Events at the Host-Microbial Interface of the Gastrointestinal Tract

V. Paneth cell α-defensins in intestinal host defense

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α-DEFENSINS, POTENT WEAPONS IN THE PANETH CELL ARSENAL

Paneth cells reside at the base of the small intestinal crypts of Lieberkühn. These epithelial cells exhibit the ultrastructural hallmarks of active secretory cells, including abundant, large secretory granules. Three decades ago, lysozyme was reported in the granules of Paneth cells, providing the initial evidence for their role in host defense. Subsequently, Paneth cells were found to express other antimicrobials, including type II secretory phospholipase A2 (sPLA2), CRS peptides, angiogenins, and α-defensins (for reviews, see Refs. 3, 13, and 16). Of all of these antimicrobial peptides, studies of Paneth cell α-defensins have provided some of the best insights into the role of these cells in host defense.

Defensins are a group of cationic antimicrobial peptides containing three intramolecular disulfide bonds that function by disrupting the membrane integrity of target microbes (10). They are grouped into categories (α and β) based on the positioning of their cystine linkages. Ouellette and colleagues (21) discovered that α-defensins are expressed in murine Paneth cells [where they are called cryptidins (crypt defensins)]. α-Defensins have subsequently been identified in the Paneth cells of human and other mammals. In addition to their expression in Paneth cells, α-defensins are also expressed by mammalian neutrophils and some other epithelial cells (10). Defensins typically have a broad spectrum of antimicrobial activity that often includes antibacterial, antifungal, and/or antiviral activity (10). Estimates of the quantities of α-defensins stored and secreted by Paneth cells indicate that micromolar levels are achievable in the lumen of mice (1). From studies of isolated crypts, it appears that over one-half of the total antibacterial activity secreted from mouse Paneth cells stems from the α-defensins (1). Subsequent investigations of human Paneth cells indicate that their two α-defensins, human defensin (HD)-5 and HD6, are the most abundant antimicrobial peptides in these cells (Fig. 1). Microbicidal concentrations of these α-defensins can also accumulate in the human intestinal lumen (6).

Several partially overlapping functions have been proposed for α-defensins and the other antimicrobial peptides of Paneth cells. The antimicrobial activity of these peptides likely targets both the endogenous (resident microflora) and exogenous (pathogenic) microbes. Because preserving stem cell viability is vital to maintaining epithelial monolayer integrity, the close proximity of Paneth cells and their antimicrobials appears to be an effective protective arrangement against potential pathogens. By shaping the composition of the endogenous flora, Paneth cell antimicrobials might also contribute indirectly to host defense because endogenous microbes can compete for nutrients with exogenous microbes and also elaborate bacteria-derivants antimicrobials (e.g., bacteriocins). Thus Paneth cell antimicrobials are proposed to protect stem cells and host
Fig. 1. Expression of antimicrobial peptides in human Paneth cells. A: estimate of the relative amounts of the 4 major antimicrobial peptides expressed in human Paneth cells by quantitative real-time PCR. Analysis of mRNA-encoding antimicrobial peptides in the human ileum, with plotted area corresponding to relative fraction of the total copy number. The mRNA copy counts were determined from standard curves using specific primers corresponding to human defensin (HD)5, HD6, sPLA2, and lysozyme (J. Wehkamp, R. W. Feathers, H. Chu, and C. L. Bevins, unpublished data). B: in situ hybridization analysis of HD5 mRNA expression in human ileum. Silver grain overlying Paneth cells (arrow) indicates localization of HD5 mRNA in these cells. Bar equals 40 μm [data from D. E. Jones and C. L. Bevins (8) reproduced with permission].

Regulation of Paneth cell α-defensins

Many epithelial defensins are controlled by transcriptional induction in response to infectious and inflammatory stimuli (10). However, α-defensins are Paneth cells are expressed continually, although levels are low in fetal/neonatal development and may vary in some disease states. Rather than transcriptional induction, granule secretion and proteolytic processing are the mechanisms that appear to regulate expression of Paneth cell α-defensins. In studies of isolated murine crypt preparations, Paneth cell secretion was observed in response to both bacteria and bacterial products (such as muramyl dipeptide, a component of bacterial peptidoglycan) but not by fungal or protozoal stimuli (1). Cholinergic agonists can also stimulate secretion by a mechanism that appears to involve both increased cytosolic Ca2+ (20) and mIKCa1 potassium channels (2). These investigations suggest that control of Paneth cell secretion in vivo may be linked to microbial sensors and cholinergic signaling pathways.

An important step in the expression of active Paneth cell α-defensins is proteolytic processing. In mice, rats, rhesus macaques, and humans, Paneth cell α-defensin propeptides of 90–100 amino acids are formed after removal of a signal sequence. However, there are dramatic differences in the enzymatic processing of these propeptides into mature, active antimicrobials when comparing rodents and primates. In mice, matrix metalloprotease (MMP)-7 (matrilysin), a protease expressed in mouse Paneth cells, is essential for further processing of the cryptdin propeptide to active mature peptides (26). In vitro, MMP-7 processes cryptdin propeptides to active mature peptides at precisely the same cleavage site as identified in vivo (17, 21, 22). Interestingly, one of the procryptdins of C57Black/6 mice contains an amino acid substitution at the MMP-7 processing site, rendering the propeptide resistant to cleavage (22). Thus polymorphic isoforms of α-defensins resulting from mutations at MMP-7 cleavage sites exist between mouse lines for some cryptdins and influence posttranslational processing (22).

Most unexpectedly, MMP-7 is not detected in human Paneth cells, and the processing of Paneth cell α-defensins is quite different from that of mice. In humans, trypsin is the protease responsible for processing of α-defensin propeptides (6). The human α-defensin propeptides are cleaved on the C-side of Arg-62, to produce mature HD5, and on the C-side of Arg-68, to generate HD6 (6). Trypsin is expressed by human Paneth cells and is stored in secretory granules as a propeptide (thezymogen trypsinogen). The propeptide form of HD5 is also stored in these same Paneth cell granules (6). Current hypotheses hold that trypsinogen is activated after secretion and then converts pro-HD5 into mature HD5 in either the crypt or intestinal lumen. The protease responsible for activation of trypsinogen is unknown. Characterization of Paneth cell α-defensins in rhesus macaques supports that trypsin-mediated processing is likely responsible for processing in these primates as well (23). Because proteolytic processing is central to the biology of Paneth cell α-defensins, it will be fascinating to better understand how and why rodents and primates diverged in their mechanisms for achieving this important posttranslational modification.

Paneth cell α-defensins in infectious disease

A genetically engineered knockout of the MMP-7 gene renders mice unable to produce mature defensins, and they are far less effective at clearing orally administered noninvasive Escherichia coli (26). These MMP-7 knockout mice are also more susceptible to virulent Salmonella enterica serovar Typhimurium (26). Although enzymatic activity of MMP-7 directed at substrates other than prodefensins might also contrib-
ute to the observed phenotype, these elegant investigations still provide compelling evidence for the key role of Paneth cell α-defensins in host defense.

Salzman et al. (18) reported more recently that transgenic mice expressing a human α-defensin (HD5) in Paneth cells are less susceptible to orally administered challenges with virulent S. enterica serovar Typhimurium (18). In this model, Paneth cell expression of HD5 was observed in transgenic mice that inherited an HD5 minigene containing its two exons and 1.4 kb of 5′-flanking sequence. The HD5 mRNA levels in the transgenic mice were comparable with expression of the endogenous mouse cryptdin-4. Additionally, HD5 peptide was detected in the distal small intestine, and peptides recovered from the transgenic mice are the same forms detected in the human intestinal lumen (18). After orally administered challenges, the transgenic mice were resistant to titers of virulent S. enterica that were lethal to wild-type controls (Fig. 2A). The protective effect was detectable at 6 h after inoculation. In these two groups of mice, fewer Salmonella bacteria were cultured from the distal small intestine of the transgenic mice (Fig. 2B). As a control experiment, no differences were seen in the transgenic mice and controls when lethal doses of bacteria were administered systemically versus orally (Fig. 2C). These data support an important role for Paneth cell α-defensins in the protection from food and water-borne bacterial pathogens.

Several pathogenic bacteria, including enteric pathogens, have evolved mechanisms aimed to evade the activity of defensins and other cationic antimicrobial peptides (for review, see Ref. 14). For example, S. enterica can resist antimicrobial peptides by reducing the anionic charge of its surface molecule lipopolysaccharide. Recently, Salzman and colleagues reported that oral inoculation of mice with virulent S. enterica serovar Typhimurium decreases the expression of Paneth cell α-defensins and lysozyme (19). No decreases in Paneth cell antimicrobials were observed in experiments using heat-killed Salmonella, mutants lacking either the PhoP regulon (MS7953s) or the pathogenicity island SPI-1 (TK93), or the enteric pathogen Listeria monocytogenes. The experiments with the SPI-1 mutants suggest that the type III secretion system is important for this effect. These bacterial resistance mechanisms, which are emerging as a widely distributed virulence mechanism of bacterial pathogens, may prove to be valuable targets for future therapeutics.

**PANETH CELL α-DEFENSINS IN CROHN’S DISEASE**

Crohn’s disease is a chronic idiopathic inflammatory bowel disease that commonly affects the small intestine and colon. Although disease pathogenesis remains elusive, mounting evidence points to the important role of intestinal bacteria in initiating and perpetuating chronic inflammation in genetically susceptible individuals (15). NOD2, an intracellular peptidoglycan receptor for muramyl dipeptide, was the first susceptibility gene identified for Crohn’s disease. Mutations in NOD2 are likely responsible for the genetic predisposition to disease in approximately one-third of patients with Crohn’s disease (for review, see Ref. 4). NOD2 is expressed in several cell types, including cells of the monocyte/macrophage lineage, where the protein appears to attenuate the Toll-like receptor 2-mediated production of proinflammatory T helper 1 cytokines (24). Therefore, defective function of NOD2 in these cells is thought to enhance the T helper 1-mediated inflammatory response characteristic of Crohn’s disease (24). In the small intestinal mucosa, however, NOD2 is expressed principally in Paneth cells, leading to a proposed central role of Paneth cell α-defensins in Crohn’s disease (5). Two recent studies provide strong support for this idea (9, 25).

In the first study, Wehkamp et al. (25) used quantitative real-time PCR analysis of ileal biopsy specimens and found lower levels of Paneth cell α-defensin mRNA in Crohn’s disease of the ileum, as compared with specimens from controls. Interestingly, the decrease was more pronounced in patients harboring NOD2 mutations, whereas expression of mRNA encoding the proinflammatory cytokines TNF-α and IL-8 were unaffected by NOD2 status. Furthermore, no decrease of Paneth cell α-defensins was seen in ileal biopsies from patients with Crohn’s disease of the colon, where the ileum was not affected (25). Thus impaired α-defensin-mediated antibacterial defense may predispose an individual to Crohn’s disease. In the second study, Kobayashi et al. (9)
report a decrease of Paneth cell α-defensin (cryptdin)-related sequences in NOD2-knockout mice. The NOD2-knockout mice had a significantly impaired response to muramyl dipeptide and several deficits in mucosal immune responses. Compared with wild-type controls, the NOD2-knockout mice were more susceptible to gastric, but not systemic, challenges with the Gram-positive bacterium *L. monocytogenes*. The decreased expression of Paneth cell cryptdin-related sequences in the NOD2-knockout mice was proposed to underlie the increased susceptibility. Together, these two studies seem to solidify a link among NOD2, α-defensins, and Crohn’s disease. Defining this link more precisely will clearly yield insights into Crohn’s disease and should identify potential therapeutic targets.

In conclusion, Paneth cell α-defensins are important mediators of innate host defense in the small intestine. A key role in protection from enteric bacterial pathogens is evident from the study of transgenic and knockout mouse models. Paneth cell α-defensins also likely contribute to host defense by influencing the composition and limiting the numbers of resident commensal microbes. Complementary lines of investigation suggest that impaired α-defensin expression may predispose to Crohn’s disease of the ileum. There is suggestive evidence that Paneth cell α-defensins may also play a role in the pathophysiology of other human diseases, such as necrotizing enterocolitis and the intestinal manifestations of cystic fibrosis (for review, see Ref. 3). Many aspects of Paneth cell α-defensin structure, function, and regulation remain poorly understood and should be fruitful areas of future investigations. Because genetic mutations, developmental immaturity, and concurrent systemic disease likely alter the effective expression of Paneth cell α-defensins, linking these alterations to disease susceptibility should further help clarify the physiological role(s) of these molecules.

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REFERENCES


