Distribution, Proliferation, and Function of Paneth Cells in Uncomplicated and Complicated Adult Celiac Disease

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Abstract

Paneth cells, granulated epithelial cells located at the base of small bowel crypts, have a crucial role in innate immunity. Because controversies remain concerning Paneth cell numbers and function in celiac disease (CD), we quantified Paneth cells and human α-defensin (HD)-5 and HD-6 in 28 patients with uncomplicated CD, 8 patients with complicated CD (3 with ulcerative jejunoileitis, 2 with refractory sprue, and 3 with enteropathy-associated T-cell lymphoma), and 14 control subjects. Paneth cell numbers and proliferation did not differ in uncomplicated untreated and treated CD and control cases. However, the number of Paneth cells was significantly reduced in complicated CD. Mucosal HD-5 and HD-6 were comparable in uncomplicated untreated and treated CD and control cases. Ex vivo gliadin challenge of treated CD biopsy specimens had no effect on mucosal HD-5 and HD-6 transcripts. Paneth cell numbers and α-defensins are unchanged in the mucosa in uncomplicated CD. Further studies are needed to clarify the implications of reduction of numbers of Paneth cells in complicated CD.

Paneth cells fulfill a crucial role in innate immunity in the gut by secreting antimicrobial peptides, including lysozyme, secretory phospholipase A2, and defensins.1,2 Because Paneth cells are also involved in linking innate and adaptive immunity,3 their role in human disease is becoming increasingly topical. Celiac disease (CD), an enteropathy caused in genetically susceptible people by a T-helper cell–type 1 immune response to gluten,4 represents a disease in which innate and adaptive responses are thought to contribute to immune-mediated gut injury.5

A number of controversial studies have been published during the last 40 years concerning Paneth cell distribution and function in the celiac mucosa.6-10 However, because Paneth cells are thought to be involved in innate immunity to gut bacteria,2 the recent evidence provided by Forsberg et al11 of rod-shaped bacteria attached to the small bowel epithelium in patients with active CD encouraged us to reexamine Paneth cell numbers and function in CD.

The aims of the present study were to enumerate Paneth cells in celiac duodenal mucosa by using a multiple histochemical and counting approach and to explore Paneth cell function in in vivo and ex vivo experiments by determining mucosal transcripts of human α-defensin (HD)-5 and HD-6, 2 major peptides in human Paneth cell granules responsible for antimicrobial activity in the intestinal lumen.2

Materials and Methods

Patients and Tissue Samples

Well-oriented endoscopic biopsy specimens were obtained from the second part of the duodenum in 14 patients with uncomplicated, untreated CD who were positive for...
antiendomysial antibodies (mean age, 36.4 years; range, 20-67 years). The histopathologic diagnosis was based on typical lesions with crypt hyperplasia, villous atrophy (8 patients had a Marsh IIIc lesion, and 6 had a Marsh IIIb lesion), and increased intraepithelial lymphocytes. Biopsy specimens were also obtained from 20 patients with uncomplicated CD who had been following a gluten-free diet for at least 12 months (mean age, 41.5 years; range, 19-65 years) and were negative for antiendomysial antibodies. All patients with CD were HLA-DQ2+. In addition, biopsy specimens were obtained from 14 consenting subjects who were negative for antiendomysial antibodies (mean age, 43.2 years; range, 21-70 years) and were undergoing upper gastrointestinal endoscopy for functional dyspepsia and from 8 patients with complicated CD (mean age, 57 years; range, 42-70 years). Of these patients, 3 were affected by ulcerative jejunoileitis, 2 by refractory sprue, and 3 by enteropathy-associated T-cell lymphoma. Clinical and pathologic features of patients with complicated CD are reported in Table 2. In the group of patients with complicated CD, duodenal biopsy specimens were obtained at the time of diagnosis of the complication except for 3 patients (cases 1, 3, and 5; Table 2) from whom biopsy specimens were obtained after this diagnosis.

Some tissue samples were immediately fixed in 10% neutral buffered formalin and embedded in paraffin within 24 hours. Consecutive 4-µm-thick sections were cut from the selected blocks, mounted on electrostatic slides (Super Frost Plus, Menzel-Glaser, Braunschweig, Germany), and dried overnight. After dewaxing and rehydration, sections were processed for traditional histologic examination, histochemical detection of Paneth cells, or immunohistochemical analysis. Some other biopsy specimens were homogenized and used for quantitative reverse transcription–polymerase chain reaction (PCR) determination of defensins or used in organ culture experiments. All patients with CD and control subjects gave informed consent to the study.

Histochemical Detection of Paneth Cells

Sections were stained with phloxine-tartrazine to visualize Paneth cells, according to the Lendrum reaction. Briefly,  

<table>
<thead>
<tr>
<th>Authors/ Publication Date</th>
<th>No. of Patients Studied</th>
<th>Detection Method</th>
<th>Counting Method</th>
<th>No. of PCs</th>
<th>Supplementary Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creamer and Pink 1967</td>
<td>7 with refractory disease</td>
<td>Phosphotungstic acid–hematoxylin staining and electron microscopy</td>
<td>Semiquantitative evaluation</td>
<td>Reduced in 5 cases; absent in 2</td>
<td>In the 5 cases with reduced numbers, PCs looked abnormal with sparse granules Reduced jejunal lysozyme activity</td>
</tr>
<tr>
<td>Ward et al 1979</td>
<td>30 (18 untreated and 12 treated)</td>
<td>H&amp;E staining</td>
<td>No. of PCs per crypt</td>
<td>Reduced regardless of GFD</td>
<td>—</td>
</tr>
<tr>
<td>Scott and Brandtzaeg 1981</td>
<td>30 (15 untreated and 15 treated)</td>
<td>Hematoxylin, asphloxine, and saffron staining</td>
<td>Lysozyme immunofluorescence staining</td>
<td>Reduced regardless of GFD</td>
<td>Normal</td>
</tr>
<tr>
<td>Elmes et al 1983</td>
<td>12 (8 untreated and 4 treated)</td>
<td>Lendrum phloxine-tartrazine staining</td>
<td>PC granule area in square micrometers per crypt</td>
<td>Normal</td>
<td>—</td>
</tr>
<tr>
<td>Kelly et al 2004</td>
<td>8 untreated</td>
<td>H&amp;E staining</td>
<td>No. of PCs per crypt</td>
<td>Normal at the crypt base; increased in the middle crypt region</td>
<td>—</td>
</tr>
</tbody>
</table>

CD, celiac disease; GFD, gluten-free diet; PC, Paneth cell.
sections were treated with alun hematoxylin (1 minute), 0.5% phloxine in 0.5% aqueous calcium chloride (20 minutes), and finally differentiated with a saturated solution of tartrazine in Cellosolve (Biocompare, San Francisco, CA). With this technique, Paneth granules stain bright red. For $p$-dimethylamino-benzaldehyde (DMAB)-nitrite staining, sections were placed in 5% DMAB solution (Sigma-Aldrich, Poole, England) for 1 minute, transferred to 1% sodium nitrite solution for 1 minute, and then washed with tap water and acid alcohol. The DMAB-nitrite method gives an intense blue staining of the cytoplasm depending on the high tryptophan content of Paneth cell granules, and it has been reported to be the most suitable method for double-labeling procedures of Paneth cells. The same sections had been previously stained with an anti-Ki-67 antibody to evaluate the Paneth cell proliferative rate (see “Immunohistochemical Analysis for Ki-67”).

Counting of Paneth Cells

For cell counts, 8 to 24 crypts were evaluated only if aligned along the longitudinal axis such that the lumen of the crypt could be seen along its length. Observation of crypt epithelial cells positive for phloxine-tartrazine and DMAB-nitrite was performed by using conventional light microscopy in a blinded manner by the same expert observer. Counts were performed at a constant magnification ($\times 1,000$) in 3 ways: (1) by counting the number of Paneth cells per crypt; (2) by a differential count of at least 500 crypt cells with the results expressed as Paneth cells per 100 crypt cells; and (3) by counting with an ocular linear graticule the number of Paneth cells placed in the epithelial layer overlying a fixed area of muscularis mucosae defined by the length of the graticule (60 µm) and the thickness of the section (4 µm). The 3 counting approaches were applied to phloxine-tartrazine- and DMAB-nitrite-stained sections.

Immunohistochemical Analysis for Ki-67

After blocking endogenous peroxidase with 0.3% hydrogen peroxide for 8 minutes, proteolytic digestion was obtained with 2% trypsin in 0.1% calcium chloride, pH 7.8, for 5 minutes. Sections were then immersed in 0.01 mol/L of citrate buffer (pH 6.0) and microwave treated for 10 minutes at 4°C and then treated with a secondary biotinylated antibody and peroxidase-conjugated streptavidin (Biogenex, San Ramon, CA). Cells with only dark brown nuclei (Ki-67+ crypt enterocytes) and cells with dark brown nuclei surrounded by blue cytoplasm (Ki-67+/DMAB+ Paneth cells) were counted in crypts with optimal orientation.

Organ Culture

Biopsy specimens from patients with treated CD were placed on grids in the central well of an organ culture dish and positioned in an airtight container with 95% oxygen/5% carbon dioxide at 37°C. Biopsy specimens were cultured for 24 hours in the presence or absence of 1 mg/mL of peptic tryptic digest of gliadin (PT-gliadin, Frazer III fraction, Sigma-Aldrich). After culture, tissue samples and supernatants were snap frozen at –70°C before RNA extraction and interferon (IFN)-γ determination by enzyme-linked immunosorbent assay, respectively.

Enzyme-Linked Immunosorbent Assay

IFN-γ levels were measured in organ culture supernatant by enzyme-linked immunosorbent assay using the Quantikine Human IFN-γ Immunoassay (R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions.

RNA and Reverse Transcription

Frozen duodenal biopsy specimens were disrupted mechanically, and total RNA was extracted by the use of Trizol (Invitrogen, Paisley, Scotland) following the manufacturer’s instructions. Extracted RNA was treated with RQ1 RNase-free DNase (Promega, Southampton, England) and reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Promega) using an oligo-$\left(d\right)_{12-18}$ primer according to the supplier’s protocol (Promega).

Quantitative Real-Time PCR

Serial dilutions of HD-5 and HD-6 gene-specific plasmids (kindly provided by Charles Bevins, University of California Davis) were used in real-time PCR to generate standard curves for each reaction. Real-time PCR was performed using complementary DNA from tissue, or gene-specific plasmids, with specific oligonucleotide primer pairs Table 3. Complementary DNA corresponding to 100 ng of
RNA served as a template in a 20 µL reaction containing 0.5 µmol/L of each primer and 2x QuantiTect SyBr green mix (Qiagen, Crawley, England). All reactions were performed in duplicate. The PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles with each cycle consisting of denaturation, 95°C for 20 seconds; annealing at 60°C for 20 seconds; and extension at 72°C for 20 seconds. The cycle-to-cycle fluorescence emission was analyzed by using RotorGene software (version 6.0; Corbett Research, Sydney, Australia). To confirm PCR amplification of the intended product, representative samples were analyzed by electrophoresis on a 2% agarose gel. The products were visualized by ethidium bromide staining and compared with DNA size standards to confirm product size. Product fidelity was confirmed by using melt-curve analysis.

Statistical Analysis

Data were analyzed in the GraphPad Prism statistical PC program (GraphPad Software, San Diego, CA) by using the Mann-Whitney U test and the paired t test. Correlations were studied by using the Spearman rank correlation test. A P value less than .05 was considered statistically significant.

Results

Paneth Cell Numbers

We identified Paneth cells in the duodenal mucosa of 28 cases of uncomplicated CD (14 untreated and 14 treated), 8 cases of complicated CD, and 14 control cases by using 2 histochemical methods, ie, phloxine-tartrazine that stains Paneth granules in bright red and DMAB-nitrite that gives an intensely blue stain to Paneth cell cytoplasm. The latter was used in a double-labeling procedure with Ki-67 staining to analyze the proliferative behavior of Paneth cells.

As shown in Table 4, sections stained with phloxine-tartrazine showed no significant differences in the frequency of Paneth cells in uncomplicated untreated and treated CD and control cases, regardless of the counting method used (per crypt, per 100 crypt cells, or per unit length of muscularis mucosae). However, the number of Paneth cells was significantly lower (P < .001) in the duodenal mucosa of patients with complicated CD in comparison with the other 3 groups. In 2 of 8 complicated CD cases (both patients had enteropathy-associated T-cell lymphoma), no Paneth cells were observed.

In keeping with the results obtained through phloxine-tartrazine staining, analysis of sections treated with DMAB-nitrite showed no significant differences in the frequency of Paneth cells in uncomplicated untreated and treated CD and control cases, regardless of the counting method used. Again, the frequency of Paneth cells was significantly lower (P < .001) in the complicated CD group in comparison with the aforementioned groups.

In complicated CD, no significant correlation was found between the number of Paneth cells per crypt and the degree of mucosal damage, evaluated through the Marsh classification, or the duration of gluten-free diet. In all 36 patients with CD and 14 control subjects, a significant positive correlation (r = 0.69; P < .0001) between the number of Paneth cells per crypt stained with the DMAB-nitrite method and the number of Paneth cells per crypt stained with phloxine-tartrazine staining was found.

Paneth Cell Proliferation

We investigated the proliferative pattern of Paneth cells by analyzing the immunohistochemical expression of Ki-67, an antigen expressed exclusively in proliferating cells. The median percentage of Ki-67+ Paneth cells was not statistically different in uncomplicated, untreated CD (11.9%; range, 7.4%-18.9%), uncomplicated, treated CD (11.2%; range, 8.1%-19.6%), and control cases (12.0%; range, 7.7%-22.1%). On the contrary, the median percentage of Ki-67+ crypt enterocytes was significantly higher (P < .01) in uncomplicated, untreated CD (47.6%; range, 39.0%-62.5%) in comparison with treated CD (26.8%; range, 20.7%-32.5%) and control cases (27.6%; range, 19.8%-37.0%). Owing to the low number of Paneth cells found in the mucosa of patients with complicated CD, we were not able to assess the percentage of Ki-67+ Paneth cells in this condition. However, we determined the median percentage of Ki-67+ crypt enterocytes that was higher, although not significantly, in complicated CD (37.7%; range, 22.1%-53.5%) in comparison with treated CD and control cases. No significant difference was found between complicated CD and uncomplicated, untreated CD.

In Vivo and Ex Vivo Mucosal HD-5 and HD-6 Transcripts

To explore Paneth cell function, we performed in vivo and ex vivo experiments to quantify mucosal transcripts of 2 major α-defensins in human Paneth cells, ie, HD-5 and HD-6. We first determined HD-5 and HD-6 messenger RNA (mRNA) levels in the duodenal biopsy specimens of 19 uncomplicated CD cases (10 untreated and 9 treated) and 10 control cases. No significant difference was found in the number of HD-5 and HD-6 transcripts in the above-mentioned groups. Because most of the tissue from complicated CD cases was used for diagnostic purposes (CD8/CD4/CD3 staining or clonality of T-cell receptor γ-chain gene studies), we did not have sufficient material to determine defensin transcripts in the group.
A-E, Histochemical detection of Paneth cells by the phloxine-tartrazine method, which stains Paneth granules bright red (arrows), showed comparable numbers of Paneth cells in the duodenal mucosa of an uncomplicated case of celiac disease (A, ×200), an uncomplicated case of treated celiac disease (B, ×200), and in a control case (C, ×200). The higher magnification detail shows Paneth cells in normal mucosa (D, ×400). In contrast, Paneth cells were absent in the duodenal mucosa of a patient with complicated celiac disease (E, ×200).

F-I, The p-dimethylaminobenzaldehyde–nitrite method, which gives an intense blue stain to Paneth cell cytoplasm, was used in a double-labeling procedure with Ki-67 staining to study the proliferative behavior of Paneth cells. Ki-67+ Paneth cells appeared as crypt epithelial cells with dark brown nuclei surrounded by blue cytoplasm (F, ×1,000; arrow). No difference was evident in the frequency of Ki-67+ Paneth cells in the mucosa of uncomplicated, untreated celiac disease (G, ×100), in the mucosa of uncomplicated, treated celiac disease (H, ×100), and in control mucosa (I, ×100). Conversely, Ki-67+ crypt enterocytes, identified as epithelial cells with only dark brown nuclei, were increased in mucosa in untreated celiac disease in comparison with mucosa in treated celiac disease and control mucosa.
with complicated CD, except in 1 case (case 8; Table 2) that showed the lowest level of HD-5 mRNA.

To further study Paneth cell function, we determined HD-5 and HD-6 transcripts in mucosal biopsy specimens from patients with uncomplicated, treated CD that were cultured ex vivo in the absence or presence of PT-gliadin. We observed that gliadin challenge, although effective in inducing a significant ($P < .0001$) up-regulation of IFN-$\gamma$ production (from a mean of 28.9 ± 8.4 to 111.6 ± 19.1 pg/mL), did not significantly change the mRNA levels of HD-5 and HD-6 in organ culture biopsy specimens #Figure 1C, Figure 1D, and Figure 1E#.

**Discussion**

A number of studies report conflicting findings concerning Paneth cell frequency in the celiac mucosa (Table 1). The earliest reports described the disappearance of Paneth cells from small intestinal mucosa of patients with refractory CD$^6$ and a significant decrease in patients with untreated and treated CD.$^7$ However, these observations were not confirmed in later studies, in which no numeric reduction of celiac Paneth cells was shown.$^{8,10}$ More recently, it has been hypothesized that Paneth cells would be markedly increased in active CD, given the high level of $\alpha$-defensins found in untreated celiac mucosa.$^{11}$

These discrepancies might be related to the staining procedure used, such as conventional light microscopy,$^{6,8,10}$ lysozyme immunofluorescence,$^8$ and Lendrum phloxine-tartrazine staining,$^{13}$ or to the counting method, which in the aforementioned studies mostly consisted of the enumeration of Paneth cells per crypt.$^{6,8,10}$ It must be noted that the accuracy of this counting method depends on the correct orientation of sections because crypt alignment along the longitudinal axis is required for reliable counting.$^{10}$

In the present study, we adopted a multiple histochemical approach by comparing Lendrum phloxine-tartrazine staining with the DMAB-nitrite technique. The latter, being the most suitable method in double-labeling procedures,$^{15}$ gave us the chance to assess in parallel the proliferative rate of Paneth cells through the immunohistochemical detection of Ki-67 antigen.$^{19}$ Furthermore, to reduce counting bias, we enumerated Paneth cells exclusively in well-oriented biopsy specimens by comparing the number of Paneth cells per crypt with the proportion of Paneth cells per 100 crypt cells or per unit length of muscularis mucosae.

Phloxine-tartrazine staining showed no change of Paneth cell numbers in the duodenal mucosa of uncomplicated untreated and treated CD in comparison with control cases with all 3 counting methods used. Similar results were obtained when sections were processed for the DMAB-nitrite staining, regardless of the counting method used. Good agreement was found between the 2 histochemical methods, regardless of the counting method used. Good agreement was found between the 2 histochemical methods, regardless of the counting method used.

<table>
<thead>
<tr>
<th>Paneth Cell Counting Method</th>
<th>Complicated CD</th>
<th>Untreated CD</th>
<th>Treated CD</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per crypt</td>
<td>0.1 (0.0-1.9)$^\dagger$</td>
<td>3.2 (2.5-4.1)</td>
<td>3.2 (2.4-4.3)</td>
<td>2.8 (2.2-4.1)</td>
</tr>
<tr>
<td>Per 100 crypt cells</td>
<td>0.2 (0.0-4.3)$^\dagger$</td>
<td>6.2 (4.2-11.5)</td>
<td>7.5 (5.1-13.0)</td>
<td>7.7 (3.3-11.1)</td>
</tr>
<tr>
<td>Per unit length of muscularis mucosae</td>
<td>0.4 (0.0-6.1)$^\dagger$</td>
<td>9.2 (4.7-14.0)</td>
<td>10.2 (4.7-15.2)</td>
<td>7.8 (4.1-14.7)</td>
</tr>
</tbody>
</table>

CD, celiac disease; DMAB, $p$-dimethylaminobenzaldehyde.

$^\dagger$ The uncomplicated group included 14 untreated and 14 treated patients. Results are given as median (range).

$^\dagger$ $P < .001$ vs patients with untreated CD, patients with treated CD, and control subjects.
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Crypt enterocytes in active CD was significantly higher than in control cases.\(^{20,21}\)

It is interesting that, in keeping with the findings of previous studies,\(^ {7,8}\) we observed a significant decrease of Paneth cells in patients with complicated CD and even an absolute lack in 2 of these patients with enteropathy-associated T-cell lymphoma. Of note, this decrease did not correlate with the degree of mucosal damage or the duration of gluten-free diet. Further studies are needed to clarify the implications of Paneth cell loss in complicated CD. The intriguing hypothesis that Paneth cell secretion might be involved in the control of...
neoplasia, thus accounting for the low incidence of neoplasms in the small bowel, encourages us to further investigate the relationship between Paneth cell deficiency and premalignant and malignant complications of CD.

We are aware that using 2 histochemical techniques to identify Paneth cells through staining their characteristic granules may conceivably result in underestimation of the number of Paneth cells because degranulation following stimuli will make them more difficult to recognize. Therefore, our histochemical approach may potentially underestimate the number of Paneth cells in celiac mucosa in which an increased proportion of Paneth cells with a low lysozyme content has been shown. Hence, to better clarify Paneth cell behavior in CD, we decided to investigate Paneth cell function by measuring mucosal transcripts of Paneth cell α-defensins by using in vivo and ex vivo experiments.

Defensins are a family of small (3.5-4.5 kDa) cationic antimicrobial peptides containing 3 intramolecular disulfide bonds that function by disrupting the membrane integrity of target microbes through formation of multiple pores. Based on the position of their cysteine linkages, they are grouped into 2 categories (α and β). Unlike β-defensins, which are primarily expressed in the epithelia of intestine, skin, kidney, trachea, and bronchi, α-defensins are produced by neutrophils and Paneth cells. The α-defensins have a broad spectrum of antimicrobial activity against bacteria, fungi, and some enveloped viruses. Among the α-defensins, HD-5 and HD-6 are expressed by Paneth cells, where they are stored in secretory vesicles as propeptides and cleaved by Paneth cell trypsin, which acts as a prodefensin convertase.

Several investigations indicate that HD-5 and HD-6 expression is increased in the colonic mucosa of patients with inflammatory bowel disease through the appearance of metaplastic Paneth cells. In contrast, Wehkamp et al showed reduced expression of Paneth cell α-defensins in Crohn ileitis. It is interesting that because Paneth cells are the most prominent cells expressing NOD2 gene products, Crohn disease–associated NOD2 mutations may affect Paneth cell α-defensin expression, thus increasing the susceptibility to bacterial invasion that, in turn, could trigger inflammation and loss of tolerance against luminal flora. These findings, together with the evidence that Paneth cells and the secretory granule area are preserved or even increased in ileal Crohn ileitis, suggest that Paneth cell function rather than number may be compromised in this condition. These findings led us to suspect that celiac Paneth cells, although not affected in number, might have an impaired function, as occurs in Crohn disease. However, we found that HD-5 and HD-6 transcript levels did not differ in the mucosa of untreated and treated uncomplicated CD and control cases. Our results are in keeping with those of Taha et al, who demonstrated that mucosal HD-5 transcripts are unchanged in active CD and are not influenced by the normalization of mucosa after gluten withdrawal. These findings contrast with those of Forsberg et al, showing that mRNA levels of HD-5 and HD-6 were increased in active CD but returned to normal in treated CD. However, in that study, α-defensin expression was not tested on whole mucosal tissue but on isolated crypt enterocytes, and it is not possible to exclude that the reported HD-5 and HD-6 increase might depend on the higher Paneth cell activation state induced by the isolation procedure per se or by the exposure to lipopolysaccharide contaminating collagenase used in the tissue digestion.

Owing to the limited tissue available in complicated CD cases, we were not able to determine Paneth cell α-defensins in this group, with the exception of 1 case complicated by ulcerative jejunoileitis with a considerably low level of HD-5 mRNA. However, in ongoing studies, we are investigating whether Paneth cell depletion is associated with reduced α-defensin expression in complicated CD.

The function of Paneth cells has not yet been clearly defined, and because of the lack of an appropriate human cell culture model for Paneth cells, it is difficult to study directly the regulation of human Paneth cell defensins. Here we used the organ culture model of celiac biopsy specimens grown ex vivo with gliadin to explore the function of Paneth cells in CD. We found that gliadin challenge, although effective in inducing a significant up-regulation of IFN-γ after a 24-hour culture, did not influence the mucosal expression of HD-5 and HD-6. This finding is again in contrast with the results of Forsberg et al, who found a significant correlation between IFN-γ mRNA levels in intraepithelial lymphocytes and mRNA levels of HD-5 and HD-6 in CD.

We acknowledge the inherent technical difficulties in detecting and counting Paneth cells in mucosal biopsy specimens. However, we consider that the unaffected gene transcription of Paneth cell α-defensins and preserved Paneth cell Ki-67 proliferative rate suggest that we were able to document the absence of real biologically and physiologically relevant changes in Paneth cells in uncomplicated CD. Further studies are needed to confirm and clarify the implications of reduction in the numbers of Paneth cells in complicated CD.
References


