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# Lineage Development in a Patient without Goblet, Paneth, and Enteroendocrine Cells: A Clue for Intestinal Epithelial Differentiation

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## ABSTRACT

We report a patient who presented with severe enterocolitis and apparent absence of Paneth, goblet, and enteroendocrine lineages from the small bowel and colon. The absorptive enterocyte seemed to be normal morphologically and functionally. Because normal enterocytes were present, we hypothesized that this patient had a developmental block in the differentiation of a common stem cell precursor for Paneth, goblet, and neuroendocrine lineages. By using antibodies to protein markers of each cell line, including some that are expressed early in the differentiation process, we aimed to study lineage development in this patient. From our data, we surmise that there may be a two-step process in lineage commitment. The stem cell may commit to an absorptive cell or a granule-containing cell. The daughter cell that is committed to the granule lineage then further commits to a goblet, enteroendocrine, or Paneth cell lineage. **(*Pediatr Res* 58: 492–498, 2005)**

**Abbreviations**
**CMG,** Chromogranin A
**HD,** human defensin
**H&E,** hematoxylin and eosin
**IBD,** inflammatory bowel disease
**MUC,** mucin
**PAS,** periodic acid-Schiff
**TFF,** Trefoil factor family

The intestinal crypts contain a population of multipotential stem cells from which all of the epithelial cell lineages are derived. On the basis of work in mice, a common progenitor cell located near the base of the crypt of Lieberkühn is thought to give rise to all four intestinal cell lines: absorptive enterocytes as well as Paneth, goblet, and enteroendocrine cells (1–4). Much work has been done to understand the differentiation and development from stem cells to the various terminally differentiated cell types of the intestinal epithelium and the factors that play a part in this process. Many factors have been implicated in this process, such as Wnt/β-catenin signaling (5); Notch signaling, including the transcription factors Math1 (6) and Hes1 (7); homeobox transcription factors Cdx1 and Cdx2 (8); Kruppel-like factors KLF4 and KLF5 (9–11); transcription factor Elf3 (12); platelet-derived growth factor (PDGF) A and its receptor, PDGF-Ra; the winged helix transcription factor Fkh6; the homeodomain transcription factor Nkx2–3; and *Hox* and *ParaHox* cluster genes, Sonic hedgehog, and bone morphogenetic proteins (13).

Intestinal differentiation is difficult to study because of the technical challenge of growing nonmalignant epithelial cells *in vitro*. Although much has been learned from the use of normal, chimeric, and transgenic mice models, the process is still very ill defined. The work of Cheng and Leblond (2) on mouse models introduced the idea that the stem cell is the direct progenitor of each of the terminal lineages.

We report a patient who presented with severe enterocolitis and apparent absence of Paneth, goblet, and enteroendocrine lineages from the small bowel and colon. The absorptive enterocyte seemed to be normal morphologically and functionally. Because normal enterocytes were present, we hypothesized that this patient had a developmental block in the differentiation of a common stem cell precursor for Paneth, goblet, and neuroendocrine lineages. These lineages have in common

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the packaging of proteins into secretory granules before regulated secretion. By using antibodies to protein markers of each cell line, including some that are expressed early in the differentiation process, we aimed to study lineage development in this patient. From our data, we surmise that there may be a two-step process in lineage commitment. The stem cell may commit to an absorptive cell or a granule-containing cell. The daughter cell that is committed to the granule lineage then further commits to a goblet, enteroendocrine, or Paneth cell lineage.

## CASE REPORT

The patient presented at 14 y of age with severe watery, predominately nonbloody diarrhea; some lower abdominal cramping; and an 11-kg weight loss over a 2- to 3-mo period. He had experienced mild intermittent diarrhea since the age of 2 y and more persistent diarrhea over the last 2 y. Laboratory values at presentation are shown in Table 1.

A double-contrast barium small bowel follow-through demonstrated nodularity and some polyposis of the jejunum and terminal ileum. A gallium scan was normal. A computed tomography scan of the abdomen demonstrated thickening of the colon, consistent with pancolitis, and small nodes in the mesentery, porta hepatis, and para-aortic areas.

Multiple cultures for enteric pathogens including *Escherichia coli* 0157:H7, *Clostridium difficile*, and parasites were negative. Tuberculin skin testing was negative, and a chest x-ray was normal. Extensive immunologic testing was performed (Table 1). Results of immunohistologic studies are reported in Table 2.

***Stomach.*** The stomach showed mild gastritis. Both staining and culture for *Helicobacter pylori* were negative. Antral sections stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS)/Alcian Blue suggested that mucus-secreting cells were present in normal amounts. Sections were also examined by immunohistochemistry to determine whether secretory products of neuroendocrine and mucus-secreting cells were normally expressed. Figure 1 compares the distribution of Chromogranin A (CMG), an early marker of neuroendocrine cells, and MUC5AC (gastric mucin) in antral sections from the case with a histologically normal control biopsy specimen. CMG-containing cells were present in both biopsy specimens in the basal one third of the antral glands (Fig. 1*a* and *b*). MUC5AC-containing cells (Fig. 1*c* and *d*) occupied similar superficial areas of antral glands in biopsy sections from our case and normal control sections. Trefoil factor 1 (TFF1), part of the intestinal trefoil family, was normally expressed in superficial portions of antral glands from our patient (data not shown). There was also no evidence of either MUC2 or TFF3, products of small intestinal and colonic goblet cells, thus providing no evidence of aberrant expression of intestinal antigens.

**Table 1.** *Laboratory values at the time of presentation*

| Test | Patient | Normal values |
|---|---|---|
| Hemoglobin | 121 g/L | 120–16 g/L |
| MCV | 71 | 80–94 fL |
| White cell count | 5.5 | $4.0–10.0 \cdot 10^9$/L |
| Platelet count | $276 \times 10^9$/L | $150–400 \times 10^9$/L |
| Serum Albumin | 28 g/L | 33–58 g/L |
| IgA, M, G, E and C3, C4 and CH50 | Normal | |
| Antibodies: ASMA, ANA, AMA, APCA | Negative | |
| ANCA and EMA, | Negative | |
| Anti-enterocyte Antibody | Negative | |
| Anti-goblet cell antibodies | Positive | |
| Serum gastrin | <25 ng/L | <90 ng/L |
| Urine VMA screen | Normal | |
| HIV serology | Negative | |

Abbreviations: ANCA, antineutrophil cytoplasmatic antibody; ANA, anti nuclear antibody; AMA, anti mitochondrial antibody; ASMA, anti smooth muscle antibody; APCA, anti parietal cell antibody; EMA, endomesial antibody; MCV, mean cell volume; VMA, vanilmandelic acid.

***Small intestine.*** The duodenum showed diffuse duodenitis. Duodenal biopsy sections are shown in Fig. 2. Sections from the patient that were stained with H&E (Fig. 2*a*) showed a moderate increase in inflammatory cells within the lamina propria, some villus blunting, but no atrophy, with a slightly increased crypt:villus ratio. The outstanding feature, however, is the absence of goblet cells in both villi and crypts, similar to a case described by Moore *et al.* (14). This feature is even more striking in sections that were stained by PAS/Alcian Blue, which reveal an intact brush border without goblet cells (Fig. 2*b*), and is contrasted with sections similarly stained from a control biopsy that show multiple Alcian Blue–positive goblet cells in a similar area (Fig. 2*c*). The intact brush border on villus cells from the patient's duodenal biopsies was confirmed by electron microscopy. Figure 2*d* shows that the brush border surface of villus enterocytes stained normally for sucrase, confirming the chemical evidence that biopsies contained normal quantities of brush border enzymes. In addition, enterocyte disaccharidase levels (trehalase, lactase, sucrase, maltase, and cellobiase) were normal.

In contrast to the apparent preservation of enterocytes, there was no evidence that duodenal biopsies from the case study expressed the mucus cell antigens MUC2, MUC5AC, TFF1, and TFF3; enteroendocrine products CMG, gastrin, and serotonin; or the early Paneth cell marker human defensin 5 (HD5). Figure 2*e* depicts, as an example, a biopsy specimen from a control biopsy showing the localization of CMG within typical enteroendocrine cells in both crypts and villi, whereas a similar area from the patient shows no staining (Fig. 2*f*). Similarly, Fig. 2*g* shows no staining for the MUC2 core antigen throughout the mucosa from the patient, whereas a control biopsy (Fig. 2*h*) reveals the normal distribution of this antibody within the endoplasmatic reticulum (ER)/Golgi zone of goblet cells.

The absence of Paneth, goblet, and enteroendocrine cells was confirmed by electron microscopy (data not shown). A few cells were noted that to contain dense granules that resembled neurosecretory granules, but CMG could not be detected within these granules using immunogold staining.

We further studied the small bowel proliferation using MIB1 (KI-67), a nuclear antigen that is associated with proliferation and found throughout the cell cycle and is absent in resting cells (15). The staining showed normal proliferation pattern (staining in crypts only) in the patient's duodenum compared with normal and Crohn's control subjects.

**Table 2.** *Results of immunostaining in the gastrointestinal tract*

| | MUC2 | TFF3 | MUC5AC | TFF1 | HD5 | Sucrase | CMG |
|---|---|---|---|---|---|---|---|
| Antrum | | | | | | | |
| Control | Neg. | Neg. | Pos. | Pos. | Neg. | NA | Pos. |
| Patient | Neg. | Neg. | Pos. | Pos. | Neg. | NA | Pos. |
| Duodenum | | | | | | | |
| Control | Pos. | Pos.(‡) | Neg. | Neg. | Pos.(‡) | Pos.(‡) | Pos. |
| Crohn's | Pos. | Pos.(‡‡) | Neg. | Pos. | Pos.(‡‡) | Pos.(‡‡) | Pos. |
| Patient | Neg. | Neg. | Neg. | Neg. | Neg. | Pos.(‡‡‡) | Neg. |
| Colon | | | | | | | |
| Control | Pos. | Pos. | Neg. | Neg. | Neg. | NA | Pos. |
| Patient | Pos.¶ | Pos.¶ | Pos.§ | Pos.§ | Neg. | NA | Neg. |

MUC2, The major intestinal mucin includes results of core and glycosylated MUC2; MUC5AC, The major gastric mucin; TFF3 (ITF), Trefoil factor 3, The intestinal trefoil factor; TFF1 (pS2), Trefoil factor 1, The gastric trefoil factor; HD5, Human defensin 5 (A Paneth cell marker); CMG, Chromogranin A (also serotonin and gastrin) Enteroendocrine markers.

‡ Implicates stain intensity.

¶ Unlike the normal staining with core MUC2 antibody, was expressed diffusely in cells in some areas.

§ Was expressed diffusely in cells in several areas, some co-localized with MUC2.

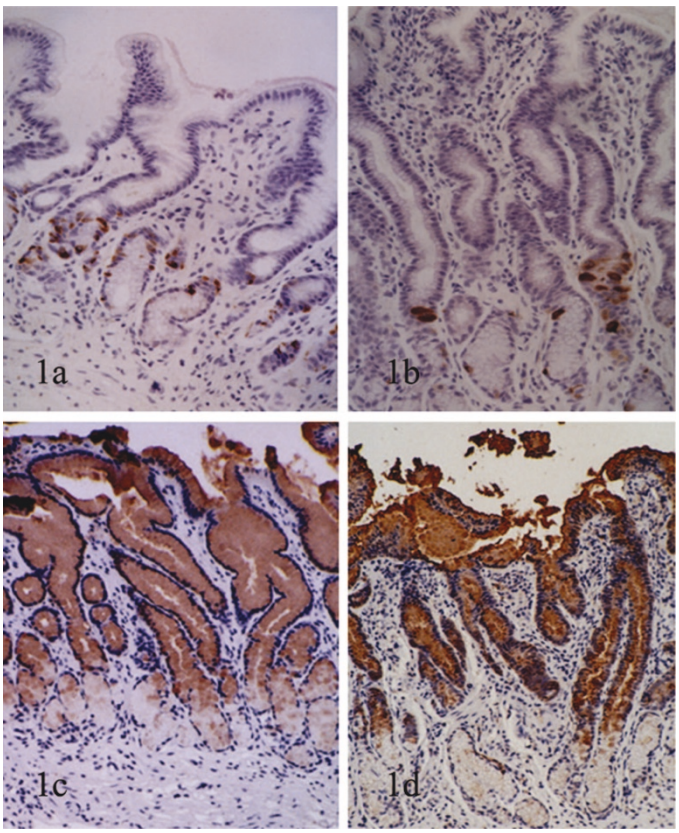


**Figure 1.** Antral biopsy showing the distribution of CMG, an early marker of neuroendocrine cells, showing stained cells in the basal one third of the antral glands in both normal control (*a*) and our patient (*b*). MUC5AC-containing cells occupied similar superficial areas of antral glands in biopsy sections from normal control (*c*) and our patient (*d*).

***Large intestine.*** The colon showed pancolitis with relative sparing of the rectum. Initial surveys of H&E-stained sections from colonic biopsies showed severe inflammation and a universal absence of goblet cells. Surprisingly, however, PCR from rectal biopsies revealed MUC2 mRNA, indicating that the colon, unlike the duodenum, expressed mucin mRNA (Fig. 3). As shown in a representative section typical of most of the colonic mucosa (Fig. 4), most of the tissue consisted of inflammatory cells with a few glands and a flattened cuboidal surface epithelium. Some of the surface and glandular cells contained PAS/Alcian Blue–positive cells (Fig. 4*a*). These cells expressed both MUC2 (Fig. 4*b*) and MUC5AC (Fig. 4*c*) antigens, suggesting that they represented mucus epithelial cells that had lost their goblet cell phenotype. Co-expression of gastric and intestinal antigens is a feature of gastric metaplasia (16). Although not seen in the mucosa distal to the cecum, patches of gastric metaplasia were present in cecal biopsies (data not shown).

None of the biopsies from any area ever had granulomata. Immunostaining for cytomegalovirus was negative. The lymphocytic infiltrate was predominately composed of T cells. PCR indicated a clonal T cell population. Southern blot analysis was negative for rearrangement of the T cell receptor gene, in keeping with an inflammatory process rather than lymphoma.

***Follow-up.*** Repeated endoscopic evaluation of the upper and lower gastrointestinal tract continued to show a pancolitis without goblet cells and further development of gastric metaplasia in the colon. The duodenal histology remained unchanged. No granulomata were ever seen. After a third trial of high-dose i.v. steroids, a few mature-looking goblet cells appeared in some colonic biopsies. Although upper intestinal inflammation improved, there was still no evidence of any type of secretory cells in the small intestine. Unfortunately, there was not significant clinical improvement, and the patient remains on parenteral nutrition.

## METHODS

Approval to obtain biopsies or use archival tissue as control biopsies was received from the Research Ethics Committee of the Hospital for Sick Children. An informed consent was obtained from the patient and his legal guardians. Control biopsy sections were from age and sex-matched normal individuals (NC) or patients with either ulcerative colitis (UC) or Crohn's disease (CD). A single senior pathologist with expertise in gastrointestinal pathology (E.C.) reviewed all slides.

***Immunohistochemical methods.*** Biopsies were fixed in 3.7% formalin, embedded in paraffin, and stained with H&E and PAS/Alcian blue. Other specimens were prepared by antigen retrieval technique for the immunostaining (17). Immunostaining was done to detect CMG (Sera Lab, Leicestershire, England), serotonin (Sera Lab), gastrin (Dako, Fort Collins, CO), MUC2 core (Novocastra Labs., New-Castle, England), glycosylated MUC2 [an antibody

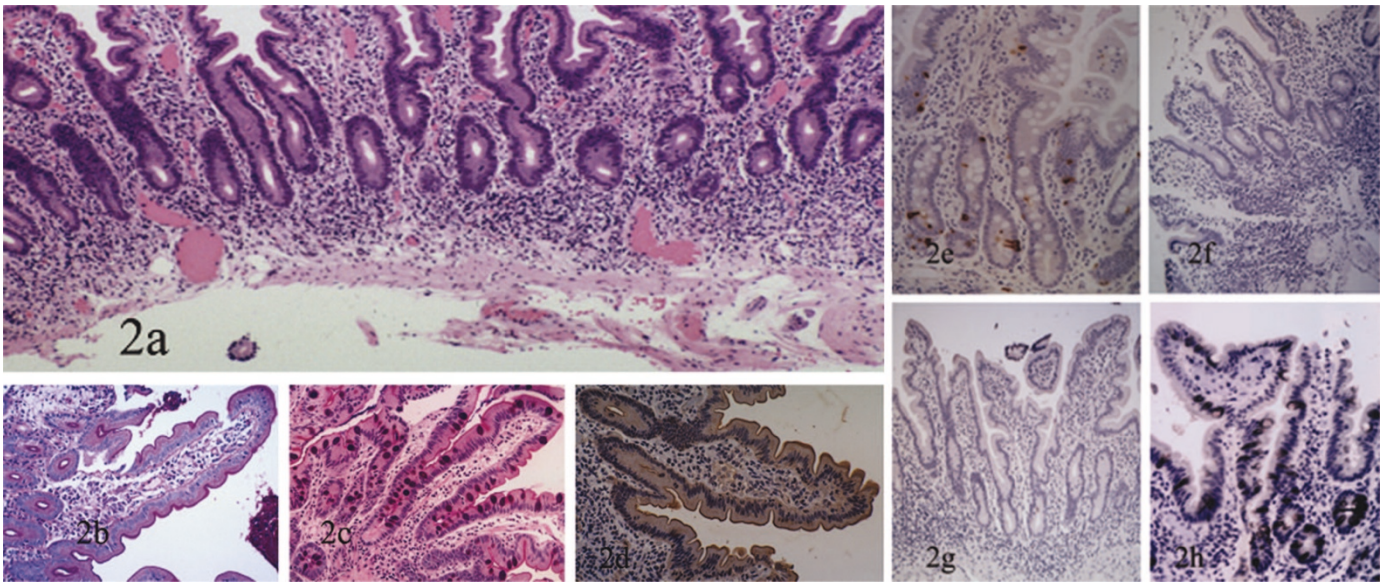


**Figure 2.** Sections from the patient's duodenum stained with H&E (*a*) show a moderate increase in inflammatory cells within the lamina propria and some villus blunting with a slightly increased crypt:villus ratio. Absence of goblet cells in both villi and crypts is noted. This feature is even more striking in sections that were stained by PAS/Alcian Blue, which reveal an intact brush border without goblet cells (*b*) and is contrasted with sections that were similarly stained from a control biopsy that show multiple Alcian Blue–positive goblet cells in a similar area (*c*). (*d*) Normal sucrase staining of patient's brush border. (*e*) A biopsy specimen from a control biopsy showing the localization of CMG within typical enteroendocrine cells in both crypts and villi, whereas a similar area from patient shows no staining (*f*). Similarly, *g* shows no staining for the MUC2 core antigen throughout the mucosa from the case, whereas a control biopsy (*h*) reveals the normal distribution of this antibody within the ER/Golgi zone of goblet cells.

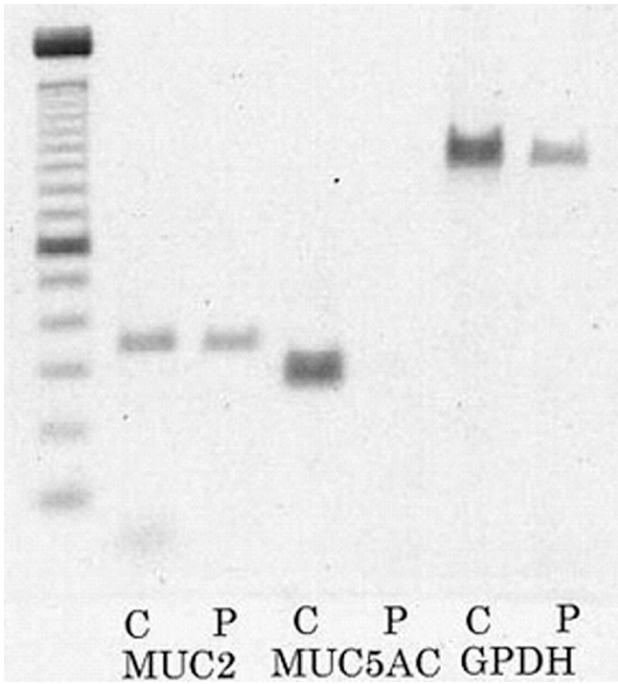


**Figure 3.** RT-PCR of a rectal biopsy from the patient (P) showing MUC2 expression and no MUC5AC expression. The control sections (C) were from the HT29A1 cell line, which expresses both MUC2 and MUC5AC.

developed in our lab (18)], MUC5AC (19), TFF1 (Biomeda, Foster City, CA), TFF3 (ITF) (20) (given by Dr. D. Podolsky, Massachusetts General Hospital, Boston, MA), rat sucrase (21) (given by Dr. A. Moore, HSC, Toronto), HD5 (22) (given by Dr. T. Ganz, UCLA, Los Angeles, CA), and MIB1 (15) (Beckman Coulter, Fullerton, CA).

***Reverse transcription–PCR.*** RNA was extracted from six rectal biopsies from the patient. The HT29A1 cell line served as control. One microgram of total RNA was reverse-transcribed with 50 U of Moloney murine leukemia virus reverse transcriptase (Perkin Elmer, Foster City, CA), 2.5 $\mu$M random hexamer, 1 mM dGTP, 1 mM dATP, 1 mM dTTP, 1 mM dCTP, and 20 units of RNase inhibitor in a total volume of 20 $\mu$L for 30 min at 42°C. PCR was performed in a 50-$\mu$L reaction that contained 0.5 unit of *Taq* polymerase (Perkin Elmer), 125 $\mu$M each dNTP, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.1 mM primers. Primers for MUC2, MUC5AC, and GPDH were synthesized according to published sequences (23–25). PCR was carried out according to Hong *et al.* (26).

## DISCUSSION

The lack of any normal-appearing granule that contained epithelial cells in the small intestine of this case suggests a failure of normal epithelial lineage differentiation. The gut epithelium is normally composed of four different cell types, which are thought to be derived from a single multipotential stem cell that lies in the crypts of Lieberkühn (1–4). This multipotential stem cell divides into a daughter cell that then, through an ill-defined process, finally becomes one of four cell types, a Paneth cell, a goblet cell, a neuroendocrine cell, or an enterocyte. Three of the lines (goblet, Paneth, and neuroendocrine) eventually develop secretory granules.

The finding in this case raises several questions. The lack of any mature-appearing granule containing epithelial cell suggests that there could be a second branching point where the daughter cell further divides into an enterocyte or a granule-containing epithelial cell. The fact that there is evidence of mucin-containing cells in the colon, but not in the small intestine further supports the evidence that regulatory factors for epithelial cell differentiation may not be the same for all of the length of the gastrointestinal tract.

Differentiation studies have been undertaken using colonic adenocarcinoma cell lines. In particular, the HT29 cell line has been of interest because it is the only cell line of intestinal

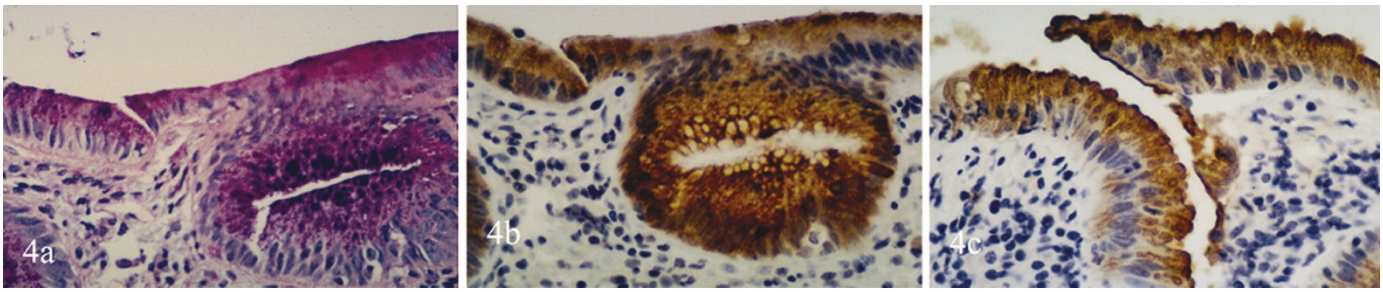


**Figure 4.** A representative section typical of most of the colonic mucosa. Most of the tissue consisted of inflammatory cells with a few glands and a flattened cuboidal surface epithelium. Some of the surface and glandular cells contained PAS/Alcian Blue–positive cells (*a*). These cells expressed both MUC2 (*b*) and MUC5AC (*c*) antigens.

origin to reversibly display structural and functional features of mature intestinal epithelial cells (27). In culture, these cells are heterogeneous with a small proportion of mucous cells and columnar absorptive cells (28). Subclones of the HT29 exhibit characteristic absorptive and goblet cells (27,29). Agents such as sodium butyrate (30,31), retinoic acid (32,33), 1,25 dihydroxyvitamin $D_3$ (34), suramin (35,36), spermine (37), and methotrexate (38) can induce changes suggestive of a more differentiated phenotype. Thus, exposure of these cells, which have a potential to develop along one or another lineage, to agents that might be found in the fecal stream may promote differentiation.

Intestinal growth, differentiation, and adaptation are likely affected by various host cell factors, including cell–cell interactions (13,39); extracellular matrix and mesenchymal cells (13,21); hormones and peptides (40–42); luminal factors, such as diet (39,41); and factors that are produced by endogenous bacteria, such as butyrate (43), and secreted products from salivary glands, gall bladder, and pancreas (42,44) also play a part in regulating differentiation. Other important participants include PDGF A and its receptor, PDGF-Ra; the winged helix transcription factor Fkh6; the homeodomain transcription factor Nkx2–3; and *Hox* and *ParaHox* cluster genes, Sonic hedgehog, and bone morphogenetic proteins. Intraepithelial lymphocytes and microbes may be additional modulators (13). A recent study of *Hes1* knockout mice indicated that, as in other systems, notch signaling is an important contributor to lineage specification (7). Taking together all of the recent advances in the field of small bowel differentiation, the bottom line is that no one really knows what the exact interplay between these factors and stem cell differentiation is (13,45). Thus, any single factor or combination of these factors could have influenced the further differentiation to a mature goblet cell in the colon.

Paneth cells were originally thought to play a major role in the regulation of proliferation and differentiation of the small bowel, but a study by Garabedian *et al.* (4) showed that ablation of the Paneth cell lineage did not affect the development of the other three lineages. In the same paper, ablation of the Paneth cells resulted in an intermediate cell somewhere between a Paneth cell and a granule-containing goblet cell, which subsequently became a mature goblet cell. So the lack of mature Paneth cells alone cannot explain the failure of stem cell development into goblet and neuroendocrine cells. The presence of an intermediate cell type also suggests that there may be a second major branching point, where the first daughter cell may be able to become either a Paneth or a goblet cell, depending on the right circumstances.

In their important paper, Bjerknes and Cheng (46) found that the crypt contains both short-lived (days) and long-lived (months) unipotential columnar cell progenitors or mucous cell progenitors and bipotential progenitors of the enterocytic and goblet cell lineages, as well as long-lived multipotent stem cells that are capable of giving rise to all epithelial cell types. We could not find in our patient any evidence to these mix progenitors.

Katz *et al.* (9) showed that Klf4 (formerly GKLF), a zinc-finger transcription factor, is a goblet cell–specific differentiation factor in the colon. This suggests a distinction between differentiation of colon *versus* small intestinal goblet cells and might explain the discrepancy of MUC2 production in the colon and not in the small bowel.

A recent study by Yang *et al.* (6) found that loss of *Math1*, a basic helix-loop-helix transcription factor that is expressed in the gut, leads to depletion of goblet, enteroendocrine, and Paneth cells without affecting enterocytes. Colocalization of *Math1* with Ki-67 in some proliferating cells suggests that secretory cells (goblet, enteroendocrine, and Paneth cells) arise from a common progenitor that expresses *Math1*, whereas absorptive cells (enterocytes) arise from a progenitor that is *Math1* independent. These findings support our theory of a common progenitor for the secretory granules cell lineage progenitor.

Protein products of the various cell lineages are often expressed early during embryonal development (47). Detection of these proteins using various markers has been used to try to follow cells through the differentiation process. These markers may be present long before the mature phenotype is evident and possibly suggest the future potential of the cell.

MUC2, the major intestinal mucin, appears as early as 12 wk gestation and is expressed in individual cells throughout the intestinal epithelium (48). In the case study, there was evidence of mRNA for MUC2 from the rectal biopsies. This was not the case for his small intestine. Thus, one suspects that he had at least the genetic ability to produce MUC2 and then goblet cells in the large intestine. This actually happened later in his course. The lack of any evidence, even at the level of mRNA, for MUC2 in the small intestine supports the theory that the insult was at a very basic level. Thus, it seems that the ability for differentiation and end product formation, even along one line, varies from one area to another.

Darmoul *et al.* (49) found cryptdin gene expression in the immature intestinal epithelium of newborn mice despite an absence of differentiated crypts or recognizable Paneth cells. Cryptdin is the mouse analogue of HD, a Paneth cell product. HD5 and HD6 are present at 13.5 wk gestation in human fetuses and localize to crypts (50). In our study, normal control small intestine was positive, as one would expect, for HD5. In the disease controls, there was overexpression of this marker, possibly in response to inflammation. The case study small intestine had no positive staining, even when severely inflamed.

CMG and TFF3 are also used as markers of early differentiation. CMG is currently the most sensitive marker for endocrine differentiation in normal and neoplastic tissue (51). TFF3 is a member of the trefoil factor family and eventually locates to Brunner's gland acini and goblet cells in both the large and small intestines (52). The trefoil proteins are secreted by mucin-secreting epithelial cells and are believed to be important for mucosal protection and repair. Normal controls, as expected, had positive staining for both of these markers. Inflamed control duodenum again had overexpression of these markers. The case study was negative, which correlated with the lack of neuroendocrine, Paneth, or goblet cells. In fact, given the results in the disease controls, one might actually expect overexpression of these markers in the case study in response to the inflammation in the intestinal biopsies.

Several patients with Crohn's disease with inflamed duodenum served as control to ensure that the case findings were not simply a local response to inflammation. All of the markers studied (MUC2, HD5, CMG, TFF1, and TFF3) were present in both healthy and disease control subjects. In fact, we found overexpression of TFF3 and TFF1 in Crohn's disease (Prof. D. Podolsky, Gastrointestinal Unit, Department of Medicine, Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, personal communication June 1997), suggesting that the changes in our patient's duodenum did not simply reflect response to inflammation.

Although the patient's colon did not express goblet cells early on, there was MUC2 and TFF3 expression in colonocytes. This is in contrast to the small bowel findings. The distribution of core MUC2 in the colon epithelial cells suggests an incomplete mucin processing. Colonic biopsies from patients with inflammatory bowel disease (IBD) exhibit similar findings in areas of goblet cell depletion (53).

Although Paneth cells are usually not present in the colon, there is often Paneth metaplasia in patients with colitis (54,55). HD5, a marker for Paneth cells, was not present in our patient's colonic biopsies. Given the degree of inflammation, including the presence of gastric metaplasia, one would have expected to observe Paneth cell metaplasia in this case. As well, trefoil factors have been shown to be overexpressed in both the large and small bowel in Crohn's disease (52). Their presence is believed to be important for repair of the intestinal mucosa. Gastric metaplasia is seen frequently in the inflamed colon of patients with IBD (56). TFF1 has been shown to be expressed in IBD in areas of ulcers and in areas of gastric metaplasia (57). The expression of MUC5AC and TFF1 in some areas of the colon in this patient may be a part of gastric metaplasia secondary to the inflammatory process and may reflect an ability to start differentiation in the colon, unlike the small bowel. Both intestinal mucus and trefoil factors are believed to have a mucosal protective effect in the intestine (58). We postulate that a lack of luminal factors that would have been produced by the absent mature small intestinal epithelial cells may play a decisive role in the health and response to inflammation in the colon.

Although inflamed, antral epithelial differentiation seemed normal on the basis of morphology and expression of the markers. The stomach, unlike the colon, may not be dependent on factors from the small bowel for normal differentiation, or, simply, the modulators for epithelial cell differentiation may be different in the stomach.

Positive anti–goblet cell antibodies were present in serum in both our patient (in low titer) and a patient described by Moore *et al.* (14). They described a similar case of childhood-onset diarrhea and absence of colonic and intestinal goblet cells. On the basis of the presence of the anti–goblet cell antibodies, increased class II antigen expression by epithelial cells, and IgA deficiency as well as some response to immunosuppressive therapy, the authors postulated an autoimmune cause. We reviewed the biopsies from this case and discovered that there were also no Paneth or neuroendocrine cells present. Some goblet and enteroendocrine cells were observed in small bowel biopsy after immunosuppressive treatment. This has never happened in our patient. Since the publication of this case, anti–goblet cell antibodies have been described in many patients with IBD (59,60), and a positive anti–goblet cell antibody may simply be secondary to the inflammation.

## CONCLUSION

In conclusion, our current knowledge suggests a common precursor for all intestinal cell lines. This differentiation process is highly complex and controlled by multiple factors. We speculate that our findings, mainly in the small bowel, are the result of an early block in lineage development at a step common to all three granule-producing cell lines. This "human model" confirms the mouse small bowel differentiation model presented by Bjerknes and Cheng (46). We believe that this mistake of nature can add to our understanding of the normal small bowel differentiation.

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