J. I. (1994) *Am. J. Physiol.* **266**, G987–G1003
- Falk, P. G., Lorenz, R. G., Sharon, N., and Gordon, J. I. (1995) *Am. J. Physiol.* **268**, G553–G567
- Falk, P. G., Bry, L., Holgersson, J., and Gordon, J. I. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 1515–1519
- Lee, E. R., Trasler, J., Dwivedi, S., and Leblond, C. P. (1982) *Am. J. Anat.* **164**, 187–207
- Hermiston, M. L., and Gordon, J. I. (1995) *J. Cell Biol.* **129**, 489–506
- Hermiston, M. L., and Gordon, J. I. (1995) *Science* **270**, 1203–1207