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Review

Multi-layered regulation of intestinal antimicrobial defense

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Abstract. The gastrointestinal tract of mammals is heavily colonized with a complex and dynamic microbial community. To cope with this complex microbial challenge, multiple epithelial lineages, such as enterocytes and Paneth cells, elaborate a diverse repertoire of protein antibiotics. The gut antimicrobial arsenal encompasses multiple protein families, including defensins, cathelicidins, and C-type lectins. These antimicrobial peptides and proteins play a key role in protecting the host against pathogen challenge, and likely also function to limit invasion of indigenous microbes. It is becoming increasingly apparent that expression of mucosal antimicrobial defenses is tightly controlled. This occurs at multiple levels, including transcriptional regulation in response to bacterial cues, post-translational proteolytic processing, and bacterial regulation of Paneth cell degranulation. Impaired antimicrobial peptide expression has also been implicated in inflammatory bowel disease, underscoring the essential role of antimicrobial defenses in maintaining intestinal homeostasis.

Keywords. Paneth cell, defensin, cathelicidin, C-type lectin, inflammatory bowel disease.

Microbial challenges at the intestinal mucosal surface

The human intestine is home to approximately 100 trillion indigenous microorganisms, and thus is one of the most densely populated microbial ecosystems on the planet [1, 2]. This microbial community makes a number of essential contributions to human health and development. A key function of the microbiota is to significantly increase host digestive efficiency [2, 3]. Gut bacterial societies are metabolically active, degrading dietary substances that are otherwise indigestible by the host [4]. Bacteria that are indigenous to the gut, such as *Bacteroides thetaiotaomicron*, produce an extensive repertoire of glycosylhydrolases that metabolize plant polysaccharides and thus liberate simple carbohydrates for uptake by the host [2, 5, 6]. In an environment where nutrients are in short supply, natural selection would likely favor such host-microbial interactions, which may explain how these associations evolved. Given the co-evolution of mammals with their microbiota, it is not surprising that these microbial communities have a broad impact on many aspects of host physiology, including immune system development [7], blood vessel development [8], and regulation of fat storage [9].

The mammalian intestinal microbiota is also one of the most complex microbial communities on earth. The development of molecular profiling techniques has provided tools which are allowing the acquisition

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of a comprehensive view of the composition of gut microbial communities. To this end, the National Institutes of Health have recently launched the Human Microbiome Project with the aim of exploring and sequencing the entire bacterial genome inside the human gut. Molecular profiling studies using ribosomal DNA sequencing methods have already revealed that the human colonic microbiota consists of more than 400 distinct bacterial species, with prominent representation of both Gram-positive and Gram-negative bacteria [10]. This complexity is not static, and the membership of the intestinal microbiota can vary widely between individuals [10], and in response to developmental stage and dietary changes [11]. An additional layer of complexity is the continual challenge by microbes that are not members of the indigenous flora but gain entry to the intestinal ecosystem through ingested food and water.

This dense and complex microbial community is separated from the internal intestinal tissues by a single epithelial layer that is only ~20 μm thick, but which encompasses about 200 m^2 of surface area in humans. Gut epithelial surfaces are composed of several distinct cell types, each of which contributes in a unique way to mucosal defense and the maintenance of barrier integrity (Fig. 1A). The enterocyte is the most abundant cell type at both small and large intestinal epithelial surfaces. Enterocyte membranes, as well as the tight junctions that are formed between these cells, form an important impermeable physical barrier to microbial penetration. However, enterocytes also assume a more active role in defending epithelial surfaces by secreting a variety of antimicrobial proteins [12]. Goblet cells, found in both the small and large intestines, secrete large quantities of mucin, which is composed of highly glycosylated proteins that form a protective layer of gel-like mucus over the surface epithelium. Paneth cells constitute an epithelial lineage which is unique to the small intestine. This lineage is located at the base of crypts of Lieberkuhn and produces a large proportion of the small intestinal antimicrobial output.

Intestinal epithelial antimicrobial proteins: diverse weaponry for a complex microbial challenge

The intestinal epithelium is in direct contact with the intestinal microbiota and is thus faced with the unique challenge of coping with enormous microbial numbers as well as a diverse and dynamic microbial community. While these microbes perform essential functions for their hosts, they nevertheless pose the constant threat of invasion due to their sheer numbers and the large surface area of the intestinal epithelium. Excessive bacterial penetration across mucosal surfaces can lead to potentially damaging inflammatory responses or even sepsis.

Epithelial antimicrobial proteins play a key role in allowing epithelial surfaces to cope with these enormous microbial challenges. These natural antibiotics constitute an evolutionarily ancient defense system and are present in virtually all multicellular organisms, including plants, worms, flies, and mammals. The mammalian gut epithelium produces an especially rich repertoire of antimicrobial proteins, reflecting the complexity of the microbial challenges faced by the mucosal surface. This diverse battery of protein antibiotics likely plays a pivotal role in preventing microbial invasion of intestinal surfaces, thus promoting homeostasis between mammalian hosts and their associated complex microbial communities. As each of the major classes of antimicrobial proteins has been well reviewed in detail elsewhere, we will discuss each only briefly.

Enzymatically active antimicrobial proteins

Antimicrobial peptides and proteins generally target essential cell wall structures of microorganisms, making it improbable that microbes will develop resistance. A key group of antimicrobial proteins encompasses enzymes that kill bacteria through enzymatic attack on microbial cell walls. One such enzymatic protein, lysozyme, is present in large concentrations in a number of secretions, including tears and saliva. It is also abundantly produced by Paneth cells and secreted into the luminal environment. Lysozyme is a glycosidase that hydrolyzes the 1,4-β-glycosidic linkages between the N-acetylglucosamine and N-acetylmuramic acid moieties that make up peptidoglycan, an essential constituent of the bacterial cell wall. Lysozyme is more effective against Gram-positive bacteria, whose peptidoglycan is on the outer cell wall surface, and therefore more easily accessible than the peptidoglycan that is present in the periplasmic space of Gram-negative bacteria [13].

Secretory phospholipase A_2 ($sPLA_2$) is a ubiquitous enzyme that kills bacteria by hydrolyzing bacterial membrane phospholipids, thus compromising the integrity of the microbial cells surface [14]. $sPLA_2$ is highly basic, which allows it to penetrate the bacterial cell wall and gain access to the bacterial membrane [15]. Like lysozyme, $sPLA_2$ is produced abundantly by Paneth cells [16] and macrophages [17], and is also found in other secretions such as tears and inflammatory fluids.

Defensins

Another mechanism by which antimicrobial proteins kill bacteria is through membrane disruption. Mem-

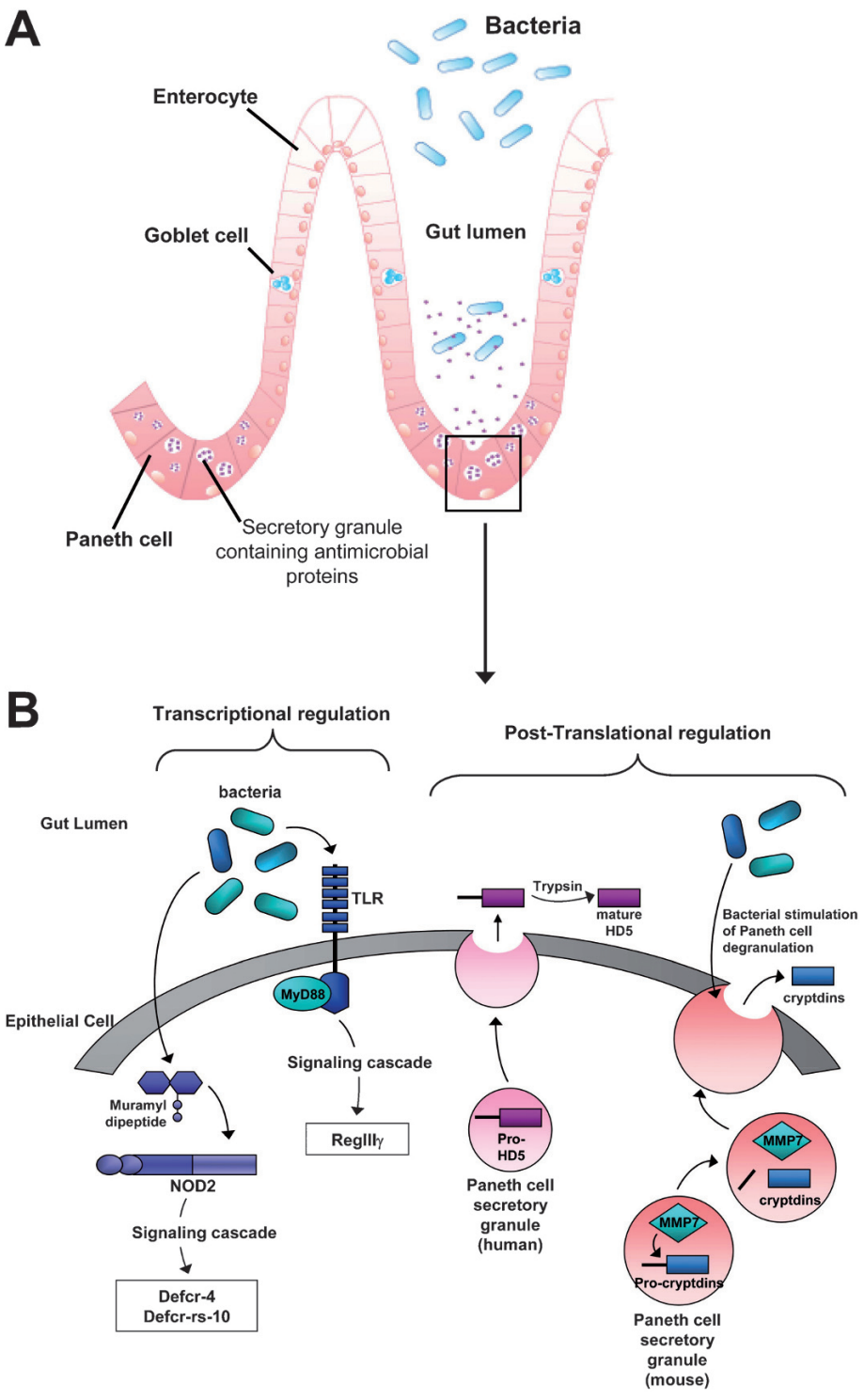

Figure 1. (*A*) Gut epithelia are comprised of several distinct cell lineages, including enterocytes, goblet cells, and Paneth cells. Paneth cells are located at the base of small intestinal crypts and actively secrete a number of antimicrobial proteins in response to bacterial signals. (*B*) Intestinal antimicrobial defenses are subject to multi-layered mechanisms of regulatory control. Expression of key antimicrobial responses is regulated by bacterial signals. α-defensins, including Defcr-4 and Defcr-rs-10, are governed by the pattern recognition receptor NOD2. Expression of RegIIIγ is regulated through MyD88-dependent TLR signaling. Note that it is not yet clear whether activation of antimicrobial gene expression in gut epithelial cells occurs in an epithelial cell-intrinsic manner. In humans, generation of fully functional α-defensins, including human defensin 5 (HD5), requires processing of the N terminus by trypsin following release of Paneth cell granule contents into the gut lumen. Mouse α-defensins are processed inside Paneth cell secretory granules by matrilysin (MMP7). Secretion of Paneth cell granular contents requires bacterial stimulation via an unknown mechanism.

brane disruption leads to breakdown of membrane potential, loss of metabolites and ions, and osmotic lysis [18]. Defensins constitute the major family of membrane-disrupting peptides in mammals, and represent one of the most diverse and highly expressed protein families in the gut. The defensins are small peptides that range from 2 to 6 kDa in size and are expressed in a variety of cells, including epithelial cells, neutrophils, and macrophages. They harbor conserved cysteine residues that form disulfide bridges, creating a conserved three-dimensional structure [13]. On the basis of their disulfide bond arrangements and spacing of the cysteine residues, defensins are classified into three major groups, α, β, and θ [19].

The spectrum of antimicrobial activity varies for each protein, but in general defensins exhibit a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, and in some cases are active against fungi, viruses, and protozoa [19].

A key characteristic of all defensins is that they are highly basic. As a result, they bind to bacterial surfaces via electrostatic interactions with negatively charged phospholipid groups in the bacterial membrane. Once a critical concentration of defensin molecules is bound to the bacterial surface, they form transient pores which promote osmotic lysis of the targeted microorganism [20–22]. The selectivity of defensins for microbial membranes stems in part from the unique positioning of negatively charged phospholipid headgroups in the outer leaflet of bacterial membranes [23]. By contrast, negatively charged phospholipids are not found in the outer leaflet of eukaryotic cell membranes [23], making them less vulnerable to attack by cationic antimicrobial peptides.

The expression of α-defensins in the gastrointestinal tract is highly restricted to Paneth cells, and is lacking from other epithelial lineages [19]. The function of α-defensins *in vivo* was elucidated through the development of a novel animal model in which a human α-defensin 5 (HD-5) minigene was expressed under the control of its own promoter, resulting in Paneth cell-specific expression [24]. Mice expressing HD-5 were remarkably resistant to oral challenge with *Salmonella typhimurium*, suggesting a critical role for α-defensins in protecting against microbial challenge in the gastrointestinal tract *in vivo* [24].

Endogenous mouse α-defensins are termed 'cryptdins.' In addition to cryptdins, mice harbor a diverse family of cryptdin-related sequence (CRS) peptides. CRS peptides have four intramolecular disulfide bridges and further form covalent dimers by an additional intermolecular disulfide bridge [25]. As CRS peptides can form both heterodimers and homodimers, the potential for combinatorial diversity is high. These dimeric peptides exhibit potent antimicrobial activity against both Gram-positive and Gram-negative bacteria [25].

In contrast to α-defensins, β-defensins are expressed in enterocytes of the large and small intestine [26]. There are so far 28 human β-defensins that have been identified in the human genome, 8 of which are known to be expressed [27]. A limited subset of these is expressed in intestinal epithelial cells [28–30]. In addition to their antibacterial activities, β-defensins may also act as chemoattractants for immune cells such as dendritic and T cells [31].

Cathelicidins

Cathelicidins are a second general class of epithelial antimicrobial peptides that kill microorganisms by membrane disruption. They are cationic, α-helical peptides with a conserved 14-kDa N-terminal 'cathelin' (cathepsin L inhibitor)-like domain and a variable C-terminal region. Both humans and mice have only a single cathelicidin gene that encodes the proteins LL-37/hCAP18 and CRAMP (cathelin-related antimicrobial peptide), respectively. Originally discovered in neutrophils [32, 33], cathelicidins are also expressed by epithelial cells of the colon [34], lung [35], skin [36], and urinary tract [37].

Cathelicidins exhibit biological activities that are similar to those of the defensin family. They kill bacteria by first binding to bacterial membranes via electrostatic interactions, followed by membrane insertion and disruption [38]. Both LL-37 and CRAMP exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungi [38]. Like β-defensins, LL-37 has also been shown to have biological functions that are independent of its bactericidal activity. For example, it has been shown to be chemotactic *in vitro* for immune cells, including monocytes, macrophages, and T cells [39]. LL-37 can also induce Th1 cytokine secretion by dendritic cells [40]. This suggests that combined microbicidal and immune modulatory properties may be a common feature of antimicrobial peptides.

RNases and C-type lectins

The molecular mechanisms underlying the antibacterial activity of several other intestinal microbicidal proteins remain a mystery. Angiogenin-4 (Ang-4) is a member of the ribonuclease family and is expressed exclusively by Paneth cells. Ang-4 exhibits broad-spectrum bactericidal activity against both Gram-positive and Gram-negative bacteria [41]. In this way, it is similar to other bactericidal RNases, including the skin protein RNase 7 [42] and eosinophil cationic protein [43]. Although Ang-4 has the ability to hydrolyze RNA, it is still unclear whether this enzymatic activity is related to its bactericidal function. C-type lectins constitute a second class of directly antibacterial proteins whose mechanism of action remains poorly understood. RegIIIγ and its human counterpart, HIP/PAP (hepatocarcinoma-intestine-pancreas/pancreatic-associated protein) are expressed in multiple small intestinal epithelial lineages, including enterocytes and Paneth cells [44, 45]. Both proteins bind to peptidoglycan and mediate direct bacterial killing [44]. In contrast to defensins, cathelicidins, and Ang-4, the bactericidal activity of RegIIIγ is highly selective for Gram-positive bacteria [44]. This is consistent with the fact that peptidoglycan

is accessible for binding on the outer surfaces of Gram-positive bacteria but is buried in the periplasmic space of Gram-negative bacteria. The bactericidal action of RegIIIγ is accompanied by disruption of cell wall integrity [44]. Whether this occurs via enzymatic activity, membrane disruption, or some other mechanism remains to be clarified. Because they are the first examples of directly bactericidal C-type lectins, RegIIIγ and HIP/PAP represent both a novel family of antimicrobial proteins and a novel biological activity for the C-type lectin family. As several members of the Reg family of C-type lectins are expressed in gastrointestinal tissues [46], it seems likely that lectins represent a general mechanism of antibacterial defense at the mucosal surface.

Regulation of intestinal antimicrobial defense

The diverse repertoire of antimicrobial proteins is likely a key factor in allowing intestinal mucosal surfaces to maintain homeostasis with the diverse bacterial populations colonizing the intestinal lumen. In the following sections, we discuss the varied ways in which these antimicrobial responses are regulated. These include transcriptional regulation in response to microbial cues, post-translational proteolytic processing to generate bactericidal mature proteins, and bacterially regulated exocytosis of antimicrobial components.

Regulation of antimicrobial protein expression

Understanding the molecular foundations of intestinal host-microbial relationships is an exceedingly challenging problem due to the complexity of the gut microflora, the complexity of the intestinal mucosal surface, and the lack of good *in vitro* models for studying the complex interplay between host and microbe. Overcoming these challenges has necessitated the development of unique *in vivo* experimental approaches. One of the key tools for studying these interactions is gnotobiotics ('known life'), a technology involving the use of microbiologically sterile ('germ-free') animals. Germ-free animals have provided critical experimental systems for examining which host intestinal functions are strictly genetically encoded and which require interactions with gut microbes for full expression [47]. Such studies have revealed that while a subset of intestinal antimicrobial proteins is expressed independently of the microbiota, others are governed by bacterial signals.

Studies of α-defensin expression in germ-free mice have revealed that the majority of enteric α-defensins are expressed independently of the microbiota [48]. Likewise, lysozyme and $sPLA_2$ are expressed independently of microbial signals [41, 49]. Certain β-defensins, such as human β-defensin-1, are also expressed constitutively, and do not require bacterial or inflammatory signals for their expression [26, 50]. The cathelicidin LL-37 was found to be expressed in human epithelial cells independently of the presence of a microbiota, although it was modestly upregulated by enteroinvasive microorganisms [34]

In contrast, a distinct subset of antimicrobial proteins is expressed under the control of microbial cues. Comparison of germ-free and conventionally raised mice revealed that members of the CRS family of peptides show marked increases under conventionally raised conditions, suggesting that the intestinal microbiota directs their expression [48]. Similarly, members of the human β-defensin family, including HBD-2, require bacterial or pro-inflammatory signals for their expression [26]. Experiments in germ-free mice have revealed that Ang-4 is expressed under the control of the microbiota. Its expression is essentially absent in germ-free mice and is upregulated upon colonization with a conventional microbiota, or by a single intestinal microbe such as *B. thetaiotaomicron* [41]. Likewise, the bactericidal C-type lectin RegIIIγ is expressed under the control of microbial cues from the intestinal microflora [44].

Insight into the host mechanisms that dictate expression of key members of the defensin family was obtained through study of mice deficient in the pattern recognition receptor Nod2. Nod2 is expressed in Paneth cells as well as macrophages [51, 52], and is involved in the recognition of muramyl dipeptide (MDP), a constituent of peptidoglycan (Fig. 1B) [53, 54]. Nod2 recognition of MDP activates signaling cascades that lead to activation of the master pro-inflammatory transcription factor NFκB [55]. Mice that lack Nod2 are susceptible to oral challenge with the gastrointestinal pathogen, *Listeria monocytogenes*; however, they do not show altered susceptibility to systemic challenge [56]. This decreased resistance to oral pathogenic challenge correlates with decreased expression of a subset of α-defensins and defensin-related cryptdins in Nod2-deficient mice. These include defensin-related cryptdin-4 (defcr-4), as well as defcr-related sequence-10 (defcr-rs-10). The lowered expression of a key subset of antimicrobial genes could in part explain the enhanced susceptibility to infection by a gastrointestinal pathogen. However, it is not yet established whether Paneth cell-intrinsic Nod2 regulates antimicrobial peptide expression or whether this expression relies on Nod2 expression in a bone marrow-derived lineage.

Toll-like receptors also regulate the expression of key subsets of antimicrobial proteins in gut epithelial cells.

These membrane-bound pattern recognition receptors are activated by conserved microbial molecular patterns, such as lipopolysaccharide, lipoteichoic acid, or flagellin [55], and activate signaling cascades that result in NFκB activation. For example, HBD-2 is normally low in cultured epithelial cells and can be induced in response to bacterial infection or pro-inflammatory stimuli in a TLR-2 dependent manner [28, 29, 57]. Furthermore, studies in mice lacking MyD88, an adaptor molecule that is common to several distinct Toll-like receptors, indicate that members of the Reg family of antibacterial C-type lectins are also expressed under the control of Toll-like receptors *in vivo* [58, 59].

Together, these findings reveal that different subsets of antimicrobial proteins are regulated via distinct mechanisms. A constitutive chemical barrier is established at the mucosal surface by the subset of antimicrobial proteins that are expressed independently of bacterial signals. The regulated expression of other proteins through TLR and Nod2 activation suggests that a subset of antimicrobial responses is precisely titrated in response to microbial numbers and/or the composition of the intestinal microbial community. Such regulation may thus allow antimicrobial responses to be precisely targeted against specific microbial threats. Furthermore, strict regulation of certain antimicrobial responses by bacterial signals could ensure against overproduction of antimicrobial proteins that could interfere with intestinal ecology and thus undermine the beneficial contributions of the microbiota.

Regulation of antimicrobial activity by proteolytic processing

A common feature of bactericidal peptides that kill through membrane disruption mechanisms is that they are expressed as pro-peptides that must be activated by proteolytic processing. This post-translational regulatory mechanism allows the host a high degree of control over the expression of microbicidal activity. This may reflect a need to protect host cells from cellular toxicity due to high concentrations of proteins that function to disrupt membranes.

Both mouse and human intestinal α-defensins are stored as inactive pro-peptides in the granules of Paneth cells. In the mouse, cryptdins undergo processing mediated by matrilysin (also known as matrix metalloproteinase-7, or MMP-7) [60–62]. This metalloproteinase removes the N-terminal acidic cryptdin pro-regions, yielding mature, fully bactericidal peptides [60–62]. MMP-7 co-localizes with cryptdins within Paneth cell secretory granules, and processing occurs within the granules prior to discharge into the gut lumen (Fig. 1B) [62]. It is proposed that the acidic charge of the N-terminal pro-segment neutralizes the antimicrobial activity of cationic cryptdins, and thus removal of the pro-cryptdin N-terminal peptide results in greatly enhanced bactericidal activity [61]. *In vivo* studies of MMP-7-deficient mice showed deficient cryptdin processing and a concomitant increased susceptibility to oral challenge with the intestinal pathogen *S. typhimurium*, thus underscoring the critical role of MMP-7 in regulating immunity to enteric bacteria.

In contrast to mice, humans do not express matrilysin in their Paneth cells. The mystery of how human α-defensin processing occurs was solved when it was shown that trypsin, a well-studied digestive enzyme, cleaves human α-defensins to their mature forms. In contrast to mice, human α-defensins such as HD-5 are stored within Paneth cell secretory granules as inactive pro-forms and are processed only after secretion [63, 64]. A specific set of trypsin isoforms is also stored as inactive precursors by Paneth cells and then activated following degranulation into the gut lumen (Fig. 1B) [63]. Trypsin-mediated processing of HD-5, like matrilysin processing of mouse cryptdins, yields peptides with greatly enhanced bactericidal activity against enteric bacteria [63].

Other intestinal antimicrobial proteins also exhibit a requirement for proteolytic processing to generate their fully functional bactericidal forms. Like α-defensins, cathelicidins are synthesized as pre-pro-peptides. In human neutrophils, the anionic pro-sequence is removed by protease 3 following degranulation [65]. However, it remains unclear how processing is carried out for cathelicidins that are expressed by intestinal epithelial cells. Similarly, β-defensins are expressed as pre-pro-peptides, but the mechanism by which they are processed to their mature forms remains to be established [66]. Endogenous RegIIIγ is present in the small intestine both as a full-length form and as a more rapidly migrating form [44]. Although the processing site remains to be determined, it is interesting that all members of the Reg lectin family, regardless of species, harbor a conserved predicted trypsin site near the N terminus. This suggests that this family of antimicrobial proteins may also be regulated by proteolytic processing. As proteolytic processing is generally a hallmark of membrane disruptors, a requirement for processing to generate fully functional bactericidal proteins could provide a clue to the mechanism underlying Reg bactericidal activity.

Regulation of antimicrobial protein secretion

An additional level at which antimicrobial defenses are regulated is through microbe-induced release of Paneth cell secretory granules. As discussed above,

Paneth cells are the major producers of antimicrobial proteins in the small intestine. These cells harbor numerous cytoplasmic secretory granules that contain a number of different antimicrobial peptides and proteins, including α-defensins, lysozyme, $sPLA_2$, Ang4, and RegIIIγ. Paneth cell granules are secreted apically and the contents are discharged into the gut lumen. To delineate how Paneth cell secretion is regulated, Ayabe and colleagues developed a clever *ex vivo* system in which isolated intact crypts were exposed to various microbial stimuli [67]. Addition of live bacteria or bacterial products (e.g., lipopolysaccharide, lipoteichoic acid) elicited crypt degranulation and release of antimicrobial contents (Fig. 1B). In contrast, live fungi and protozoa did not result in degranulation, pointing to the idea that Paneth cells may specifically function in antibacterial defense. These findings thus suggest that Paneth cells may precisely regulate discharge of antibacterial substances by sensing the degree of bacterial threat to mucosal integrity.

Antimicrobial proteins in inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic inflammation of the intestine that is highly prevalent in North America and northern Europe. While the root causes of IBD remain poorly understood, it has become increasingly apparent that dysregulated interactions between intestinal microbes and the host immune system are involved in initiating and perpetuating IBD [68].

Crohn's disease (CD) is a form of IBD that is characterized by inflammation in the distal small intestine and/or colon. Genetic linkage studies in human families predisposed to CD of the ileum (distal small intestine) have disclosed that Nod2 mutations are frequently associated with the disease [69]. The naturally occurring human mutations were found to lead to a loss-of-function phenotype [70]. However, *in vivo* studies of mice harboring a knock-in human Nod2 mutant have clouded the picture by suggesting a gain-of-function phenotype characterized by enhanced intestinal pro-inflammatory responses [71].

The fact that Nod2 governs expression of a key subset of α-defensins [56] suggested the possibility that lowered α-defensin expression could be associated with CD. Analysis of α-defensin expression in human CD patients exhibiting ileal pathology indeed showed reduced HD5 and HD6 expression [72]. The correlation with disease was even more pronounced for patients harboring Nod2 mutations [73]. These findings suggest a model in which reduced α-defensin production as a result of loss-of-function Nod2 mutation leads to reduced intestinal barrier protection. This in turn would likely result in increased bacterial adherence to and perhaps penetration of the mucosal barrier, which could initiate and perpetuate the inflammation that characterizes CD.

Conclusions and perspectives

The mammalian intestinal epithelium is faced with a complex and dynamic microbial challenge that is unique among tissues. As a result, gut epithelia have evolved a diverse array of antimicrobial strategies to maintain homeostasis with the enteric microbiota and to prevent pathogen encroachment. Ongoing studies in a number of experimental systems are revealing that antimicrobial responses at the mucosal interface are tightly regulated at both transcriptional and post-translational levels. Transcription of key antimicrobial responses occurs in response to bacterial signals and can be governed by either Nod2 or MyD88-dependent TLR signaling. Future studies will be required to determine the precise nature of the bacterial signals required to elicit antimicrobial responses through each of these pathways. In addition to transcriptional regulation, the bactericidal activity of several antimicrobial protein families is regulated by proteolytic processing. A final layer of regulatory control is conferred by the requirement for bacterial signals to stimulate secretory granule exocytosis. These multilayered mechanisms of regulatory control are likely important in allowing the intestinal mucosal surface to precisely titrate innate immune responses to match the degree and type of microbial threat. This would allow appropriate responses to be mounted when intestinal barrier integrity is threatened, while avoiding perturbation of the healthy intestinal microbiota.

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