Examining the Role of Paneth Cells in the Small Intestine by Lineage Ablation in Transgenic Mice*

(Received for publication, February 12, 1997, and in revised form, May 1, 1997)

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The Paneth cell lineage is one of four epithelial lineages derived from the adult mouse small intestine's multipotent stem cell. Mature Paneth cells secrete antimicrobial peptides (cryptdins), growth factors, as well as two gene products, a secreted phospholipase A2 and matrilysin, that has been implicated as modifiers of adenoma formation in mice containing a mutation in the tumor suppressor Apc. Immature Paneth cells are located just above and below the cell layer, in intestinal crypts, that has been proposed to contain the multipotent stem cell. Paneth cells differentiate during a downward migration to the crypt base. The location and direction of Paneth cell migration, their high density and long residency time at the crypt base, and the nature of their secreted gene products, suggest that they may influence the structure and/or function of the stem cell niche. Paneth cell ablation can therefore be viewed as an experimental manipulation of the cellular microenvironment that purportedly contains the stem cell and its immediate descendants. Two types of ablation experiments were performed in transgenic mice. Nucleotides −6500 to +34 of the mouse cryptdin-2 gene (CR2) were used to express an attenuated diphtheria toxin A fragment. Light and electron microscopic immunohistochemical analyses of several pedigrees of postnatal day 28 to 180 animals established that ablation of Paneth cells is accompanied by an increase in the proportion of undifferentiated crypt base columnar cells. These cells normally co-exist with Paneth cells. The ablation does not produce a detectable effect on the proliferation or terminal differentiation programs of the other three lineages or on host-microbial interactions. The last conclusion is based on the ability of crypts to remain free of microbes detectable by Gram and Warthin-Starry stains and by retention of the normal crypt-villus distribution of components of the diffuse gut-associated lymphoid tissue. CR2-directed expression of simian virus 40 large T antigen also results in a loss of mature Paneth cells but produces a marked amplification of crypt cells having a morphology intermediate between Paneth and granule goblet cells. EM immunohistochemical analyses suggest that intermediate cells can differentiate to mature goblet cells but not to Paneth cells, as they migrate up the crypt-villus axis. Our findings suggest that (i) stemness in the crypt is not defined by instructive interactions involving the Paneth cell; (ii) expressing a Paneth cell fate may require that precursors migrate to the crypt base; (iii) antimicrobial factors produced by Paneth cells are not required to prevent colonization of small intestinal crypts; and (iv) this lineage does not function to maintain the asymmetric crypt-villus distribution of components of the diffuse gut-associated lymphoid tissue.

The structural and functional organization of the adult mouse small intestinal epithelium lends itself to studying both the regulation and integration of cellular proliferation, differentiation, and death programs. The epithelium contains four principal cell types: absorptive enterocytes (comprising >80% of the total population), enteroendocrine cells, mucus-producing goblet cells, and Paneth cells. All four lineages are derived from a multipotent stem cell that is functionally anchored near the base of each of the small intestine's 1.1 million crypts of Lieberkühn (I–4). Cell division is confined to these crypts (5). Enterocytes, enteroendocrine, and goblet cells migrate out of the crypt and up an adjacent villus. Migration is highly ordered and associated with terminal differentiation. Cell death occurs near the villus tip where cells are exfoliated into the lumen (6, 7). Proliferation, differentiation, and death take place in a spatially well-organized continuum that extends from the crypt to the apex of a villus. This sequence is completed rapidly (2–5 days in the case of enterocytes, enteroendocrine, and goblet cells; Refs. 1 and 8–10) and is recapitulated throughout the lifespan of the mouse.

The Paneth cell lineage differs from the others in a number of notable ways. It is the only lineage that executes its terminal differentiation program during a downward migration from the stem cell zone to the crypt base (11). It is the longest lived lineage, and the only one that exists entirely within the proliferative compartment. Each crypt contains 30–50 mature Paneth cells that survive for 18–23 days before degenerating and undergoing phagocytosis by their neighbors (11–13). Paneth cell age correlates with position in the crypt; the most mature cells are located at or near the crypt base (2). The size of the Paneth cell's apical secretory granules also correlates with age; larger granules are produced by older cells (2, 11).

The function of the Paneth cell has not yet been clearly defined. Residency at the crypt base places this lineage in a position to release products from its apical granules that could affect establishment and/or maintenance of the stem cell's niche or influence the properties of the stem cell's descendants. A number of factors exported by Paneth cells could regulate epithelial proliferation and differentiation programs. They include tumor necrosis factor-α (14), guanylin (15), and epidermal growth factor (16). Two Paneth cell products have been implicated as modifiers of adenoma formation in mice heterozygous for a mutation in the adenomatous polyposis coli gene, ApcMin (17). Production of matrilysin, a matrix metalloproteinase...
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ase, is limited to the Paneth cell lineage in the adult mouse intestine (18). The protein is expressed in a high percentage of early stage human colorectal neoplasms and Min adenomas (19). Min/+ mice homozygous for a null allele of the matriitin gene have 60% fewer adenomas than animals with the wild type allele, suggesting that the enzyme functions as suppressor of tumorigenesis (19). Pla2g2a encodes a phospholipase A2 that is secreted from Paneth cells (20–22). This gene is a strong candidate for Mom1, a semi–dominant modifier of adenoma size and multiplicity in Min/+ animals (23–25).

Paneth cells also export lysozyme (26, 27) and a family of defense-related anti-microbial peptides known as cryptdins (28). The intestine contains a complex microflora. Components of this microflora are able to establish stable niches at particular positions along the duodenal–ileal axis (29). The fact that different cryptdins exhibit distinct developmental and spatial patterns of expression along this axis (30) suggests that Paneth cells could play a role in modulating the composition of the microbiota or contribute to mucosal barrier functions.

We have examined the contribution of this lineage to epithelial and microbial homeostasis by generating two types of transgenic mice in which mature Paneth cells have been eliminated.

EXPERIMENTAL PROCEDURES

Construction of Transgenes—A 2.7-kb DNA fragment containing simian virus 40 large T antigen (SV40 TAg) was excised from pBluescript SK-1 (31) with BamHI and subcloned into the BamHI site of pCR-H1 (32). This yielded pCR2-TAg which contained SV40 TAg under the control of nucleotides -6500 to +34 of the mouse cryptdin-2 gene (33).

Complete digestion of pBluescript SK-1 with BamHI, followed by partial digestion with EcoRI, allowed purification of a DNA fragment containing pBluescript SK-1 (Stratagene) with nucleotides 34 linked to hGH and subcloned into the EcoRI site of pCR-H1 (34). This fragment contained BamHI/EcoRI fragment containing cryptdin-2 -6500 to -34 and SV40 TAg from pCR2-TAg were ligated together, producing pCR2-TAg-hGH. pCR2-TAg-hGH was subsequently cut with BamHI, treated with Klenow, and ligated to a 0.6-kb HindII fragment containing an attenuated diphtheria toxin A fragment (tox176; Ref. 35). The resulting plasmid, pCR2-tox176-hGH, contained tox176 immediately downstream of cryptdin-2 -6500 to -34 and immediately upstream of hGH -34 to +2150. The initiator Met and the ribosomal re-entry site to re-initiate translation at the downstream initiator were removed en bloc.

Generation of Transgenic Mice—A 9.2-kb fragment containing cryptdin-2 -6500 to -34 and SV40 TAg (CR2-TAg) was released from pCR2-TAg by digestion with NotI and EcoRI. pCR2-tox176-hGH was digested with NotI and XhoI to liberate a 9.4-kb DNA fragment containing cryptdin-2 -6500 to -34, tox176-hGH (CR2-tox176). An 8.3-kb fragment containing cryptdin-2 -6500 to -34 linked to hGH -34 to +2150 (CR2-hGH) was released from pCR2-H1 with EcoRI (32). Each fragment was purified by agarose gel electrophoresis followed by glass bead extraction (GeneClean, Bio 101) and used for pronuclear injection of FVB/N oocytes. Oocytes were subsequently transferred to pseudograft Swiss Webster females using standard techniques (36).

Live born mice were screened for the presence of transgenes by extracting tail DNA and performing polymerase chain reactions using primers that anneal to hGH DNA (CR2-tox176; CR2-hGH; 5′-AGGTGG-GCCTTTGGACACCATTACGG-3′ and 5′-TCTGTTGTGGTTGCTCCCTC-3′) or to SV40 TAg DNA (CR2-TAg; 5′-ATGAAATGGGACGAGT-GTG-3′ and 5′-GCAGACATCTATCCGTTGGTG-3′). The polymerase chain reaction mixtures (final volume = 25 μl) contained 50 mM KCl, 20 mM Tris, pH 8.4, 2.5 mM MgCl2, 200 μM dNTPs, primers (1 μM each), 0.7 unit of Taq DNA polymerase (Boehringer Mannheim), and approximately 0.5 μg of genomic DNA. Taq polymerase-based amplification reactions were used to amplify an hGH fragment from CR2-tox176 and CR2-hGH DNAs: denaturation, 1 min at 94 °C; annealing, 1.5 min at 55 °C; and extension, 2 min at 72 °C for 30 cycles. For CR2-TAg, denaturation was performed at 95 °C and annealing at 58 °C.

Four CR2-hGH founders were identified from 38 live born mice, 2 CR2-tox176 founders from 67 mice, and 10 CR2-TAg founders from 87 animals. Pedigrees were established from each of the CR2-hGH and CR2-tox176 founders and from 8 of the CR-TAg founders. All pedigrees were maintained by crosses to normal FVB/N littermates. Pedigree 61, containing CR2-hGH, has been described in an earlier publication (32).

Maintenance of Animals—Mice were housed in microisator cages under a strictly controlled light cycle (lights on at 0600 h and off at 1800 h) and given a standard irradiated chow diet ad libitum (Pico rodent chow 20, Purina Mills). Routine screens for hepatitis, minute, lymphocytic choriomeningitis, estromelia, polyoma, sendai, pneumonia, and MAD viruses, enteric bacterial pathogens, and parasites were negative. Specific pathogen-free transgenic animals and their nontransgenic littermates were sacrificed between postnatal days 28 (P28) and P180.

Histochemical Stains—Immediately after sacrifice, the small intestine was removed en bloc, flushed with ice-cold phosphate-buffered saline (PBS), fixed in 10% buffered formalin (Fisher) for 4–6 h, and then washed in 70% ethanol overnight at room temperature. The intestine was embedded in plastic (JB-4 Embedding Kit, Polysciences), and 4–μm thick sections ("thin sections") cut from its proximal, middle, and distal thirds (these segments were arbitrarily designated duode- num, jejunum, and ileum, respectively). Alternatively, after washing in 70% ethanol, the intestine was cut open along its duodenal–ileal axis, rolled into a circle, and held in this circular configuration by mounting agar (2% agar (Sigma) in 5% buffered formalin). Each of the resulting “Swiss rolls” was then placed in a tissue cassette, embedded in paraffin, and 5 μm-thick serial sections were prepared. Plastic- or paraffin-embedded sections were stained with hematoxylin and eosin, phosphine/tartaric acid, or with Alcian blue and periodic acid Schiff (PAS) using standard protocols (37).

Goblet cells were quantitated by counting Alcian blue/PAS-positive cells in all well-oriented jejunal crypt-villus units in at least two non-adjacent sections cut from Swiss rolls (sections were prepared from three T antigen animals and three normal littermates per pedi- gree). Paneth cells were likewise quantitated by counting phloxine/tartaric acid-positive cells in jejunalcrypts.

To analyze the distribution of components of the microflora along the crypt-villus units of specific pathogen-free transgenic animals and their normal littermates, mice from the various pedigrees were sacrificed at P28, P42, and P120–P180. Their small intestines were fixed 4–6 h in 10% buffered formalin with 0.7 unit of phosphine/fluoro-2deoxyuridine (12 mg/kg) and 5-bromo-2-deoxyuridine (120 mg/kg, BrdU) and 5-fluoro-2-deoxyuridine (12 mg/kg) 1.5 to 72 h before sacrifice. The small intestine was then removed from each animal, flushed with cold PBS, fixed in Bouin’s solution for 8 h at room temperature, treated with 70% ethanol, and 4–6-μm thick sections cut from paraffin-embedded Swiss rolls. Sections were then deparaffinized, rehydrated, and placed in PBS-blocking buffer (1% bovine serum albumin, 0.3% Triton X-100 in PBS) for 20 min at room temperature. Slides were incubated overnight at 4 °C with the following antibodies: (i) rabbit anti-syndecan raised against residues 34–35 of syndecan-1 (the anti sera reacts with purified syndecans 1, 2, 3, and 6 (32, 38), was supplied by Michael Selsted, University of California, Irvine, and was diluted 1:500 in PBS-blocking buffer; (ii) rabbit anti-synerin to the secreted phospholipase A2, A-20 (Supra; Ref. 39), was supplied by F. Gross (Munich, Germany); (iii) rabbit anti-human syndecan (Dako, Santa Barbara, CA); specificity in the FVB/N intestine described in Ref. 32; dilution = 1:50; (iv) rabbit anti-human syndecan (Dako, Santa Barbara, CA); specificity in the FVB/N intestine described in Ref. 32; dilution = 1:50; (v) rabbit anti-human syndecan (Dako, Santa Barbara, CA); specificity in the FVB/N intestine described in Ref. 32; dilution = 1:50; (vi) rabbit anti-human syndecan (Dako, Santa Barbara, CA).
(Dako; 39; 1:2000); (vii) rabbit anti-STV40 Tag (a generous gift of Doug Hanahan, University of California, San Francisco; 40; 1:2000); and (viii) goat anti-BrdU (Ref. 41; 1:1000). Antibody-antigen complexes were detected with indocarbocyanine (Cy3)- or indodicarbocyanine (Cy5)-conjugated donkey anti-rabbit or anti-goat immunoglobulins (Ig; Jackson ImmunoResearch; 1:500).

Sections were also incubated with a series of fluorescein isothiocyanate (FITC)-conjugated lectins (all obtained from Sigma, all used at a final concentration of 5 μg/ml PBS blocking buffer); (i) Ulex europaeus agglutinin 1 (UEA-1; carbohydrate specificity = Fuc(α1,2Gal epitopes; all obtained from Vector Laboratories and used at a 1:500 dilution in PBS-blocking buffer); (ii) rat anti-mouse CD4 (clone H129.19); (iii) rat anti-mouse α chain of CD8 (clone 53–6.7); (iv) hamster anti-mouse β-subunit of the αβ T-cell receptor (TCR; clone H57–597); (iv) hamster anti-mouse γδ TCR (clone GL3); and (v) rat anti-mouse CD45R/B220 (a B-cell marker; clone RA3–6B2). Mice were sacrificed, and the small and large intestines were flushed with PBS and then frozen in OCT (Miles). Serial sections were cut, fixed in 2% osmium tetroxide, and stained with aqueous uranyl acetate and lead. Samples were dehydrated in graded alcohols and embedded in Lowicryl (Polysciences). Fifty to seventy nanometer-thick sections were cut, placed on 100-nm Formvar-coated grids (Electron Microscopy Sciences), and floated for 30 min at room temperature on a solution of Tris-buffered saline-blocking buffer (20 mM Tris, 150 mM NaCl, pH 7.4, 0.1% normal mouse serum, 0.3% Tween 20). Grids were then incubated for 2 h at room temperature with rabbit anti-mouse cryptdin (see above, diluted 1:50 with Tris-buffered saline, 5% normal mouse serum, 0.3% Tween 20), rabbit anti-mouse Pla2g2a (1:1000), or rabbit anti-hGH (Dako, 1:100; 45). Following washes with Tris-buffered saline, 0.3% Tween 20 against-antibody complexes were detected with 15-nm diameter colloidal gold-conjugated goat anti-rabbit IgG (Jackson Immunoresearch, diluted 1:15). Grids were counterstained with aqueous uranyl acetate and lead.

RESULTS

General Comments About Paneth, Goblet, and Intermediate Cells—Paneth cells are distributed along the length of the duodenal-ileal axis in adult (postnatal day 28 to 180) FVB/N mice (32). They can be recognized based on staining of their characteristic apical secretory granules with tartrazine, by their reaction with antibodies directed against lysozyme, the secreted phospholipase encoded by Pla2g2a, and cryptdins, as well as by their production of fucosylated glycoconjugates detected by the lectins Ulex europaeus agglutinin type 1 (UEA1), peanut (Arachis hypogaea) agglutinin (PNA), and Dolichos biflorus agglutinin (DBA) (32, 42).

Approximately 10% of goblet cells in the normal adult mouse intestine contain very small electron dense cores within their mucin granules. As these “granule goblet cells” migrate up villi and differentiate, they secrete their dense core mucin granules which are then replaced by “common” mucin granules that lack electron-dense cores (8). A rare cell type has been observed in normal small intestinal crypts. Its granules contain electron-dense cores that are intermediate in size between those in granule goblet cells and those in the apical granules of young Paneth cells (Fig. 1, A–C). The granules of these rare cells also contain small amounts of mucin. Because of their ultrastructural features, they have been termed “intermediate” (13), “granulomucous” (46), or “transitional” (47, 48) cells. These cells have been proposed to be Paneth cells undergoing transformation to goblet cells; cells in this transitional group were also termed “fibrous” cells, or a precursor of both lineages.

Nucleotides —6500 to —3 of the Mouse Cryptdin-2 Gene Are Active in the Paneth, Granule Goblet, and Intermediate Cells of Adult Transgenic Mice—Light microscopic surveys of adult FVB/N small intestine disclosed that ~95% of all crypts present in a cross-section contain cryptdin-positive cells (average = 3 cells/duodenal crypt section; 5 cells/ileal crypt section). EM immunohistochemical analysis using polyclonal antibodies that recognize several members of the cryptdin family revealed that cryptdins are present in the dense core granules of goblet cells, intermediate cells, and Paneth cells (Fig. 1, E and
antibodies that react with several members of the cryptdin family. FVB/N P28 mouse. The section was incubated with rabbit polyclonal antibodies as in Antigen-antibody complexes were visualized with gold-labeled second-
particles are evident in the electron dense apical secretory granules.

hGH and gold-conjugated goat anti-rabbit Ig. The 18-nm diameter gold
transgenic mouse. The section was incubated with rabbit anti-
evidence of hGH expression in the mature Paneth cells of a P28 CR2-
hGH transgenic mouse. The section was incubated with rabbit anti-
epitope synthesis and cell death.

Fragment A of diphtheria toxin (DT-A) ADP-ribosylates
Diphtheria Toxin A Fragment Results in Paneth Cell Abla-
gene are active in Paneth, granule goblet, and intermediate
cells (see below).

to define the small intestinal patterns of expression of a human
growth hormone reporter in several pedigrees of P28 to P180
transgenic mice containing a cryptdin-2
34-directed Expression of an Attenuated
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tox176) mice were produced. There were no statistically signif-
ics are not expressed in any other intestinal epithelial cell type, including
mature goblet cells (data not shown).

We subsequently used light and EM immunohistochemistry
to define the small intestinal patterns of expression of a human
growth hormone reporter in several pedigrees of P28 to P180
transgenic mice containing a cryptdin-2
34/tox-176 (CR2-

Paneth cell ablation in transgenic mice because it is less likely than
wild type DT-A to cause death if expressed at low basal levels in nontarget cell populations (49).

Two pedigrees of FVB/N cryptdin-2
34/tox-176 (CR2-
tox176) mice were produced. There were no statistically signif-
cic granules with electron dense cores are found in this region of the cell. Bar = 3 μm. C, supranuclear region of a Paneth cell showing details of secretory granule morphology. Bar = 3 μm. D, immunohistochemical evidence of hGH expression in the mature Paneth cells of a P28 CR2-
hGH transgenic mouse. The section was incubated with rabbit anti-
hGH and gold-conjugated goat anti-rabbit Ig. The 18-nm diameter gold
gold particles are evident in the electron dense apical secretory granules. Bar = 1 μm. E and F, cryptdins are present in the dense core granules of intermediate cells (E) and granule goblet (F) cells in a normal
FVB/N P28 mouse. The section was incubated with rabbit polyclonal antibodies that react with several members of the cryptdin family. Antigen-antibody complexes were visualized with gold-labeled secondary antibodies as in D. Bar = 1 μm.

Antigen−antibody complexes were visualized with gold-labeled second-
ary antibodies as in D. Bar = 1 μm.

F). The EM study also indicated that these cryptdins are not
expressed in any other intestinal epithelial cell type, including

mature goblet cells (data not shown).

We subsequently used light and EM immunohistochemistry
to define the small intestinal patterns of expression of a human
growth hormone reporter in several pedigrees of P28 to P180
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growth hormone fusion gene (CR2-hGH). The results estab-
lished that nucleotides −6500 to +34 of the mouse cryptdin-2
gene are active in Paneth, granule goblet, and intermediate
cells (e.g. Fig. 1D) and silent in all other epithelial cell types
present in crypt-villus units.

Cryptdin-2
34-directed Expression of an Attenuated
Diphtheria Toxin A Fragment Results in Paneth Cell Abla-
tox176) mice. Ablation of Paneth cells is evident as are several
attenuated DT-A. The solid arrow points to several apoptotic cells that can be visualized with tartrazine or by dUTP nick end-labeling assay of an adjacent section. As expected from the known mechanism of action of DT-A, there was increased cell death in the crypts of transgenic mice compared with their normal littermates. Comparison of A and B with C

emphasizes the extent of the Paneth cell ablation produced by the attenuated DT-A. D, high power view showing several crypts from a CR2-TAg mouse. Ablation of Paneth cells is evident as are several apoptotic cells (arrows). Bars = 25 μm.

FIG. 1. EM immunohistochemical analysis of the cellular pattern of expression of CR2-hGH in adult FVB/N transgenic mice. A–C, transmission EM of crypt epithelial cells from the distal jejunum of a normal P28 FVB/N mouse. The distal jejunum is defined as the junction between the middle and distal thirds of the small intestine. A, view of the lower portion of the crypt containing Paneth cells (e.g. arrow) with their electron-dense apical secretory granules. Bar = 10 μm. B, transmission EM of the supranuclear region of an intermediate cell from the same mouse. Characteristic mucin-containing secretory granules with electron dense cores are found in this region of the cell. Bar = 3 μm. C, supranuclear region of a Paneth cell showing details of secretory granule morphology. Bar = 3 μm. D, immunohistochemical evidence of hGH expression in the mature Paneth cells of a P28 CR2-
hGH transgenic mouse. The section was incubated with rabbit anti-
hGH and gold-conjugated goat anti-rabbit Ig. The 18-nm diameter gold
gold particles are evident in the electron dense apical secretory granules. Bar = 1 μm. E and F, cryptdins are present in the dense core granules of intermediate cells (E) and granule goblet (F) cells in a normal
FVB/N P28 mouse. The section was incubated with rabbit polyclonal antibodies that react with several members of the cryptdin family. Antigen-antibody complexes were visualized with gold-labeled secondary antibodies as in D. Bar = 1 μm.

Antigen−antibody complexes were visualized with gold-labeled secondary antibodies as in D. Bar = 1 μm.

FIG. 2. Phloxine/tartrazine histochemical stains of FVB/N normal, CR2-tox176, and CR2-TAg transgenic small intestine. 1-μm thick sections were prepared from plastic-embedded sections of the distal jejunum of P28 normal or transgenic mice and stained with phloxine and tartrazine (P/T). A, crypt-villus units from a nontrans-
genic littermate. Note that Paneth cells contain distinctive apical tar-
trazine-positive granules (red). These cells are confined to the base of crypts. B, high power view of a crypt from the animal in A. The solid arrow points to the tartrazine-positive apical secretory granules of a Paneth cell. C, high power view of several crypts from a P28 CR2-tox176 mouse. Note the absence of Paneth cells detectable by this histochem-
astain. The solid arrows point to several apoptotic cells that can be visualized with tartrazine or by dUTP nick end-labeling assay of an adjacent section. As expected from the known mechanism of action of DT-A, there was increased cell death in the crypts of transgenic mice compared with their normal littermates. Comparison of A and B with C

emphasizes the extent of the Paneth cell ablation produced by the attenuated DT-A. D, high power view showing several crypts from a CR2-TAg mouse. Ablation of Paneth cells is evident as are several apoptotic cells (arrows). Bars = 25 μm.

Two pedigrees of FVB/N cryptdin-2
34/tox-176 (CR2-
tox176) mice were produced. There were no statistically signif-
icant differences between the growth rates and adult body
weights of CR2-tox176 mice and their normal littermates. Compar-
ably aged members of each pedigree had identical pheno-
types (see below).
A 95% reduction in the number of Paneth cells was evident throughout the length of the small intestines of transgenic animals by P28, whether defined by a loss of staining of serial sections with tartrazine (Fig. 2, A–C), a loss of cellular reactivity with the lectins UEA-1, PNA, and DBA, or the failure of antibodies to detect cryptdins, lysozyme, and Pla2g2a (phospholipase A₂) in crypt-villus units (e.g. Fig. 3, A and B). The ablation of Paneth cells was verified using transmission EM (Fig. 4A).

The reduction in Paneth cell number within the small intestines of CR2-tox176 mice persists from P28 through at least P180, although the magnitude of the reduction is less at later time points (e.g. 82% at P42). Granule goblet and intermediate cells can only be defined using EM methods that are not useful for broad surveys of rare cell populations. Nonetheless, EM studies indicated that these cells were reduced in number, but not as markedly as Paneth cells.

The space normally occupied at the crypt base by Paneth cells was occupied in CR2-tox176 mice by “crypt base columnar cells” (Fig. 4, A–C). Crypt base columnar cells are normally interspersed among Paneth cells and constitute 60–70% of the cells that populate the bottom three cell layers of duodenal, jejunal, and ileal crypts (9). Previous [³H]thymidine labeling/EM radioautography studies indicated that: (i) their residence time at the crypt base after entering S-phase is just a few hours; (ii) they migrate up and out of duodenal, jejunal, and ileal crypts within 3–4 days; and (iii) they differentiate into enterocytes (9). The morphologic features of crypt base columnar cells present in CR2-tox176 transgenic mice and in their normal littermates were indistinguishable. In each case, they resembled the undifferentiated, proliferating transit cell population located in the mid-crypt (see Fig. 4C and legend).

To define the effects of Paneth cell ablation on epithelial cell proliferation, P28, P42, and P120–180 CR2-tox176 mice and their age-matched normal littermates were pulse-labeled with BrdU 1.5 h prior to sacrifice. The number of S-phase cells was counted in sections of distal jejunal crypts (n = 3 mice/pedigree/time point). There were no statistically significant differences between the total number of S-phase cells in the middle and upper thirds of normal and CR2-tox176 transgenic crypts. Since the fractional representation of crypt base columnar cells absent as judged by the lack of UEA-1- or cryptdin-positive cells at the crypt base. BrdU-positive crypt base columnar cells (e.g. open arrow) occupy the space where Paneth cells normally reside. C and D, standard light micrographs of crypt-villus units from a P28 normal mouse (C) and its CR2-tox176 littermate (D). Mice received BrdU 48 h prior to sacrifice. The sections were stained with goat anti-BrdU (detected as red with Cy3-donkey anti-goat Ig) and rabbit anti-cryptdin (visualized as dark blue with AMCA-conjugated donkey anti-rabbit Ig). The leading and trailing edges of the crypts of BrdU-positive cells are correspondingly positioned in normal and transgenic mice, indicating that loss of Paneth cells does not affect cell movement and that crypt base columnar cells are able to exit the crypt along with former members of the transit cell population. E, confocal micrograph of crypts from a P28 CR2-TAg mouse. The section was incubated with rabbit anti-SV40 TAg (detected as orange with Cy3-donkey anti-rabbit Ig) and FITC-UEA1. The open arrow points to a crypt devoid of UEA1-positive Paneth cells. The closed arrow points to a SV40 TAg-negative, UEA-1-positive Paneth cell in the adjacent crypt. Paneth cells were rarely encountered in these mice. When present they were invariably SV40 TAg-negative. F, confocal micrograph of crypt-villus units from a P28 CR2-TAg mouse that had been labeled 1.5 h before sacrifice with BrdU. The section was incubated with antibodies to BrdU (visualized as red with Cy3-donkey anti-goat Ig), FITC-conjugated UEA1 (green), and SV40 TAg (detected as blue with Cy3-donkey anti-rabbit Ig). Villus epithelial cells expressing SV40 TAg are UEA-1-positive. Members of this amplified goblet cell lineage contain purple nuclei (e.g. solid arrows) indicating that they had entered S-phase within the 1.5-h period before sacrifice. Open arrows point to BrdU-positive, SV40 TAg-negative crypt base columnar cells. Bars = 55 μm.
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complex variations in its pattern of glycoconjugate production along the crypt-villus and duodenal-ileal axes. These variations are a sensitive marker of the lineage’s differentiation program and are definable with a panel of lectins and in situ histochemical assays (42). Histochemical surveys using three of these lectins (UEA1, DBA, and PNA) indicated that terminal differentiation of goblet cells is unaffected by loss of Paneth cells (Fig. 3A, B plus data not shown). Based on results obtained with antibodies to chromogranin A and serotonin plus the three lectins, we concluded that there were no significant changes in enteroendocrine cell number or differentiation. The enteroendocrine lineage also appears unaffected, as judged by the number and distribution of villus epithelial cells that react with antibodies to intestinal fatty acid binding protein and with lectins that recognize fucosylated glycoconjugates (data not shown).

Loss of Paneth Cells Does Not Have Any Appreciable Effect on the Crypt-Villus Distribution of Components of the Microflora—Paneth cells are not present in adult FVB/N colonic crypts. Bacterial density increases along the duodenal-colonic axis of mice and man (50). The colonic crypts of many mammals, including mice, are colonized by bacteria. Microbial colonization is rarely seen in the small intestinal crypts of healthy animals. The secretion of cryptdins and lysozyme from the apical secretory apparatus of Paneth cells may help prevent colonization.

We used a prominent and easily detectable member of the normal FVB/N intestinal microflora to determine whether loss of Paneth cells affected the distribution of bacteria along the crypt-villus and duodenal-ileal axes. A segmented filamentous bacterium (SFB), thought to belong to the Gram-positive Clostridia (51), colonizes the normal small intestine after weaning (52) and reaches very high densities in the ileum of adult animals. The SFB cannot be recovered from the intestine and cultured in vitro (53, 54), but can be easily seen in the ileum of normal FVB/N mice using the Warthin-Starry silver stain. SFB adheres to ileal enterocytes located in the upper half of the villus (Fig. 6, A and B). It is not present in crypts (Fig. 6A). Unperfused small intestines from P28 CR2-tox176 transgenic mice and their normal littermates (housed in the same microisolator cages) were subdivided into 1–2-cm segments along the length of the duodenal-ileal axis. Warthin-Starry stains of sections prepared from each segment indicated that loss of Paneth cells had no effect on the duodenal-ileal or crypt-villus distribution of SFB (Fig. 6, A and C). Gram stains provided independent confirmation that duodenal, jejunal, and ileal crypts of P28-P180 CR2-tox176 mice were free of detectable microbes (data not shown).

Ablation of Mature Paneth Cells Is Not Associated with a Change in the Crypt-Villus Distribution of Components of the Diffuse Gut-associated Lymphoid Tissue (GALT)—Luminal antigens and microbes are delivered by M-cells to submucosal lymphoid tissues (e.g. Peyer’s patches). These components of the organized GALT can serve as inductive sites for initiation of immune responses (56). Analysis of intestines harvested from specific pathogen-free P28-P180 transgenic mice and their normal littermates indicated that loss of Paneth cells is not accompanied by changes in the size, number, distribution, or histochemical features of Peyer’s patches (data not shown).

Complex, dynamic, and often subtle interactions occur between the gut epithelium and components of the diffuse GALT. For example, mice homozygous for a null allele of the γ subunit gene of the T-cell receptor (TCR) lack γδ T-cells and exhibit a reduction in crypt cellularity as well as a reduction in epithelial cell migration rates up the villus. Mice homozygous for a null allele of the β subunit gene of the TCR lack αβ T-cells but do not manifest these abnormalities (57).

Fig. 4. Transmission EM of a distal jejunal crypt from a P28 CR2-tox176 mouse showing ablation of the Paneth cell lineage. A, section showing the entire crypt. The base of the crypt is occupied by poorly differentiated columnar epithelial cells that lack secretory granules. Bar = 4 μm. B, higher power view of three adjacent crypt base columnar cells from this mouse. Bar = 1 μm. C, two crypt base columnar cells adjacent to an enteroendocrine cell. The closed arrowhead points to a secretory granule in this enteroendocrine cell located on the right-hand portion of the panel. The centrally positioned, cylindrically shaped crypt base columnar cell contains a basal nucleus, a plasma membrane that is smooth and does not interdigitate with adjacent cells, numerous supranuclear mitochondria (e.g. closed arrows), and a prominent supranuclear Golgi apparatus (e.g. open arrows). Bar = 1 μm.

was markedly increased in transgenic mice, it was not surprising that there was a greater number of BrdU+ cells in the lower third of transgenic crypts (Fig. 3B).

Surveys of sections of duodenal, jejunal, and ileal villi prepared from normal and CR2-tox176 mice that had received BrdU 1.5 h before sacrifice failed to reveal villus epithelial cells in S-phase (Fig. 3B). Transgenic mice and their normal littermates were subsequently sacrificed at later time points after receiving BrdU. There were no differences in the rate of clearance of BrdU-positive cells from normal or transgenic crypts; by 24 h, BrdU+ cells were no longer present in the lower two-thirds of duodenal, jejunal, or ileal crypts; by 36 h, crypts were free of these labeled cells; and by 48 h and 72 h, the location of the trailing edge of BrdU+ cells on duodenal, jejunal, or ileal villi was similar in normal and transgenic animals (e.g. Fig. 3, C and D; n = 2–6 animals/time point/pedigree). These results indicate that the columnar cells that populate the base of transgenic crypts are not abnormally retained at this location in the absence of Paneth cells. The data are also consistent with the notion that the production and subsequent upward migration of crypt epithelial cells are not grossly perturbed by the lineage ablation.

Loss of Paneth cells had no demonstrable qualitative or quantitative effects on the other three small intestinal epithelial cell lineages. A comparison of CR2-tox176 mice and their age-matched normal littermates did not reveal any statistically significant differences in the number of their Alcian blue/PAS-positive goblet cells per duodenal, jejunal, or ileal villus section (e.g. Fig. 5, A–C). The goblet cell lineage normally exhibits
The normal distribution of components of the diffuse GALT along the crypt-villus axis has not been extensively characterized because of the difficulty in identifying cellular markers with conventional immunohistochemical detection methods. We used a protocol employing tyramide signal amplification to identify these components in serial sections of jejunum prepared from age-matched FVB/N CR2-tox176 mice and their normal nontransgenic cagemates.

In normal P42 animals with a conventional microflora ($n = 3$), CD4$^+$ T-cells are largely confined to the lamina propria and are present throughout the length of the crypt-villus axis (Fig. 7B). CD8$^+$ intraepithelial T-cells have been postulated to serve cytotoxic as well as immunosuppressive functions (58). In normal P42 animals, CD8$^+$ T-cells, unlike CD4$^+$ T-cells, are predominantly intraepithelial and restricted to the villus (Fig. 7D). αβ T-cells are distributed along the length of the crypt-villus axis and populate the intraepithelial and lamina propria compartments (Fig. 7F). In contrast, γδ T-cells are limited to the villus where they are predominantly located within the epithelium (Fig. 7H). B-cells, defined using CD45R/B220 as a marker, are confined to the lamina propria and are distributed from the base of the crypt to the villus tip (data not shown). These results are summarized in Fig. 7A.

Comparable immunohistochemical surveys of P42 CR2-tox176 mice ($n = 3$) indicated that ablation of Paneth cells was not associated with any detectable alteration in the distribution of these components of the diffuse GALT (Fig. 7, C, E, G, and H). Moreover, hematoxylin and eosin stains of sections prepared along the length of P28-P180 CR2-tox176 small intestines failed to disclose any evidence of acute or chronic inflammatory changes ($n = 40$ animals). Together, these results suggest that Paneth cells do not have a direct or indirect organizing function for the diffuse GALT. Furthermore, they are consistent with the notion that loss of Paneth cells does not produce marked perturbations in host-microbial interactions in pathogen-free mice.

**Cryptdin$^{6500-6544}$-directed Expression of SV40 TAg Blocks Paneth Cell Differentiation but Amplifies Intermediate and Granule Goblet Cells**—Promoter-targeted expression of simian virus T antigen (SV40 TAg) in the progenitor cells of specific
lineages has been exploited to generate transgenic mouse models of specific physiologic deficiency syndromes. Physiologic deficiencies result because differentiation of these progenitors is blocked; the “entrapped” progenitors do not have the functional capacities of their terminally differentiated descendants and therefore cannot compensate for their loss (59). SV40 TAg-stimulated amplification of normally rare progenitors also provides an opportunity to study their intrinsic properties and/or the consequences of their increased representation (e.g. Refs. 59 and 60).

With these thoughts in mind, nucleotides 6500 to +34 were used to direct expression of SV40 TAg in FVB/N transgenic animals. Three lines of CR2-TAg mice were analyzed from P28 to P180. All pedigrees had identical intestinal phenotypes. The growth rates and adult body weights of transgenic mice were not significantly different from those of their normal littermates.

Staining with tartrazine, UEA1, and antibodies to cryptdins, lysozyme, and enhancing factor revealed a decrease in the number of mature Paneth cells in CR2-TAg mice comparable to the decrease observed in similarly aged CR2-tox176 animals (e.g. 90–95% at P28) (Figs. 2D and 3F). EM confirmed the Paneth cell ablation (Fig. 8A). The few mature Paneth cells observed in scattered duodenal, jejunal, and ileal crypts did not contain detectable levels of SV40 TAg (Fig. 3E). The mouse cryptdin gene family includes at least 17 members whose expression varies both as a function of developmental stage and cellular position along the crypt-villus and duodenal-ileal axes (30). Although we do not have antibodies specific for cryptdin-2, it seems likely that this small population of residual mature SV40 TAg-negative Paneth cells was able to complete its terminal differentiation because its members do not support expression of the endogenous cryptdin-2 gene or transgenes under the control of cryptdin-2 promoter (Fig. 8).

SV40 TAg-positive epithelial cells were distributed along the length of the crypt-villus axis. These cells were most abundant in the crypts and lower half of the villus. SV40 TAg levels decreased as cells moved to the upper half of the villus (Fig. 3F). SV40 TAg-positive villus epithelial cells were also UEA-1-positive (Fig. 3F). The UEA-1/SV40 TAg-positive cells were members of the goblet cell lineage. Unlike CR2-tox176 mice, CR2-TAg animals exhibit a statistically significant 2–3-fold increase in the number of Alcian blue/PAS-positive goblet cells per duodenal, jejunal, or ileal villus section (p < 0.05; reference control = age-matched normal littermates) (Fig. 5, D and E). Pulse labeling with BrdU 1.5 h prior to sacrifice revealed that production of SV40 TAg is associated with re-entry of these villus goblet cells into S-phase (Fig. 3F).

EM immunohistochemical studies provided further insights about the origins of this amplified goblet cell population. Analyses of distal jejunal crypts from CR2-TAg mice and their normal littermates disclosed a marked amplification of crypt-
CR2-TAg mice contain an amplified population of cryptdin- and phospholipase A₂-producing intermediate and granule goblet cells. A, transmission EM of a distal jejunal crypt showing ablation of mature Paneth cells. Intermediate cells are amplified (e.g., solid arrowheads) as are granule goblet cells (e.g., open arrowhead). The open arrow points to an apoptotic cell. Bar = 4 μm. B–D, EM immunohistochemical demonstration of Pla2 in the dense core granules of intermediate cells located in the lower and middle thirds of the crypt (D and C, respectively) and in a granule goblet cell from the upper third of the crypt (B). The sections were incubated with rabbit anti-phospholipase A₂ (Pla2g2a) and gold-labeled goat anti-rabbit Ig. Bar = 1 μm. E–G, EM immunohistochemical demonstration of cryptdin accumulation in the secretory granules of intermediate cells located in the lower and middle thirds of the crypt (G and F, respectively), and in a granule goblet cell positioned in the upper crypt (E). Sections were treated with rabbit anti-cryptdin and gold-labeled goat anti-rabbit Ig. Note how the diameter of the electron dense cores diminishes as cells move up the crypt–villus unit. As long as these cores are present, cryptdin and enhancing factor are detectable. Control experiments using non-immune serum gave no signal (data not shown). Villus goblet cells with common mucin globules that lack electron dense cores do not contain detectable levels of the phospholipase A₂ or cryptdin (data not shown). Bars = 1 μm.

**DISCUSSION**

Paneth Cells Do Not Appear to Be Necessary to Establish and Maintain a Functional Stem Cell Niche—The precise location of the multipotent stem cell in the adult mouse small intestinal crypt has not been established. However, titrated thymidine labeling/radioautographic analyses of cell proliferation, movement, and differentiation programs have led to speculation that stem cells are positioned in the fifth cell stratum from the crypt base (1–3, 5).

Immature Paneth cells are located just above and below the presumptive stem cell niche. They differentiate during a downward migration to the crypt base where they comprise ~50% of the cell population in the first through fourth cell layers (9). The location and direction of Paneth cell migration, the concomitant acquisition of the ability to produce growth factors, their potential for providing instructions to neighboring cells during their downward descent, and their high density and the long residency time at the crypt base raise the possibility that Paneth cells may influence the structure and/or function of the stem cell niche. Paneth cell ablation by an attenuated diphtheria toxin A fragment represents the first reported experimental manipulation of the cellular microenvironment that purportedly contains the stem cell and its immediate descendants.

The gut provides a unique system in which to perform these types of experimental manipulations because the stem cell is contained in a readily detectable anatomic unit, i.e. the crypt. Furthermore, each adult mouse crypt contains a monoclonal population of cells (62–64). The stem cell's descendants undergo 4–6 rounds of rapid cell division, generating a steady state population of ~250 crypt epithelial cells of which ~150 are cycling at any given moment (5). Thus, perturbations that change the biological properties of the stem cell and its immediate descendants may be inferred by noting changes in the behavior and/or composition of their amplified progeny.

The results of tox176-mediated ablation of Paneth cells suggest that this lineage is not essential for establishment of a functional stem cell niche, at least based on the observations that (i) proliferative activity is maintained in crypts over the first 6 months of life and (ii) the composition of terminally differentiated members of the intestine's other self-renewing epithelial lineages is not perturbed. Further analysis of the longer term effects of Paneth cell loss in this model is limited by the gradual re-appearance of members of this lineage. This could reflect time-dependent changes in CR2-mediated expres-
sion of tox176. If the level rather than the generality of CR2-tox176 expression in Paneth cells is the cause of this phenomenon, then using wild type DT-A may produce a longer lasting ablation.

Changes at the crypt base in CR2-tox176 animals include loss of mature Paneth cells and an increase in the proportion of crypt base columnar cells. Therefore, we can also conclude that the augmented representation of crypt base columnar cells does not have a demonstrable effect on the functional properties of the gut stem cell.

We do not know whether ablation of Paneth cells is associated with a change in the physical location of stem cells in the crypt or whether that location is fixed even in normal animals. Although the properties of “stemness” in the crypt may not be defined by instructive interactions involving neighboring Paneth cells, signals derived from other neighboring epithelial cell populations and/or components of the underlying extracellular matrix/mesenchyme may play critical roles.

Directional Migration and the Differentiation of Paneth and Goblet Cell Lineages—The first through fourth cell strata in normal adult mouse crypts also contain a small percentage (1–2%) of oligomucous and intermediate cells (9). A relationship between Paneth and goblet cell differentiation has been suggested but never proven. The morphologic features of intermediate cells had suggested to others that they may be very young granule goblet cells or a precursor of differentiated Paneth and granule goblet cells (13). To explore this possible precursor-product relationship, various groups have attempted to detect lysozyme in intermediate cell granules by light microscopic immunohistochemistry. Some workers have reported success (65), others failure (66, 67). Because of the high background of nonspecific cellular staining observed when commercially available antibodies to lysozyme were used for EM immunohistochemistry, we have also been unable to determine whether intermediate cells contain lysozyme.

Although lysozyme has long been considered part of the immunohistochemical definition of a Paneth cell, cryptdins and the secreted phospholipase A2 encoded by Pla2g2a have been recently identified as markers of this lineage. We have now demonstrated that cryptdins are produced in the intermediate and granule goblet cells of normal adult FVB/N small intestinal crypts. In addition, cells with morphologic and immunohistochemical features of the ordinarily rare crypt intermediate cells abound in CR2-TAg crypts. These intermediate cells produce SV40 TAg, proliferate, and support expression of cryptdins as well as the phospholipase A2. As these SV40 TAg-positive cells emerge from the crypt and move up the villus, they undergo a sequence of cellular alterations that includes a decrease in the size and number of their electron dense granules and an increase in their mucin content. Immunoreactive cryptdins and the phospholipase A2 are retained until these granules are lost and cellular SV40 TAg expression is suppressed in mature or common goblet cells.

These observations suggest that SV40 TAg is expressed in an intermediate cell precursor with shared features of immature Paneth and goblet cells. Terminal differentiation of this or other precursors into Paneth cells appears to be blocked throughout the crypt. In contrast, the upper crypt and lower half of the villus appear capable of supporting proliferation, survival, and differentiation of the intermediate cell to granule goblet and then mature common goblet cells but not to Paneth cells.

These contrasting responses of the Paneth and goblet cell lineages to SV40 TAg expression may reflect the effects of the microenvironment along the crypt-villus axis. Adopting and expressing a Paneth cell fate may require that precursors migrate to the crypt base rather than to the villus. Such a notion is consistent with a recent study in which epithelial cell migration from the crypt to the villus tip was slowed in transgenic and chimeric-transgenic mice by forced expression of E-cadherin (68). Despite the slowing of cell migration out of the crypt, terminal differentiation markers that are normally only expressed on the villus were not produced in the crypt. This finding suggests that terminal differentiation of the principal small intestinal epithelial lineages is largely cell nonautonomous and apparently dependent upon instructions obtained at specific positions along the crypt-villus axis. An intriguing question raised by these considerations is what determines whether a cell migrates out of a crypt or down to its base.

Paneth Cells and Intestinal Neoplasia—A Leu → Stop substitution at codon 850 in the 2845-residue mouse Apc protein is associated with the development of multiple intestinal neoplasms (Min; Refs. 17 and 69). Comparable germ line mutations in the human APC gene also leads to multiple gut adenomas (familial adenomatous polyposis). Mom1 is a semidominant modifier of tumor multiplicity in Min/+ animals located on mouse chromosome 4 (70). As noted in the Introduction, genetic studies indicate that Pla2g2a is a candidate gene for Mom1 (23–25). Two Pla2g2a alleles have been described. One allele contains a frameshift mutation (Mom1f) and is encountered in C57BL/6J and 129/Sv-Pas mice. The other allele does not contain the mutation (Mom1s) and is found in AKR/J, MA/Myl, BALB/cByJ, and Mus castaneus animals (25). Mom1f is associated with a 4–8-fold greater number of intestinal adenomas.

Current evidence indicates that a Min adenoma arises within a small intestinal crypt and that the initiated cell may be the stem cell or one of its immediate descendants (e.g. Ref. 71). The mechanism by which the secreted phospholipase A2 encoded by Pla2g2a could influence initiation or progression is unclear at present. Our experiments indicate that neither a marked reduction in Pla2g2a in the crypt (CR2-tox176 mice) nor an expansion of the population of intermediate and granule goblet cells that produce this phospholipase A2 (CR2-TAg mice) were associated with apparent changes in the properties of the crypt stem cell or its descendants.

Although the Mom1 allele of FVB/N mice has not been characterized, crosses of FVB/N CR2-tox176 and their nontransgenic littermates to Min/+ animals with Mom1s or Mom1f alleles illustrate how Paneth cell ablation can be used as a preliminary test of the effects of gene products produced in this lineage on tumorigenesis. Targeting potential regulators of tumorigenesis to the apical secretory apparatus of Paneth cells using nucleotides −6500 to +34 of the mouse cryptdin-2 gene could result in their export to the stem cell zone, thereby testing their effects in Min/+ or other mouse models. These latter experiments illustrate how the Paneth cell can be used as a tool for delivering a variety of molecules to a critical region of the crypt where decisions about proliferative status and lineage allocation are made.

Ablation of the Paneth Cell Lineage Does Not Appear to Affect Host-microbial Interactions or the Spatial Organization of the Diffuse GALT—Small intestinal crypts are normally devoid of detectable micro-organisms. Surprisingly, our lineage ablation experiments suggest that the anti-microbial factors produced by Paneth cells may not be required to prevent colonization of these crypts. Nonetheless, Paneth cells may play other roles in host-microbial interactions. For example, the anti-microbial
products released from Paneth cells could function to define the composition and/or density of the intestinal microflora. This idea is consistent with the fact that bacterial density in the small intestine is generally 3 to 4 orders of magnitude less than in the cecum and colon (50) which lack Paneth cells. Members of the microflora are able to establish niches at various positions along the duodenal-colonic axis even in the face of the epithelium’s continuous replacement. Colonization of this open ecosystem begins soon after birth and progresses through a series of stages resulting in complex but stable climax communities predominated by Gram-negative anaerobes (29). “Autochthonous” members of the flora represent indigenous species that normally inhabit a given ecological niche. “Allochthonous” members are colonizers that do not normally occupy a given niche. Their existence in the ecosystem either results from a transient “passing through” or from conditions that significantly disrupt the stability of the autochthonous flora (e.g. starvation or treatment with antibiotics). The composition and flux of the autochthonous flora play a significant role in the ability of pathogens to gain a foothold in the ecosystem. Cephalocaudal differences in Paneth cell cryptdin expression together with the differential sensitivity of various microorganisms to different cryptdins may help define the gut’s autochthonous (and allochthonous) flora. With these thoughts in mind, it will be important to evaluate the effects of Paneth cell ablation on the density, composition, and cephalocaudal distribution of components of the gut’s microbiota. This can be done using conventional mice or animals that have been raised under germ-free conditions and then inoculated with one or more species of bacteria that normally reside in the gut.

CR2-tox176-mediated ablation of Paneth cells in mice that are free of pathogens had no demonstrable effect on the crypt-villus distribution of members of the diffuse GALT. This finding not only provides another piece of evidence that host-microbial interactions are not markedly deranged but also indicates that this lineage does not function, directly or indirectly, in establishing or maintaining the asymmetric distribution of some critical components of the diffuse GALT.

While previous genetic experiments have shown that ablation of components of the diffuse GALT (e.g. intraepithelial γδ T-cells) affects epithelial homeostasis (57), reciprocal experiments designed to test the effects of ablating epithelial cell lineages on GALT homeostasis have not been described. This likely reflects prior difficulty in defining the spatial organization of the diffuse gut-associated immune system, a problem that can now be overcome by using tyramide signal amplification to increase the sensitivity of immunohistochemical surveys.

There are several notable asymmetries in the crypt-villus distribution of components of the diffuse GALT in adult FVB/N CR2-tox176 transgenic mice and their normal littermates. First, CD8⁺ cells are principally intraepithelial and are restricted to the villus, whereas CD4⁺ cells are distributed along the length of the crypt-villus axis where they reside principally in the lamina propria. Second, γδ T-cells are limited to the villus and its intraepithelial compartment. In contrast, αβ T-cells are not restricted to the intraepithelial compartment and are distributed along the length of crypt-villus units. The absence of CD8⁺ and γδ T-cells from the crypt may reflect, at least in part, the lack of microbial colonization of this region of the crypt-villus axis. If true, these cells may be useful markers to follow if and when colonization occurs.

Our studies suggest that further assessment of the contributions of Paneth cells to the regulation of host-microbial and microbial-immune interactions might require provocative tests. Possibilities include introduction of pathogens, with or without members of normal flora, into germ-free CR2-tox176 animals and their germ-free nontransgenic littermates or crosses between conventionally raised CR2-tox176 animals and mice that are genetically predisposed to develop inflammatory bowel disease. An example of the latter would be interleukin-10 knockout mice who develop small intestinal mucosal inflammation when housed in a conventional animal facility but not when they are raised in a specific pathogen-free state (73).
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Examining the Role of Paneth Cells in the Small Intestine by Lineage Ablation in Transgenic Mice

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J. Biol. Chem. 1997, 272:23729-23740.
doi: 10.1074/jbc.272.38.23729

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