

Expression and regulation of antimicrobial peptides in the gastrointestinal tract

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Abstract: The gastrointestinal (GI) tract is exposed to a wide range of microorganisms. The expression of antimicrobial peptides has been demonstrated in different regions of the GI tract, predominantly in epithelial cells, which represent the first host cells with which the microorganisms have to interact for invasion. The intestinal epithelial monolayer is complex, consisting of different cell types, and most have a limited lifespan. Of the GI antimicrobial peptides, α - and β -defensins have been studied the most and are expressed by distinct types of epithelial cells. Enteric α -defensin expression is normally restricted to Paneth and intermediate cells in the small intestine. However, there are important differences between mice and humans in the processing of the precursor forms of enteric α -defensins. Parasite infection induces an increase in the number of enteric α -defensin-expressing Paneth and intermediate cells in the murine small intestine. In the chronically inflamed colonic mucosa, metaplastic Paneth cells (which are absent in the normal colon) also express enteric α -defensins. Epithelial expression of β -defensins may be constitutive or inducible by infectious and inflammatory stimuli. The production of some members of the β -defensin family appears to be restricted to distinct parts of the GI tract. Recent studies using genetically manipulated rodents have demonstrated the likely *in vivo* importance of enteric antimicrobial peptides in innate host defense against microorganisms. The ability of these peptides to act as chemoattractants for cells of the innate- and adaptive-immune system may also play an important role in perpetuating chronic inflammation in the GI tract. *J. Leukoc. Biol.* 75: 49–58; 2004.

Key Words: epithelial cells · defensins · Paneth cells · cryptdin

INTRODUCTION

The gastrointestinal (GI) tract is unique, as it represents the largest area of the body that is constantly exposed to microorganisms. This exposure occurs in association with oral intake (which may be contaminated with microorganisms) and the resident microbial flora, varying in distinct proximal to distal

regions of the GI tract. Of the resident flora in the oral cavity, *Streptococci* predominate. In the stomach, although the secreted gastric acid aims to keep the lumen largely sterile, *Helicobacter pylori* are resident in the mucus layer of many individuals. The proximal small intestine (duodenum, jejunum, proximal ileum), which mediates the important functions of digestion and absorption of nutrients, is also relatively sterile. In the distal ileum and the colon, there is an extensive resident bacterial flora (total $\sim 10^{14}$) consisting of ~ 400 different species of anaerobic and aerobic bacteria [1].

Gastric and other secretions, motility, and secretory immunoglobulin A (a component of mucosal adaptive immunity) have been known for many years to provide protection against microorganisms in the GI tract. In recent years, there has been increasing appreciation of the likely importance of antimicrobial peptides and proteins as components of innate immunity against microorganisms. The antimicrobial peptides/proteins are expressed predominantly by epithelial cells, which have distinct characteristics in different regions of the GI tract.

Numerous factors may regulate the expression of antimicrobial peptides in the GI tract. These include intra- and extracellular processing of biologically inactive precursor forms of a peptide, epithelial interactions with pathogenic and resident luminal microorganisms, and the presence of acute or chronic inflammation (e.g., inflammatory bowel disease). Additional factors include the type and number of antimicrobial peptide-expressing epithelial cells and their state of differentiation. Nonepithelial cell types may also be important in diseased tissue. Thus, in the inflamed tissue, there is infiltration from the circulation by antimicrobial peptide/protein-expressing polymorphonuclear leukocytes, which contribute to innate mucosal host defense.

CELL BIOLOGY OF INTESTINAL EPITHELIAL CELLS AND ITS RELEVANCE TO THE EXPRESSION AND REGULATION OF ANTIMICROBIAL PEPTIDES

A highly dynamic monolayer of epithelial cells lines the small and large intestine, which is largely replaced every 2–5 days in

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mice and other species [2]. In the small intestine, there are four main epithelial cell types: absorptive enterocytes, goblet cells, enteroendocrine cells, and Paneth cells, which are derived from multipotent stem cells [3, 4]. Following their origin from stem cells present near the crypt base, the epithelial cells (apart from Paneth cells) differentiate as they migrate up the villus tip. They are subsequently lost into the lumen via exfoliation and/or apoptosis, which not only facilitates the removal of adherent bacteria but is also associated with the release of antimicrobial activity [5, 6]. In contrast to the other epithelial cell types, Paneth cells are long-lived, residing at the crypt base for ~20 days [2, 3, 7]. Paneth cells have generated considerable interest as mediators of innate immunity in the GI tract, as they express a number of antimicrobial peptides and proteins [8]. In addition to the antimicrobial proteins lysozyme [9] and secretory phospholipase A₂ (PLA₂) [10, 11], Paneth

cells have also been shown to express members of the α -defensin family in mice (designated cryptidins [12–14]) and humans [15–19]. Paneth cells are normally restricted to the small intestine, where they may play an important role in maintaining the relative sterility of the lumen and/or provide protection to stem cells (which are located close to Paneth cells) in the crypt [20]. Definitive studies to confirm these functions of Paneth cells are awaited. Cells with morphological features of Paneth and goblet cells, designated intermediate cells [21–23], are infrequently present in the normal intestinal mucosa and have recently been shown to express α -defensins (Figs. 1 and 2) [17, 24].

In contrast to enteric α -defensins, human β -defensin 1 (HBD1), a member of the β -defensin family, appears to be expressed by most epithelial cells of the small and large intestine [25]. Human cathelicidin LL-37/human cationic an-

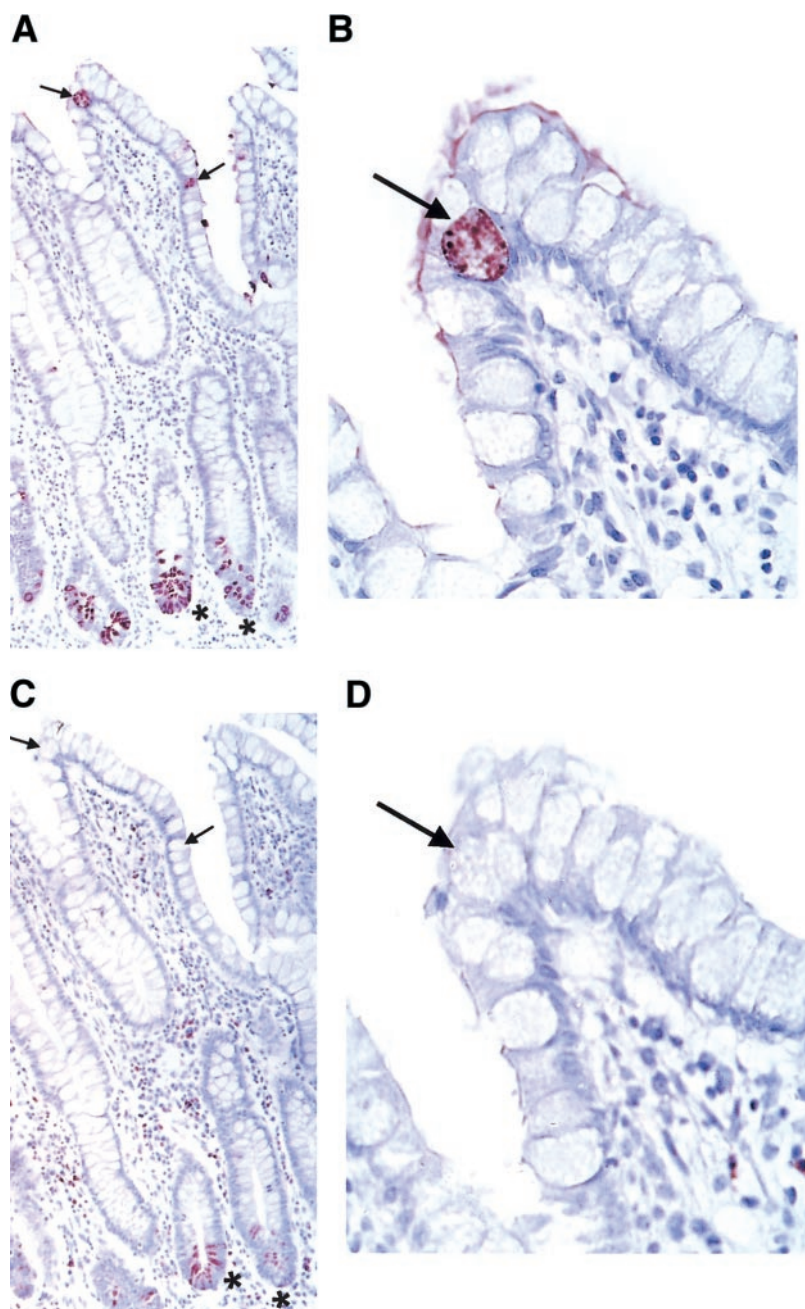


Fig. 1. Expression of human defensin (HD)-5 by human Paneth and intermediate cells. Sequential sections of normal human terminal ileal mucosa, immunolabeled using anti-HD-5 antiserum (A and B) and antilysozyme antiserum (C and D). Paneth cells in the crypts express HD-5 and lysozyme, but HD-5 immunoreactive-intermediate cells (arrowed) do not express lysozyme. Reproduced from ref. [17] with permission from BMJ Publishing Group.

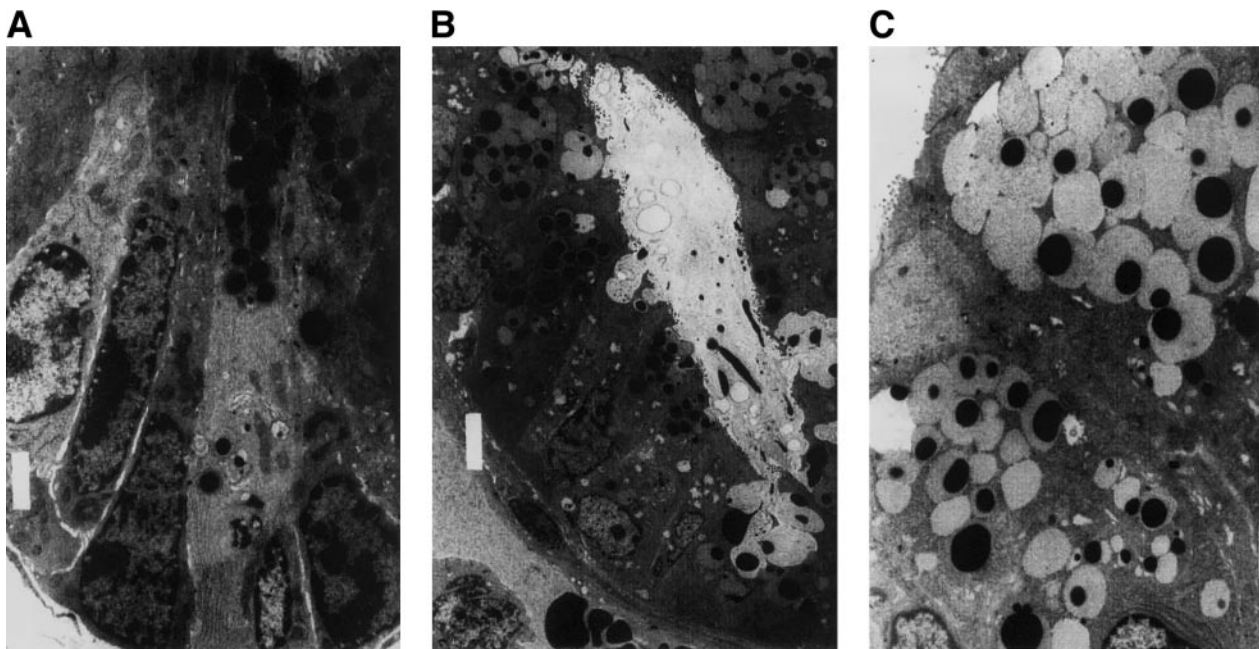


Fig. 2. Transmission electron micrographs of duodenal crypts of control (A) and mice infected with the nematode *Trichinella spiralis* (B). At the base of the control crypt (A), two Paneth cells containing electron-dense granules are present. In the *T. spiralis*-infected duodenum (B), Paneth cells occupy most of the crypt, and some of these cells have discharged their granular contents into the lumen. (C) Two intermediate cells containing granules with electron-dense cores and pale halos are present at the side of the villus of a duodenal section of a *T. spiralis*-infected mouse. Reproduced from ref. [24] with permission from Blackwell Publishing Ltd.

timicrobial protein 18 is expressed by mature enterocytes in the intestine, but its expression is absent in crypts [26, 27]. In vitro studies in intestinal epithelial cell lines have shown that expression of LL-37 is increased by factors that induce differentiation of epithelial cells [26, 27]. Bactericidal/permeability-increasing protein, an antibacterial and endotoxin-neutralizing protein known to be produced by neutrophils, has recently been shown to be expressed by colonic epithelial cells, predominantly in the crypt and surface epithelial cells, with reduced expression in cells in the intermediate zone [28].

Following infection by pathogenic bacteria, epithelial cells secrete chemokines that induce the migration of α -defensin-expressing neutrophils from the circulation into the intestinal mucosa [29–31]. Intestinal infection with parasites and chronic inflammation induces changes in epithelial cell differentiation, which may affect the expression of antimicrobial peptides. Changes in the murine small intestinal epithelium following parasite infection are characterized by an increase in goblet, Paneth, and intermediate cells (Fig. 2) [24, 32–34]. The Paneth and intermediate cells express cryptdins, and an increase in the number of these cells in *T. spiralis*-infected mice appears to be mediated by a unique population of mucosal T cells [24]. Activation of murine T cells by anti-CD3 has also been shown to induce an increase in the number of Paneth cells, which follow apoptosis in crypt cells [35]. In contrast to normal, the colonic mucosa in patients with the chronic inflammatory bowel disease often contains Paneth cells [36], which likely arise from stem cells in the crypt. These metaplastic Paneth cells have also been shown to express HD-5 [17, 37], lysozyme [9, 38–40], and secretory PLA₂ [41]. In the inflamed intestinal mucosa, epithelial expression of members of the defensin family of antimicrobial peptides may also be

induced in mature enterocytes and is discussed below. To date, defensins are the most abundant and best characterized family of antimicrobial peptides in the GI tract.

α - AND β -DEFENSINS

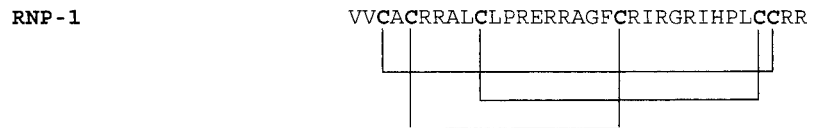
Defensins are small (29–45 amino acid residues), cationic peptides that contain six conserved cysteine residues, which form three disulfide bonds. On the basis of the position of the cysteine residues and the disulfide bond-pairing pattern, defensins may be divided into two main families, the α - and β -defensins. In humans and mice, both families are encoded by a cluster of genes on chromosome 8, suggesting that all defensins evolved from a common ancestral gene [42, 43]. Defensins are synthesized as prepropeptides and are post-translationally processed into mature, active peptides. Structural studies have shown that both families consist of rigid β -sheets stabilized by disulfide bonds and that they have similar three-dimensional structures in solution. The microbicidal activity of defensins is believed to be mediated via the formation of a microbial-selective, membrane-spanning pore, which leads to dissipation of electrochemical gradients and cell lysis [44]. The structure of several α - and β -defensins, illustrating the conserved cysteine residues and disulfide-bonding pattern, is shown in **Figure 3**. A third family of defensins, the θ defensins, has recently been discovered in monkeys [45, 46].

Six α -defensins have been identified in humans; these comprise four neutrophil defensins (HNPI, -2, -3, -4) and two Paneth cell enteric defensins (HD-5, -6). Enteric α -defensins are also found in rodents, but surprisingly, leukocyte α -defensins are not. A number of human and murine β -defensins

α-DEFENSINS

Cysteine residue:	1	2	3	4	56																															
HNP-1	A	C	R	I	P	A	C	I	A	G	E	R	R	Y	G	T	C	I	Y	Q	G	R	L	W	A	F	C	C								
HNP-2	C	Y	C	R	I	P	A	C	I	A	G	E	R	R	Y	G	T	C	I	Y	Q	G	R	L	W	A	F	C	C							
HNP-3	D	C	Y	C	R	I	P	A	C	I	A	G	E	R	R	Y	G	T	C	I	Y	Q	G	R	L	W	A	F	C	C						
HNP-4	Y	C	S	C	R	L	V	F	C	R	R	E	L	R	V	G	N	C	L	I	G	G	V	S	F	T	Y	C	T	R	V					
HD-5	A	T	C	Y	C	R	T	G	R	C	A	T	R	E	S	L	S	G	V	C	E	I	S	G	R	L	Y	R	L	C	C	R				
HD-6	F	T	C	H	C	R	R	-	S	C	Y	S	T	E	Y	S	Y	G	T	C	T	V	M	G	I	N	E	R	F	C	C	L				
Cryptdin-1	L	R	D	L	V	C	Y	C	R	S	R	G	C	K	G	R	E	R	M	N	G	T	C	R	K	G	H	L	L	Y	T	L	C	C	R	
Cryptdin-2	L	R	D	L	V	C	Y	C	R	T	R	G	C	K	R	R	E	R	M	N	G	T	C	R	K	G	H	L	M	Y	T	L	C	C	R	
Cryptdin-3	L	R	D	L	V	C	Y	C	R	K	R	G	C	K	R	R	E	R	M	N	G	T	C	R	K	G	H	L	M	Y	T	L	C	C	R	
Cryptdin-4	G	L	L	C	Y	C	R	K	G	H	C	K	R	G	E	R	V	R	G	T	-	-	G	-	I	R	F	L	Y	C	C	P	R	R		
Cryptdin-5	L	S	K	K	L	I	C	Y	C	R	I	R	G	C	K	R	R	E	R	V	F	G	T	C	R	N	L	F	L	T	F	V	F	C	C	S
Cryptdin-6	L	R	D	L	V	C	Y	C	R	A	R	G	C	K	G	R	E	R	M	N	G	T	C	R	K	G	H	L	L	Y	M	L	C	C	R	

Fig. 3. Structure of known, mature human α [human neutrophil peptide (HNP)-1, -2, -3, -4, HD-5, -6] and HBD1 and -2, along with mouse cryptdins and rabbit neutrophil peptide-1 (RNP-1). Amino acid sequence is shown in single-letter code. Conserved cysteine residues are shown in bold, and numbered and disulfide pairing is shown.



β-DEFENSINS

Cysteine residue:	1	2	3	4	56																																		
HBD-1	D	H	N	C	V	S	S	G	G	Q	C	L	S	A	C	P	I	F	T	K	I	Q	G	T	C	Y	R	G	K	A	C	K	C						
HBD-2	G	I	G	D	P	V	T	C	L	K	S	G	A	I	C	H	P	V	F	C	P	R	R	Y	K	I	G	T	C	G	L	P	G	T	K	C	K	K	P

(MBDs) have been identified in various epithelial cells, including those of the GI tract, and many more probably exist [47–49]. Epithelial β-defensin expression has also been identified in a wide variety of other animals [50].

EXPRESSION OF α-DEFENSINS IN THE GI TRACT

Enteric α-defensins were the first defensins to be identified in cells other than leukocytes. The first of these was discovered in Paneth cells of the mouse small intestine and was termed cryptdin (“crypt defensin”) [51]. Six of the Paneth cell-derived cryptdins have been characterized and studied in detail [52, 53]. They have extended N-termini in comparison with human neutrophil α-defensins, and there is some evidence that the N terminus is important in determining antimicrobial activity [54]. Cryptdins have also been identified in rat small intestinal Paneth cells, but the peptides remain to be characterized [55, 56].

In marked contrast to the situation in the mouse, only two enteric α-defensins (HD-5 and -6) have been identified in humans [15]. These, like cryptdins, are predominantly expressed in Paneth cells of the small intestine, and HD-5 has recently been isolated from ileal tissue and characterized [17–19]. The primary structures of cryptdins, HD-5 and HD-6, are

shown in Figure 3. Recent studies have shown that in addition to Paneth cells, a further type of small intestinal epithelial cell in mice and humans also expresses enteric α-defensins [17, 24]. This cell type has characteristics of goblet and Paneth cells and has been variously termed the granular-mucous or intermediate cell [7]. The function of these rare cells is unknown, but their expression of defensins suggests that they may be involved in intestinal mucosal defense.

Enteric α-defensins exhibit a broad-spectrum antimicrobial activity. The antimicrobial potential of murine cryptdins has been extensively studied. Cryptdins are active against a defensin-sensitive *phoP* mutant of *Salmonella typhimurium* [57], *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Giardia lamblia* [13, 14, 52, 53, 58]. Individual cryptdins exhibit antimicrobial activity of varying range and potency. Thus, cryptdin 4 is highly active against *E. coli*, and cryptdin 2 has limited activity against *E. coli* but is active against *G. lamblia* [53]. In addition, differential expression of mouse cryptdin genes within the small intestine has been observed. The highly potent cryptdin 4 is not expressed in the duodenum but reaches maximal levels of expression in the distal ileum [59, 60]. This may have a functional implication, as maximal levels of this peptide are expressed in an area of the small intestine that is in close proximity to the colon, whose lumen contains a large population of resident bacteria.

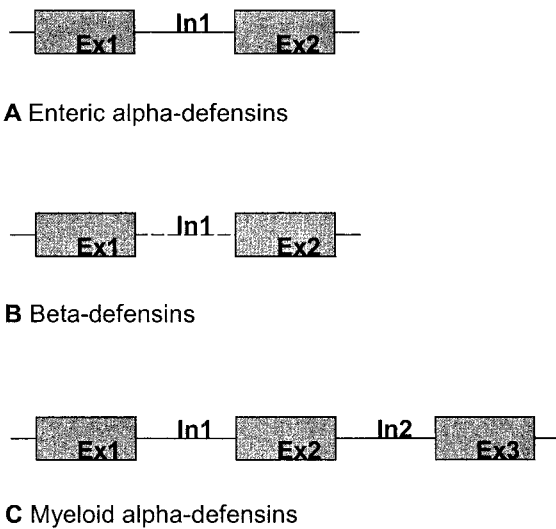


Fig. 4. Defensin gene structure. Enteric α -defensins and enteric β -defensins (EBDs) have similar gene structure with two exons (Ex1 and Ex2). Exon 1 encodes the 5'-untranslated region, signal sequence, and propeptide, and exon 2 encodes the mature peptide. β -Defensin genes vary further according to whether they are inducible (e.g., HBD2), in which case the intron (In1) is ~ 2 kb or constitutive (e.g., HBD1) where the intron is ~ 10 kb. The myeloid α -defensins have three exon genes: Exon 1 encodes the 5'-untranslated region; exon 2, the signal sequence and propeptide; and exon 3, the mature peptide.

REGULATION OF α -DEFENSIN EXPRESSION IN THE GI TRACT

Enteric α -defensins are encoded by genes that consist of two exons (**Fig. 4**): Exon 1 encodes the 5'-untranslated region, signal sequence, and propeptide, and exon 2 encodes the mature peptide [53, 61, 62]. In contrast, myeloid α -defensins have three exons: Exon 1 encodes the 5'-untranslated region; exon 2, the signal sequence; and exon 3, the mature peptide. Enteric α -defensins are found in Paneth cells in fetal intestine [61], and they are also present in Paneth cells in germ-free mice [63], suggesting that their expression is truly constitutive and that bacterial stimuli are not required for their production. Up-regulation of HD-5 mRNA has been observed in the Paneth cells of newborn infants with necrotizing enterocolitis [64]. HD-5 is also expressed in epithelial cells of the female genital tract and up-regulation of HD-5 mRNA, and peptide has been observed in inflamed fallopian tube [65]. Induction of mRNA expression of a rat cryptdin gene can be induced by haemor-

rhagic shock, a condition that may result in bacterial translocation from the intestinal lumen into the systemic circulation [56]. It thus appears that enteric α -defensin gene expression may be inducible under certain circumstances. The 5'-flanking region of the HD-5 gene contains consensus binding sites for a nuclear transcription factor, nuclear factor interleukin-6 (NF-IL-6), which may provide a mechanism whereby this up-regulation could occur in response to inflammatory stimuli [61].

Recent studies have characterized the mechanisms by which precursor forms of Paneth cell defensins are processed into mature, active peptides, and there are important differences between mice and humans. Murine procryptidins are processed to active cryptdin peptides within the Paneth cell granules by the coexpressed matrix metalloproteinase enzyme matrilysin (MMP7) [66–68]. There is more than one cleavage site for MMP7 in the precursor segment of cryptidins, adding another level of complexity to the processing of this family of peptides [63, 67]. However, the in vivo importance of cryptdin processing has been shown by the greater susceptibility of mice lacking MMP7 to lower doses of *S. typhimurium* [66]. Processing of procryptidins does not appear to be affected by the microflora, as similar propieces have been found in germ-free and colonized mice [63]. Prosegment and mature cryptidins have been demonstrated in Paneth cell granules, and analyses suggest that the majority of the procryptidins in the granules has been activated by MMP7 [67]. Degranulation of Paneth cells therefore leads to the release of mature cryptidins, which have been reported to account for 70% of bactericidal peptide activity released by the cells [69]. Gram-negative bacteria, Gram-positive bacteria, lipopolysaccharide (LPS), lipoteichoic acid, lipid A, and muramyl dipeptide, but not live fungi or protozoa, elicited murine Paneth cell secretion [69]. Ultrastructural studies have also shown degranulation of the increased number of murine small intestinal Paneth cells in vivo in response to infection with the nematode *T. spiralis* (Fig. 2) [24].

In contrast to mice, human Paneth cell granules contain only the pro-form of HD-5 (amino acids 20–94), and they do not contain matrilysin [17, 18]. In vitro, pro-HD-5 can be processed to the mature form (amino acids 63–94) by trypsin, which together with $\alpha 1$ -antitrypsin and pancreatic secretory trypsin inhibitor (Kazal-type trypsin inhibitor), are expressed in Paneth cells [19, 70, 71]. Analyses of two luminal forms of HD-5 showed the cleavage sites to be COOH-terminal to an arginine residue (**Fig. 5**), and it has therefore been proposed

Signal (amino acids 1-19)

1 19
MRTIAILAAILLVALQAQA

Prosegment (amino acids 20-62)

20 23 29 36 56 62
ESLQERADEATTQKQSGEDNQDLAISFAGNGLSALRTSGSQAR

Mature peptide

63 94
ATCYCRTGRCATRESLSGVCEISGRLYRLCCR

Fig. 5. Predicted amino acid sequence of HD-5, based on cDNA sequence [15]. HD-5 peptide forms isolated from human ileal tissue samples: amino acids 20–94 (predominant), 23–94, and 29–94 [17–19]. Peptide forms isolated from ileal neobladder urine: 36–94, 56–94, and 63–94 [18]. HD-5 form secreted by isolated terminal ileal crypts: 36–94 [17]. Forms of HD-5 isolated from intestinal lumen: 63–94 (predominant) and 56–94 [19].

that following secretion by Paneth cells, enzymatically active trypsin processes pro-HD-5 to the mature form in vivo [19]. In addition to the mature form present in the normal small intestinal lumen [19], truncated forms of pro-HD-5 have been identified in ileal neobladder urine [18, 19]. One truncated form of pro-HD-5 (amino acids 36–94; Fig. 5) was the predominant form present in pooled, stimulated (with carbamyl choline or LPS) secretions of terminal ileal crypts obtained from five different individuals [17]. Thus, it is possible that alternative mechanisms for the processing of pro-HD-5 to the mature form exist.

In vitro, pro-HD-5 is active against *L. monocytogenes* but not *S. typhimurium*, against which mature HD-5 is active [19]. Recombinant HD-5 is active against *E. coli*, *L. monocytogenes*, *phoP* mutant of *S. typhimurium*, wild-type *S. typhimurium*, *S. aureus*, and *Candida albicans* [19, 72]. Evidence for an important in vivo role for HD-5 was provided in a recent study in which transgenic mice expressing HD-5 in small intestinal Paneth cells were protected against oral but not intraperitoneal challenge with *S. typhimurium* [73].

Following the intracellular processing of their precursor forms, human neutrophil defensins are also stored in azurophil granules as fully processed, active peptides [74, 75], which mediate their antimicrobial function in the phagolysosome. It appears that neutrophil defensins may also be expressed in intestinal epithelial cells in certain conditions. In a recent study, expression of HNP1–3 was observed in epithelial cells of the ileum and colon in cases of active inflammatory bowel disease but not in normal intestinal tissue [40]. Whether this reflects induction of gene expression or uptake by epithelial cells of peptides released by neutrophils in the vicinity remains to be determined.

Crohn's disease and ulcerative colitis are idiopathic chronic inflammatory diseases of the GI tract. In both of these conditions, expression of HD-5 (and HD-6) occurs in the colonic epithelium [17, 37, 76]. The HD-5-expressing cells are metaplastic Paneth cells, which also express lysozyme [39, 40] and secretory PLA₂ [41] and are found in other inflammatory conditions of the colon, such as diverticulitis [77]. It can be speculated that the expression of α -defensins and other antimicrobial proteins in metaplastic Paneth cells in inflammatory conditions of the colon may assist in the killing of luminal microbes to prevent invasion across the damaged mucosal surface.

EXPRESSION OF β -DEFENSINS IN THE INTESTINE

The β -defensins were discovered more recently than the α -defensins; the first tracheal, antimicrobial peptide was identified in bovine airway epithelium [78]. Subsequently, β -defensin expression has been observed in myeloid cells of cattle and poultry and at multiple epithelial surfaces in a wide variety of animals, including humans [50]. Compared with α -defensins, a wider variety of β -defensin peptides appears to exist in humans and animals.

HBD1 is expressed in epithelial cells at a variety of mucosal surfaces, including several regions of the GI tract, namely the oral mucosa, salivary gland, stomach, small intestine, colon, liver, and pancreas [25, 79–84]. HBD2 was originally identified in psoriatic keratinocytes [85], but it too is present in epithelial cells at multiple mucosal surfaces including that of the GI tract. HBD2 has been shown to be expressed in gingival epithelial cells, stomach, small intestine, colon, and pancreas [25, 83, 84, 86, 87]. In contrast to HBD1, HBD2 is present at very low levels in normal intestinal tissues, and in inflamed or infected tissue, such as cases of ulcerative colitis or *H. pylori*-associated gastritis, its expression is up-regulated (see below). HBD1 and HBD2 have been detected in airway surface fluid and saliva and are probably secreted by epithelial cells to operate at the mucosal surface [83, 86]. In in vitro testing of native and recombinant peptides, HBD1 and HBD2 are active against Gram-negative bacteria including *E. coli* and *Pseudomonas aeruginosa*. They have limited activity against *S. aureus* and other Gram-positive bacteria, but HBD2 is active against *H. pylori* [88].

HBD3 has been identified recently and in addition to skin and tonsils, is expressed in the oral cavity and esophagus [89–92]. HBD3 has potent antibacterial activity against Gram-positive bacteria including *S. aureus*. HBD4 has recently been reported to be expressed in the gastric antrum and testis [93]. Synthetic HBD4 has broad-spectrum antimicrobial activity against a variety of bacteria, including *Staphylococci*, *P. aeruginosa*, and also yeasts.

In common with the HBDs, MBDs are expressed in a wide variety of mucosal epithelial cells, including the GI tract. MBD1 is a homologue of HBD1 and is also constitutively expressed in a variety of mucosal epithelial cells. In the GI tract, it is expressed in the tongue, esophagus, and liver [94]. MBD3 appears to be a homologue of HBD2, and its expression is induced in the small intestine and liver in response to infection [95]. MBD3 is active against *P. aeruginosa* and *E. coli*. MBD4 is expressed in the tongue, esophagus, and trachea but surprisingly, no other tissues [96]. MBD6 has recently been identified and is expressed in the esophagus, trachea, and skeletal muscle and is active against *E. coli* [97].

β -Defensins are also expressed in the GI tract of a variety of other animals, and in common with HBDs and MBDs, constitutive and inducible patterns of expression are found. Lingual antimicrobial peptide (LAP) is expressed in the tongue and throughout the GI tract of cattle [98, 99]. Its expression is enhanced in chronic inflammatory lesions of the tongue and also in the ileum in cases of Johne's disease. The latter condition is caused by infection with *Mycobacterium paratuberculosis*, and it is interesting that it has histological features in common with Crohn's disease in humans [100]. EBD, with predominant expression in the intestine, has also been identified in the cow [101]. EBD is expressed in crypt epithelial cells of the small intestine and colon, and its expression is up-regulated tenfold in the intestines of calves infected experimentally with the parasite *Cryptosporidium parvum*, implying an important role in intestinal mucosal defense. β -Defensins are also found in intestinal epithelial cells of sheep [102], goats [103], and pigs [104].

REGULATION OF β -DEFENSIN EXPRESSION IN THE GI TRACT

Preproteins of β -defensins are encoded by two exon genes in a similar manner to enteric α -defensins (Fig. 4). The precursor product of the β -defensin gene is processed to a mature peptide of 36–47 amino acids by a mechanism that remains to be characterized [105]. Expression of β -defensins in the GI tract may be constitutive (e.g., HBD1) or as appears to be more commonly the case, inducible by infectious and inflammatory stimuli (e.g., HBD2). Several studies have shown that β -defensin expression is induced in the GI tract in inflammatory conditions such as naturally occurring grazing lesions of the tongue (LAP [98]), inflammatory bowel disease (HBD2 [25, 37]), and *H. pylori*-induced gastritis (HBD2 [84]). As outlined above, other studies have demonstrated induction of β -defensins in the GI tract in response to infections.

The mechanisms by which β -defensin genes may be regulated have been extensively studied in vitro. Most inducible β -defensin genes contain recognition sites for nuclear transcription factors such as NF- κ B and NF-IL-6. Induction of the genes may be brought about by a variety of stimuli including bacteria, LPS, and inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor α [25, 84, 89, 93, 106, 107]. In some cases, LPS induction of the genes is achieved via a CD14-mediated signal transduction pathway [108]. One recent study has reported that a *Salmonella enteritidis* flagella protein induces HBD2 in colonic epithelial cells via a NF- κ B-mediated, calcium-dependent pathway [109].

BIOLOGICAL RELEVANCE OF THE ANTIMICROBIAL FUNCTION OF DEFENSINS

The intestinal tract has a huge resident microbial flora that confers some beneficial effects on the host. A complex and poorly understood relationship exists between the host and this bacterial population. The widespread expression of defensins in epithelial cells of the GI tract suggests that they play an important role in the maintenance of a stable microbial population in the intestine. This includes on the one hand preventing invasion of host tissues by luminal flora and ingested pathogenic bacteria and on the other, maintaining relative sterility in certain areas such as the small intestine. Complex regulation of defensin gene expression may play a role in achieving this balance.

Potent antimicrobial defensin peptides are expressed in tissue-specific and constitutive and inducible ways in the GI tracts of humans and animals. The proposed role for Paneth cells in secreting enteric α -defensins and other antimicrobial entities to maintain sterility of the small intestine is particularly attractive. However, direct evidence for biological effect of epithelial defensins has until recently been scarce. In fact one study, in which the entire Paneth cell population was ablated in a transgenic mouse strain, reported that lack of these cells and thus cryptdins had no effect on host-microbial interactions [23].

Recent studies do suggest that defensins and other antimicrobial peptides have important roles in host defense at mucosal epithelia. Gene knockout mice that lack matrilysin and thus synthesize and secrete only inactive cryptdin precursors are more susceptible to infections with lower doses of *S. typhimurium* and are less effective at clearing infections with enteropathogenic *E. coli*, compared with wild-type littermates [66]. The effect of augmentation of epithelial innate defense with antimicrobial peptides has also been studied. Mice expressing the human cathelicidin antimicrobial peptide LL-37 following gene transfer had increased resistance to experimental respiratory and systemic bacterial infections [110]. In a further, recent study, transgenic mice that express HD-5 (in addition to cryptdins) in their Paneth cells were resistant to oral infections with *S. typhimurium* compared with nontransgenic controls, thus providing direct evidence that HD-5 is an effective luminal antimicrobial [73].

Down-regulation of antimicrobial peptide expression could also be a mechanism by which pathogenic bacteria overcome host innate defenses at the mucosal surface. Thus, down-regulation of LL-37 and HBD1 has been reported in mucosal biopsies of patients with *Shigella* infection [111]. A recent study has also shown that murine enteric α -defensin expression in Paneth cells may be down-regulated following oral infection with wild-type *S. typhimurium* but not heat-killed *Salmonella*, mutant strains, and other bacteria [112].

CHEMOTACTIC FUNCTIONS OF ANTIMICROBIAL PEPTIDES

A number of studies have demonstrated the ability of defensins to provide a link between epithelial cell-mediated innate-immune responses and adaptive immunity, by their capacity to also act as chemoattractants for dendritic cells (DCs), monocytes, and T cells [113]. HBD1 and HBD2 have been shown to be chemotactic for immature DCs and memory T cells via interaction with the chemokine receptor CCR6 [114]. Such biological effects are likely to be particularly relevant in the intestinal mucosa, where memory T cells [115] and DCs [116] are prominent. It is interesting that MBD2 has recently been reported to act as an endogenous ligand for Toll-like receptor 4, to induce maturation of DCs [117]. This suggests that β -defensins may be capable of acting as potent immunological adjuvants, but whether human β -defensins will have such activity remains to be demonstrated. LL-37 has also been reported to induce chemotaxis of human peripheral blood neutrophils, monocytes, and T cells [118].

Although their in vivo biological importance in intestinal adaptive-immune responses remains to be determined, it is likely that defensins and LL-37 make a significant contribution to chronic inflammatory responses in the GI tract. This may particularly be the case for inflammatory bowel disease in which there is involvement of host-innate and adaptive-immune responses to resident luminal microorganisms [119] and in which (as outlined above) there is also induction of epithelial expression of defensins.

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