Paneth Cells Regulate Both Chemotaxis of Immature Dendritic Cells and Cytokine Production from Epithelial Cells

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Paneth cells in the small intestine are able to sense luminal bacteria and secrete granules that contain antibacterial peptides. Human defensin (HD)-5 and -6 are antimicrobial peptides found in human Paneth cell granules, and are major bactericidal components. We investigated whether any constituents in the Paneth cell secretions showed chemotactic activity or stimulated cytokine secretion from intestinal epithelial cells, and assessed to what extent HD-5 and -6 were responsible for these activities. The secretions from human Paneth cells and recombinant HD-5 and -6 were evaluated to elucidate their effects on the chemotaxis of dendritic cells (DCs) in a migration assay. The Paneth cell secretions were chemotactic for immature DCs at concentrations ranging from 10 to 1,000 µg/ml. HD-6 was active at 100 ng/ml, but HD-5 was not. Next, the stimulation of cytokine production by the T84 intestinal cell line was assessed using ELISA and/or an antibody array. The secretions more strongly stimulated interleukin (IL)-8 production than did the defensin peptides, and induced production of various cytokines by the antibody array. The secretions were also analyzed by high performance liquid chromatography (HPLC) and mass spectrometry (MS) in order to determine the components. A large number of molecules was found in the secretions, and HD-5 was identified as an immature propeptide. In conclusion, some constituents other than defensin in human Paneth cell secretions activated the migration of DCs and induced the production of inflammatory cytokines. Therefore, Paneth cells may play a role in the innate immunity associated with adaptive immune responses.

Keywords: chemotaxis; cytokine; dendritic cell; innate immunity; Paneth cell

The mammalian intestine is exposed to numerous microbes, and the surface monolayer of intestinal epithelial cells functions as the primary barrier against microbial invasion. The mucosal immunity in the intestine can be mainly categorized into two types, innate and adaptive immunity. The roles of intestinal adaptive immunity have been vigorously studied. Recently, the role of innate immunity in the gut has also been receiving increasing investigative interest. In addition to neutrophils and macrophages, some intestinal epithelial cells provide a rapid protection against environmental microbes. Paneth cells, one of four epithelial cell lineages in the small intestine, contribute to the mucosal innate immunity by sensing luminal bacteria and secreting granules with antimicrobial peptides into the lumen (Bevins et al. 1999; Ayabe et al. 2000; Tanabe et al. 2005). The Paneth cell secretary granules are composed of an antimicrobial mixture including α-defensins, lysozyme, secretory phospholipase A2, cryptdin-related sequences, and angiogenin (Porter et al. 2002; Keshav 2006). Enteric α-defensins, which are the major components of the antibacterial activity in Paneth cell secretions, are small antimicrobial molecules that play an important role in the intestinal mucosal immunity (Ayabe et al. 2004).

Genetically modulated mouse models have been gen-
tered to examine the physiological functions of antimicrobial peptides in vivo (Wilson et al. 1999; Nizet et al. 2001). Mice containing a knockout of matrilysin, which is an activating enzyme of mouse enteric defensin, are more sensitive to enteric salmonella in the gut (Wilson et al. 1999). Human defensin (HD)-5 and HD-6 have been identified in the human small intestine, and HD-5 transgenic mice show an additional protective effect against mucosal bacterial infection (Salzman et al. 2003). HD-5, examined in vitro, is active against many bacterial strains and inhibits viral proliferation. In contrast to HD-5, HD-6 has been reported to show little or no antibacterial activity in previous studies (Ericksen et al. 2005; Tanabe et al. 2008).

In addition to their microbicidal effects, some antimicrobial peptides may contribute to the interaction between the innate and adaptive immune responses. It has been reported that neutrophil-derived human neutrophil defensins (HNP)s and skin-derived human β-defensin (HBD)-2 are chemotactic for T lymphocytes and immature dendritic cells (DCs) (Yang et al. 1999a, 2001a), suggesting that antimicrobial peptides modulate adaptive immunity by recruiting inflammatory cells to the site of bacterial invasion. Furthermore, these peptides stimulate some epithelial cells to secrete cytokines which cause subsequent inflammatory responses. Mouse Paneth cell α-defensin (cryptdin-3) induces the secretion of the proinflammatory cytokine interleukin (IL)-8 from the human intestinal cell line T84 (Lin et al. 2004). Some HBDS have also been reported to increase the production of IL-6, IL-10, interferon (IFN)-γ-inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-3α, and regulated upon activation, normal T cell expressed and secreted (RANTES) in human keratinocytes (Niyonsaba et al. 2007). We previously proposed that HD-5, which is mainly produced by the intestinal epithelia, also possesses the ability to induce the secretion of IL-8 from intestinal epithelial cells (Ishikawa et al. 2009). Therefore, some antimicrobial peptides induce the release of cytokines and consequently stimulate adaptive immunity. As a result, they may modulate mucosal inflammation by both direct and indirect pathways (Kruse and Kristensen 2008).

This study investigated whether the components in the Paneth cell secretions mediate the interactions between adaptive and innate immunity by inducing the migration of DCs and the induction of inflammation-related cytokines. Paneth cell-specific defensins, HD-5 and -6, were examined for their effects in both chemotaxis assay and by the quantification of the cytokine production from the T84 colonic epithelial cell line. Furthermore, we investigated whether human Paneth cell secretions obtained from small intestinal crypts showed these activities compared with those of HD-5 and -6, in order to determine the roles of the defensin peptides in those activities.

Materials and Methods

Isolation of small intestinal crypts and tissue preparation

Fresh normal ileal tissues of the small intestine were removed from patients who underwent ileocecal resection for colon cancer in the surgical unit of Asahikawa Medical University. Small intestinal crypts were prepared as described in a previous report (Ayabe et al. 2000). Briefly, the tissue of the ileum was shaken in calcium-magnesium-free Hank’s Balanced Salt Solution (HBSS; Invitrogen, Carlsbad, CA) containing 30 mM EDTA (Dojindo Labolatories, Kumamoto, Japan). The villi and crypts eluted during 10 min intervals were deposited by centrifugation at 500 × g, and cryt-rich fractions were resuspended in HBSS, photographed under phase contrast microscopy (Olympus, Center Valley, PA), and the numbers of the crypts were estimated by hemocytometry (Ayabe et al. 2004). Formalin fixed tissue used for the immunohistochemical analyses was obtained from the pathology unit of Asahikawa Medical University Hospital. Written informed consent was obtained from each patient.

Preparation of Paneth cell secretions and secretory granules

A total of 6,000 isolated intact crypts were stimulated with Salmonella typhimurium at 1,000 CFU per crypt for 30 min at 37°C to induce Paneth cell secretions (Bjerknes and Cheng 1981; Ayabe et al. 2000). Next, the secretions were collected and used immediately for experiments. The protein concentrations of the secretions were measured using the Bradford assay (Bio-Rad, Hercules, CA).

Paneth cell granules were prepared by the nitrogen disruption method as described previously (Ayabe et al. 2002). Briefly, crypts deposited by centrifugation at 500 × g were resuspended in HBSS and placed under N2 at 750 p.s.i. in a nitrogen cavitation bomb for 15 min (Parr Instrument Co., Moline, IL). The lysed cells were centrifuged at 700 × g for 10 min at 4°C. The granules in the supernatants were deposited by high speed centrifugation at 27,000 × g for 40 min at 4°C. The granules were stored frozen or were dissolved immediately in 30% acetic acid and extracted.

Production of recombinant HD-5, HD-6 and antisera against HD molecules

The recombinant HD-5 and HD-6 were produced using an Escherichia coli expression system using the previously reported methods (Tanabe et al. 2004, 2008). The recombinant HD-6 was produced as a peptide with a methionine residue substituted with a leucine as the 23rd amino acid. The purity of each defensin was more than 95%, as determined by high performance liquid chromatography (HPLC), and a single band was shown with polyacrylamide gel electrophoresis followed by Coomassie blue staining (Tanabe et al. 2008).

The 6His-tag fusion HD-6 peptide was rapidly purified with Ni-NTA superflow (Qiagen, Valencia, CA), dialyzed in 1% acetic acid, and lyophilized. The fusion peptides were used for the production of antisera without conjugation. A rabbit was subcutaneously immunized twice with the conjugated peptide, and the whole serum was then harvested at 7 weeks after injection. The specificity of the anti-HD-6 serum was determined by a dot blotting analysis, which showed reactivity to the HD-6 peptide, but no reactivity with the HD-5 peptide. The details of the anti-HD-5 serum have already been reported (Tanabe et al. 2007).

Immunohistochemistry

The anti-HD-5 or anti-HD-6 serum was used for the immuno-
histochemical analyses as described in a previous report (Tanabe et al. 2008). Briefly, the thin tissue sections were deparaffinized, and endogenous peroxidase was inactivated. The sections were reacted with 1:200 diluted serum at 4°C overnight, followed by sequential incubation with biotinylated goat anti-rabbit IgG (Vector, Burlingame, CA, USA), then with streptavidin-biotin-peroxidase complex (DAKO, Glostrup, Denmark). Immobilized peroxidase was visualized in a diaminobenzidine solution for a few minutes. The sections were counterstained with hematoxylin.

Quantification of HD-5 and HD-6

The Paneth cell α-defensin concentrations in the secretions were measured by an immunoblotting analysis. A secretion specimen was blotted onto a 0.2 µm pore nitrocellulose membrane (Invitrogen, Carlsbad, CA) using a BIO-DOT manifold (Bio-Rad Laboratories, Hercules, CA). The membranes were then blocked with 5% skim milk (Wako, Osaka, Japan), incubated sequentially with the anti-HD-5 or anti-HD-6 serum (1:500), horseradish peroxidase-conjugated anti-rabbit IgG (Sigma Aldrich, St Louis, MO, 1:20,000), and SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL) followed by exposure to X-ray films (Kodak, Rochester, NY). The density was quantified densitometrically using the NIH image software program (Research Service Branch of the National Institute of Mental Health, http://rsb.info.nih.gov). Recombinant HD-5 and HD-6 were used as reference peptides. To calculate the crypt luminal volume, the radius (r) and the depth of the human intestinal crypt lumen (h) were measured as described (Ayabe et al. 2000). The volume of the crypt lumen cylinder was calculated as \( \pi r^2 h \).

Preparation of DCs and chemotaxis assay

Purified monocytes from the human peripheral blood samples of healthy volunteers were cultured in RPMI1640 supplemented with 10% fetal calf serum (Biosource Invitrogen, Carlsbad, CA), granulocyte macrophage colony stimulating factor (GM-CSF, R&D systems, Minneapolis, MN), IL-4 (R&D systems, Carlsbad, CA) and transforming growth factor (TGF)-β1 (R&D systems, Carlsbad, CA) for 7 days to obtain immature DCs (iDCs). Mature DCs (mDCs) were differentiated by incubating iDCs in the same conditioned medium supplemented with tumor necrosis factor (TNF)-α for an additional 2 days as described previously (Romani et al. 1994; Yang et al. 2001b). The phenotypes of these DCs were confirmed by a FACS analysis. The cells were incubated with Cocktail Lin 1-FITC (BD Bioscience, San Jose, CA), HLA-DR-PC5 (Immunotech, Marseille, France), or CD83-PE (Immunotech, Marseille, France) for 30 min at 4°C and then were analyzed with Epics Altra (Beckman Coulter, Fullerton, CA).

Cell migration was tested using a 96-well chemotaxis chamber (Neuro Probe, Gaithersburg, MD). The lower and upper compartments were separated by a 5 mm pore filters. The iDCs or mDCs were cultured in the wells of the upper compartments at the concentration of 10^5 cells/ml, with 4 x 10^4 cells in each well. The Paneth cell secretion and the recombinant HD-5 or HD-6 were added to the wells of the lower compartments of the chamber. Stimulation with N-formyl-methionyl-leucyl-phenylalanine (fMLP, Sigma Aldrich, St Louis, MO) for iDCs and stromal cell derived factor (SDF)-1α for mDCs were used as chemotaxis controls. After incubation for 90 minutes at 37°C, the filters were removed and stained with Diff-Quick stain (Siemens Healthcare Diagnostics, Deerfield, IL). The cells that had migrated through the filter were counted at least 5 high power fields (HPF) using a CK40 light microscope (Olympus, Tokyo, Japan) (Yang et al. 1999a, 2001a).

Apical stimulation of epithelial cells and measurement of cytokine production

T84 cells (ATCC® CCL-248), an intestinal cell line originally established from human colorectal carcinoma, were purchased from the ATCC and used as a model of the intestinal epithelia. The T84 cells were grown to confluent monolayers and then were differentiated on collagen-coated inserts (Becton Dickinson Labware, Bedford, MA) as described previously (Lin et al. 2004). T84 cells on 0.3 cm² inserts were washed and incubated with 100 µl HBSS in the apical compartment and with 300 µl in the outer well at 37°C. The secretion samples or the defensin peptides were added in the apical compartment and incubated for 5 hours, and the media in the outer wells were collected as basolateral secretions. The concentrations of IL-8 and TNF-α were measured by an enzyme-linked immunosorbent assay (ELISA) kits (Pierce Endogen, Rockford, IL) following the manufacturer’s instructions. The absorbance at 450 nm was measured with an Immuno Mini NJ-2300 (Nihon InterMed, Tokyo, Japan).

The collected basolateral secretions were also assayed using an inflammation antibody array III (Ray Biotech, Inc., Norcross, GA). The array membranes were incubated in the conditioned medium according to the manufacturer’s protocol. The array enables the detection of 40 inflammatory cytokines, e.g. IL-2, -4, -6, -12, -17, and TNFa.

Separation and identification of HD-5 in Paneth cell secretions

Protein extraction and separation were performed according to our previous report (Tanabe et al. 2007). Briefly, Paneth cell secretions were extracted in 30% acetic acid and diluted for freeze-drying in a lyophilizer. Samples dissolved in 0.1% trifluoroacetic acid (TFA) were applied to a C18 reversed phase HPLC (AKTApexlolar 10S, Amersham Biosciences Piscataway, NJ) in a gradient of 10 to 60% acetonitrile in 0.1% TFA. The molecular mass of the fraction was measured by matrix-assisted laser deionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Ibaragi Japan).

Statistical analysis

The data analyses were performed using the Stat View software package (SAS institute, Cary, NC). The measured data are presented as the means ± standard deviation. The Mann-Whitney U test was used for statistical analysis. A p value of < 0.05 was considered to be significant.

Results

Quantification of HD-5 and HD-6 in the granules and secretions of human Paneth cells

We first evaluated the immunoreactivity of tissues to the anti-HD-5 and anti-HD-6 rabbit sera. HD-5 was located in the Paneth cells of the normal small intestinal crypt, as determined by an immunohistochemical analysis (Fig. 1A and B). The specific expression of the human defensins was observed at the apical site of Paneth cells (Fig. 1A and C). The cells were recognized as dense eosinophilic granules observed by Hematoxylin-Eosin staining (Fig. 1D). Although no significant differences in localization between HD-5 and HD-6 were observed, the density of the staining
was stronger for HD-5.

The mouse crypt isolation technique was applied to the human small intestine (Ayabe et al. 2000). Briefly, human crypts were successfully eluted from the submucosal layer of the small intestine by EDTA exposure for 30 to 50 min (Fig. 2A). Crypt-rich fractions were resuspended, and an average of $6 \times 10^4$ crypts were harvested from each patient. The Paneth cells with dense granules at the base of the crypts were identified by confocal microscopy (Fig. 2B). The median volume of the three representative crypts was 17 $\mu$m according to the formula, with the measured values being diameter ($2r$) = 6 to 15 $\mu$m and depth ($h$) = 180 to 200 $\mu$m (Fig. 2C).

Antimicrobial mixtures were secreted from Paneth cells which were stimulated by living bacteria, and the amount of crude protein measured $8.3 \pm 6.1$ ng per crypt. The amounts of secreted and stored defensins were measured using an immunoblotting assay (Table 1). The HD-5 and -6 secreted from single crypts and stored in granules of a Paneth cell were 1.2 ng (70 mg/ml) and 0.06 ng (3.5 mg/ml), and 4.9 and 0.09 ng, respectively.

Chemoattraction of immature dendritic cells (iDCs) by Paneth cell secretions and HD-6

Purified monocytes were cultured to obtain immature and mature forms of DCs, and their purity was determined using a FACS analysis (Fig. 3A-D). Each population of DCs was used in the chemotaxis assays. The Paneth cell secretions acted as chemoattractants for iDCs at concentrations ranging from 10 to 1,000 $\mu$g/ml. We next examined $\alpha$-defensin peptides for chemoattraction of iDCs. HD-5 was not chemotactic for iDCs in this assay system. In contrast, HD-6 showed an optimal concentration at 100 ng/ml, and the effect was not dose-dependent (Fig. 3E). These results suggest that HD-6 may play a role in the chemotraction of iDCs in the Paneth cell secretions. In contrast, mDCs were not migrated through the chemotaxis chamber filters by the secretions, HD-5, or HD-6 (Fig. 3F). The mDCs stimulated by SDF-1$\alpha$ migrated through the filter, and only 14 cells/HPF were counted. This number was significantly smaller than that in immature DCs stimulated by fMLP. The results indicated that immature DCs migrate better than mature DCs, which was consistent with a previous study (Yang et al. 2000).

Cytokine secretion from intestinal cells by Paneth cell secretions, HD-5 and HD-6

The Paneth cell secretions, HD-5 and HD-6, were tested to determine whether they could induce cytokine production from the intestinal epithelial cell line, T84. The cells were grown to confluent monolayers and exposed to the peptides at the apical surface, then the basolaterally
secreted cytokines were examined. The Paneth cell secretions induced IL-8 secretion by the T84 cells at $222 \pm 126$ pg/ml, and the response occurred in a dose-dependent manner. Moreover, both HD-5 and HD-6 also induced IL-8 secretion in a dose-dependent manner (Fig. 4A).

The TNF-α secretion from T84 cells was also determined. Paneth cell secretions elicited a weak induction of the cytokine, whereas neither HD-5 nor HD-6 led to any increase in production (Fig. 4B). Other cytokines were also tested using an antibody array kit for 40 known proinflammatory cytokines. Apically applied Paneth cell secretions induced the basolateral secretion of several proinflammatory cytokines: IL-13, TNF-α, IL-1β, RANTES and TGF-β1 (Fig. 4C). The ratios of the increased densities are 1.5 for TNF-α, 1.7 for RANTES, 1.7 for TGF-β1, and 2.0 for IL-13. The ratios for major Th1, Th2, and Th-17 cytokines are shown in Table 2.

These results suggested that Paneth cell secretions might therefore induce a general inflammatory response.

**Table 1. Quantities of proteins present in Paneth cell secretions and granules.**

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<th>HD-5</th>
<th>HD-6</th>
<th>Total protein</th>
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<tr>
<td>Secretions</td>
<td>$1.25 \pm 1.04$</td>
<td>$0.06 \pm 0.01$</td>
<td>$8.28 \pm 6.06$</td>
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<td>Granules</td>
<td>$4.89 \pm 0.80$</td>
<td>$0.09 \pm 0.00$</td>
<td>n.d.</td>
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Data are shown as the mean per crypt ± s.d. (ng); n.d., not determined.

**The Paneth cell secretion mixture and identification of HD-5**

The extracts of Paneth cell secretions were separated by HPLC, and many peaks were observed in the chromatogram (Fig. 5). A sample of each fraction was applied to MALDI-TOF MS. The MS showed one peak, at 8,136.9 m/z, which was identical to the proform of the HD-5 peptide (theoretical mass of proHD-5 with three disulfide bonds; 8,102.5).

**Discussion**

The present study demonstrated that human Paneth cell secretions derived from small intestinal crypts stimulated the migration of iDCs and induced the secretion of the pro-inflammatory cytokine IL-8, thus suggesting that Paneth cells contribute to the interaction between innate and adaptive immunity in the intestinal tract. It is worth noting that HD-6 and some other components in Paneth cell secretions, but not HD-5, could stimulate the migration of iDCs. The secretions could more strongly induce IL-8 pro-
Human peripheral monocytes were incubated with GM-CSF, IL-4, and TGF-β1 to isolate DCs. Further incubation with TNF-α activated the DCs. The purity was determined with Cocktail Lin 1-FITC and HLA-DR-PC5 using a FACS analysis. Lin 1-negative and HLA-DR-positive cells were recognized as DCs (A, iDCs; B, mDCs). CD83 was used to define DC maturation. Very few iDCs were CD83 negative (C), and 66.4% of DCs were matured using the procedure described the Materials and Methods (D). The purified DCs were used for the chemotaxis assay. Cells were cultured in the chemotaxis chamber, and the migration induced by crypt secretions or human defensins was investigated. iDCs (E) were mobilized by Paneth cell secretions and HD-6 (100 µg/ml), however, mDCs (F) were not mobilized by any stimulus except for the positive control (SDF-1α). Separate assays (n = 4 in E, n = 2 in F) were repeated, and the measured data were presented as the means ± s.d.
Paneth Cells Regulate Gut Immunity

Paneth cells regulate gut immunity by producing more IL-1β, TNF-α, IL-13, and RANTES than either HD-5 or HD-6. While the specific components of Paneth cell secretions induced IL-1β, TNF-α, IL-13 and RANTES are presently unknown, these secretions clearly have the ability to modulate the adaptive immunity in the intestinal tract. HNPs derived from neutrophils and HBD-2 from skin have been shown to modulate adaptive immunity.

Fig. 4. Cytokine production by apical stimulation with Paneth cell secretions and human defensins.
A) The colonic epithelial cell line, T84, was incubated to form confluent monolayers on collagen-coated inserts. Samples were mixed apically in the upper compartment, and the IL-8 produced in the lower chamber was measured with an ELISA kit. Both crypt secretions and defensins stimulated basolateral IL-8 production. B) No significant TNF-α production was observed. C) The medium collected after stimulation by crypt secretions was analyzed with an inflammatory antibody array. The immunofluorescence was photographed and the densities were measured with the NIH Image software program. The production of multiple cytokines was examined by comparing the stimulated and non-stimulated media, and some cytokines, including TNF-α, TGF-β1, RANTES, IL-1β, and IL-13, were upregulated.
immunity by recruiting inflammatory cells to the site of bacterial invasion (Yang et al. 1999a, 2000, 2001a,b). Taken together, these findings indicate that any antimicrobial peptides have the ability to modulate the adaptive, as well as innate, immunity in immune-related organs. Recently, the reduction of Paneth cell defensin expression has been identified in Crohn’s disease (CD) patients (Wehkamp et al. 2004). In addition, it has been revealed that variants of the ATG16L1 gene, which encodes a protein in the autophagosome pathway processing intracellular bacteria, are associated with susceptibility to CD (Hampe et al. 2007). ATG16L1 deficient mouse exhibits abnormalities in Paneth cell granule exocytosis. As a result, Paneth cell-deficiency could therefore be associated with the etiology of CD.

The current study demonstrated that Paneth cell secretions, including HD-6, could affect iDCs. DCs are professional antigen presenting cells (APCs) that can activate naïve T cells. The monoocyte-derived myeloid iDCs used in this study express C-C chemokine receptor 6 (CCR6) (Yang et al. 1999b). Myeloid DCs that express CCR6 migrate toward MIP-3α, which is the ligand for CCR6 and is expressed in follicle-associated epithelia in the gut (Iwasaki and Kelsall 2000). CCR6-deficient mice show impaired mucosal immune responses to orally administered antigens because of the lack of iDCs beneath the microfold cells (M cells), one of the cell types of follicle-associated epithelium. MIP-3α-CCR6 binding is considered to be an important mechanism that mediates the interaction of the M cells which take up antigens and the antigen-presenting iDCs (Kunkel 2003). A recent report showed that CCR6 is one of the CD susceptibility genes (Barrett et al. 2008), suggesting that abnormalities in the MIP-3α-CCR6 interaction are associated with CD. The current study suggested that Paneth cell secretions, including HD-6, possessed similar functions as MIP-3α for attracting iDCs, and enhanced the immune reaction through the activation of naïve T cells. It is notable that the expression of HDs was decreased in the intestinal epithelia of patients with Crohn disease (Wehkamp et al. 2004), suggesting the involvement of an impaired HD-6-iDC interaction in the pathogenesis of CD.

The crypt isolation technique reported by Ayabe et al. (2000) allows for the development of ex vivo experiments investigating small intestinal physiology and shows the detailed antimicrobial function of Paneth cells. These methods were applied in this study in order to determine the physiological concentration of human defensins in the small intestinal crypt lumen. This study indicated that the concentration of HD-5 in the crypt lumen was approximately 70 mg/ml, calculated from the secreted HD-5 (1.2 ng) and the internal volume (17 pl) of small intestinal crypts. The reported mouse defensin concentration is 15 to 100 mg/ml in the crypt environment (Ayabe et al. 2000). The current

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<td>Th17 IL-17</td>
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n.d., not determined.
Paneth Cells Regulate Gut Immunity

The authors have no conflict of interest to declare.

References


Ayabe, T., Satchell, D.P., Pesendorfer, P., Tanabe, H., Wilson, C.L.,...


