



Review Article

Interplay between gut microbiota and antimicrobial peptides

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ABSTRACT

The gut microbiota is comprised of a diverse array of microorganisms that interact with immune system and exert crucial roles for the health. Changes in the gut microbiota composition and functionality are associated with multiple diseases. As such, mobilizing a rapid and appropriate antimicrobial response depending on the nature of each stimulus is crucial for maintaining the balance between homeostasis and inflammation in the gut. Major players in this scenario are antimicrobial peptides (AMP), which belong to an ancient defense system found in all organisms and participate in a preservative co-evolution with a complex microbiome. Particularly increasing interactions between AMP and microbiota have been found in the gut. Here, we focus on the mechanisms by which AMP help to maintain a balanced microbiota and advancing our understanding of the circumstances of such balanced interactions between gut microbiota and host AMP. This review aims to provide a comprehensive overview on the interplay of diverse antimicrobial responses with enteric pathogens and the gut microbiota, which should have therapeutic implications for different intestinal disorders.

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1. Introduction

The mammalian gastrointestinal microbiome is composed of dynamic microbial communities that have increasingly been found to serve as key regulators of health and disease (Jandhyala et al., 2015; Luthold et al., 2017). At the genetic level, these commensal microbes outnumber host genes by 25-fold (Qin et al., 2010). The microbiome is composed of microbes that have evolved to function in mutualistic relationships with their hosts (Ayres, 2016; Bai et al., 2019), with a range of different viruses, bacteria, and fungi composing the overall gastrointestinal microbiome (Chen et al., 2018; Foca et al., 2015). Through the production of small-molecule metabolites that benefit their hosts, these microbes have also been shown to shape mammalian immunity (Moossavi

and Bishehsari, 2019). When normal microbial homeostasis is disrupted, a range of serious metabolic disorders and diseases such as cardiovascular disease and inflammatory bowel disease (IBD) can develop (Mahnic et al., 2020; Ray and Dittel, 2015; Salguero et al., 2019), indicating that microbial dysbiosis can compromise normal immunological functionality. These microbes also serve as important regulators of mucosal barrier repair in the intestines (Okumura and Takeda, 2017, 2018), further supporting anti-pathogen defenses within their hosts (Guan et al., 2019; Ubeda et al., 2017).

Antimicrobial peptides (AMP) have been identified as key regulators of interactions between commensal microbes and host tissues. AMP are a diverse array of peptides that exert a range of antimicrobial activities via sequestering key growth nutrients, permeabilizing bacterial membranes, and other related mechanisms. Antimicrobial compound production has generally been studied in the context of competition within and between bacterial species (Mitri and Foster, 2013), but there is also evidence that AMP and other antimicrobial compounds may serve other purposes such as facilitating communication or cooperation between different bacterial strains in particular contexts (Abrudan et al., 2015). Antimicrobial activities can facilitate biodiversity, which is evidenced by the variable ecological fitness of specific sensitive and resistant microbes in a given dynamic environment and reflected

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by the different metabolic inputs necessary to produce these antimicrobial compounds (Guo et al., 2020; Ladram and Nicolas, 2016).

Paneth cells and enterocytes within the gastrointestinal tract serve as the primary sources of AMP, with Paneth cells in particular being important secretory cells localized within intestinal crypts proximal to intestinal stem cells. Mucin, which creates a physical barrier between intestinal epithelial cells and gastrointestinal microbes, is also produced by goblet cells. The integrity of the intestinal epithelium is essential for the maintenance of normal gastrointestinal homeostasis, and perturbations of this integrity can have serious complications. Impaired AMP responses, for example, can increase host susceptibility to gastrointestinal infection with pathogens such as *Yersinia pseudotuberculosis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Muniz et al., 2012; Mwangi et al., 2019). IBD patients have been found to exhibit reduced gastrointestinal AMP production, potentially leading to dysbiosis within this tissue (Yao et al., 2017). Tightly regulated control of AMP secretion is therefore essential in order to prevent such dysbiosis induced by pathogens or commensal microbes. Bacterially-derived peptides can also suppress the growth of certain microbes, thereby directly constraining pathogen infections.

To better understand the gastrointestinal microbiome, in the present review we discuss recent findings pertaining to interactions between commensal microbes and AMP. In particular, we pay specific attention to the ability of host-derived AMP to modulate the composition of the gut microbiome.

2. The composition of the gastrointestinal microbiome

About 100 trillion mutualistic microbes are found within the human gastrointestinal tract wherein they serve as important regulators of host metabolism (Illiano et al., 2020). The makeup of this gastrointestinal microbiome varies throughout the gastrointestinal tract, and nutrient availability is a primary determinant of the growth ability of these microbes in this tissue (Kamada et al., 2013). The gastrointestinal microbiome is dynamic. It is initially colonized immediately following birth and varies substantially (Guo et al., 2020; Milani et al., 2017), eventually stabilizes within 1 to 3 years (Lozupone et al., 2012; Lv et al., 2018). Thereafter, the microbiome typically remains relatively stable, recovering from acute insults such as antibiotic exposure in many cases (Donaldson et al., 2016). Prolonged changes, as may occur in the context of dietary changes, however, can result in long-lived structural changes in the composition of the gastrointestinal microbiome (Sekirov et al., 2010; Tang et al., 2020).

The specific composition of the microbiome varies significantly between individuals and can be difficult to directly assess, and as such, many recent studies have instead focused upon metagenomics approaches aimed at characterizing these microbes, given that many of them can not currently be cultured or evaluated in vitro (Sekirov et al., 2010). Dominant bacterial phyla in the human gut microbiome include Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia species (Donaldson et al., 2016). These bacteria, however, are not uniformly distributed throughout the gastrointestinal tract. The local pH, oxygen level, concentration of host- and bacteria-derived antimicrobial compound, and bile acid level all contribute to heterogenous bacterial distributions in tissues. Facultative anaerobes including Firmicutes (Lactobacillales) and Proteobacteria (Enterobacteriales) are typically found within the small intestine (Wang et al., 2020; Wang et al., 2018). Microbial density is generally lower in the proximal small intestine due to the harsher environmental conditions in this site, whereas microbes flourish increasingly well in the distal small intestine proximal to the ileum. Slower gastrointestinal transit

within the colon leads to the availability of high nutrient levels, which in turn support the growth of fermentative microbes capable of breaking down dietary fiber and other complex nutrients. Microbial density is thus highest within the colon, which contains high levels of luminal Bacteroidetes (Bacteroidaceae, Prevotellaceae, and Rikenellaceae) species as well as high levels of Firmicutes (Lachnospiraceae and Ruminococcaceae) (Donaldson et al., 2016). In adult pigs, the colon has been shown to contain 35% Firmicutes, 21% Bacteroidetes, 3% Proteobacteria, and 2% Spirochetes species (Lamendella et al., 2011; Looft et al., 2012). Proteobacteria, in contrast, make up approximately 70% of ileal and jejunal microbial species, and Firmicutes make up approximately 20% of microbes in these tissue niches. This trend is reversed in the colon and cecum, wherein Firmicutes account for over 75% of bacteria, and Proteobacteria make up just 13% of the microbiome (Kim et al., 2012; Zhao et al., 2015). The high density of commensal microbes within the gastrointestinal tract is believed to limit the ability of many pathogens to colonize and damage the host gastrointestinal tract.

Understanding the composition of the gastrointestinal microbiome is important, given that dysbiosis can promote obesity or other serious disorders (Villanueva-Millan et al., 2015) such as IBD (Ostaff et al., 2013). Similarly, skin microbiome dysbiosis can promote the development of psoriasis and atopic dermatitis (Hourigan et al., 2018; Zou et al., 2019). Eliminating or disrupting the normal microbiome can seriously adversely impact host health, increasing susceptibility to pathogen infection and simultaneously causing many nutritional defects and impairing hormone signaling (Raymann et al., 2017; Zheng et al., 2017). It is thus essential that mammals be capable of regulating the composition of the gastrointestinal microbiome so as to promote homeostasis. Appropriate localization of commensal microbes within the gastrointestinal tract is also a key consideration in the maintenance of health and tissue homeostasis, given that both pathogenic microbes and commensal microbes require similar ecological niches in order to colonize the gastrointestinal tract. The co-evolution of these microbes and their hosts has led to the development of mutualistic relationships that simultaneously support microbial growth and host health (Ostaff et al., 2013). Indeed, many commensal microbes can prevent pathogen infection of the gastrointestinal tract by altering local environmental conditions (Nuding et al., 2014), whereas other commensal species are able to prevent such infections via competitive exclusion (Sekirov et al., 2010). In this setting, commensal microbes serve as an effective barrier to pathogen infection. Furthermore, these commensal microbes regulate host immunological functionality, control the production of cytokines such as IL-12, influencing the differentiation of CD4⁺ T helper cell subsets, and impact the production of host- and microbe-derived antimicrobial compounds (Jacob et al., 2019). AMP represent important host-derived antimicrobial compounds that help to regulate gastrointestinal microbiome homeostasis. Notably, these AMP are highly evolutionarily conserved and are expressed in all mammals and many other distantly-related species including cnidarians (Mergaert, 2018). Studies in cnidarians, insects, and mammals have consistently shown that host-derived AMP can influence epithelial microbial community composition (Mergaert, 2018).

3. Interactions between AMP and the gut microbiome

Interactions between host and gut microbiota are complex and can regulate metabolite production (Franzosa et al., 2019), and AMP serve as key regulators of host–microbe interactions (Dodd et al., 2017). Appropriate AMP production has been found to be dependent upon the presence of a healthy microbiota, and once these peptides are produced, the quality and quantity of the microbiome

can be adjusted. Interactions between AMP and microbiota are bidirectional, and microbe-derived metabolites in turn serve to regulate host cell functionality and AMP production (da Costa et al., 2015; Nuding et al., 2014).

3.1. Host-derived AMP and the gut microbiome

Epithelium-derived AMP are regularly and inducibly produced and contribute to barrier defenses that protect hosts against pathogenic incursions. Paneth cells and enterocytes are the primary sources of AMP in the gastrointestinal tract (Filipp et al., 2019), but immune cells such as dendritic cells, macrophages, and lymphocytes within the lamina propria can also produce these peptides (Phadke et al., 2005). The 3 primary types of AMP found in the gut include defensins, cathelicidins, and regenerating gene (Reg) III α / β / γ .

3.1.1. Defensins

Defensins are the most abundant AMP associated with the intestinal mucosa. To date, 10 types of defensins have been identified and separated into 2 groups (α - and β -defensins) according to their structural homology. Defensins are broadly antimicrobial cationic peptides that create small pores in the membranes of bacteria, resulting in a loss of membrane integrity, and eventually cell death (Cobo and Chadee, 2013). Paneth cells primarily secrete α -defensins in the small intestine, whereas epithelial cells in the colon primarily produce β -defensins. In addition, immune cells including B cells, T cells, macrophages, monocytes, and dendritic cells can also produce both α - and β -defensins (Dutta and Das, 2016).

There is evidence that the gut microbiome serves as an important regulator in α -defensin induction to protect hosts against pathogens (Miani et al., 2018). Indeed, in vitro intact intestinal crypts can be stimulated to produce these defensins by live *Escherichia coli* or *Staphylococcus aureus*, live or dead *Staphylococcus typhimurium*, lipopolysaccharide (LPS), lipid A, lipoteichoic acid, or liposomes, underscoring the ability of bacteria and bacterially-derived components to promote defensin secretion by Paneth cells (Ayabe et al., 2000). With respect to the ability of commensal microbes to control defensin production, lactic acid has been shown to suppress α -defensin transcription in vitro in Caco-2 intestinal epithelial cells (IEC), whereas cecal contents can promote the expression of α -defensin 5 (Sugi et al., 2017). In contrast, Menendez et al. (2013) determined that the administration of lactobacilli to antibiotic-treated mice was sufficient to restore α -defensin gene expression. Mice that overexpressed human α -defensin 5 (HD5; also known as DEFA5) exhibited significantly reduced segmented filamentous bacteria (SFB) colonization that was associated with decreased lamina propria Th17 cell levels (Salzman, 2010). At present, however, the role of SFB in regulating the production of α -defensins remains uncertain, and further studies of the ability of specific microbes to control the secretion of these AMP are warranted. Further studies of specific microbial metabolic pathways associated with defensin expression may offer novel insights into the mechanistic basis for host–microbiota interactions.

Compared with α -defensins, β -defensins are produced by epithelial cells in a more ubiquitous fashion. Beta-defensins have been shown to be active against a range of Gram-positive and -negative enteric pathogens such as *S. aureus*, *Streptococcus pyogenes*, *P. aeruginosa*, *E. coli*, and *Candida albicans* (Fusco et al., 2017) without causing significant toxicity to healthy host cells (Chow et al., 2012). The gut microbiome also controls β -defensin secretion, although these AMP are still produced in the intestines of germ-free mice (Putsep et al., 2000). Studies of human colon epithelial cell lines have revealed constitutive expression of β -

defensin-1 but not β -defensin-2, which is upregulated in xenografts in the context of intraluminal *Salmonella* infection (O'Neil et al., 1999). Enterotoxigenic *E. coli* O157 can also drive the expression of β -defensins in porcine intestines (Xiong et al., 2016). Pre-incubating Caco-2 cells with live *Enterococcus faecium* markedly impaired *S. typhimurium* uptake by 45.8%, whereas heat-killed *E. faecium* pretreatment did not impact pathogen internalization (Fusco et al., 2017). Miani et al. (2018) recently utilized a model of antibiotic-treated mice to explore the association between the microbiome and β -defensin production, and additionally assessed the influence of a low-affinity aryl hydrocarbon receptor (AHR) allele on impaired pancreatic β -defensin-14 secretion in non-obese diabetic mice. These authors ultimately determined that AHR ligands, butyrate, and other microbiota-derived compounds were sufficient to drive innate lymphoid cells (ILC) in the pancreas to secrete IL-22, which in turn induced β -defensin-14 secretion by endocrine cells. This suggests that both dysbiosis and low-affinity AHR alleles can influence the secretion of pancreatic β -defensin-14 in mice. Given the evidence that only live microbes can promote β -defensin production, we speculate that particular gut microbes that produce certain metabolites and/or AHR ligands may serve as the primary intestinal regulators of β -defensins. However, further in vivo research will be essential to fully explore the mechanisms whereby the gut microbiota controls β -defensin expression so as to suppress pathogen colonization and to control gut homeostasis.

3.1.2. Cathelicidins

Like defensins, cathelicidins function by disrupting the bacterial cell membrane. These cationic peptides are composed of signal peptides containing a cathelin domain and a cationic AMP produced via C-terminal proteolysis (Sorensen et al., 2001). Cathelicidins such as LL-37/cramp are expressed in humans, mice, fish, chickens, snakes, and countless other species (Bals et al., 2001; Das et al., 2006; de Mera et al., 2008; Gao et al., 2014; Scocchi et al., 1999; Shamova et al., 1999; Uzzell et al., 2003; Wang et al., 2008; Xiao et al., 2006), functioning as natural AMP derived from macrophages and colonic epithelial cells that serve to control the composition of the microbiome within the colon.

In prior analyses, we have found that cathelicidin-WA can enhance the barrier function of the intestinal epithelium, protecting hosts from enterohemorrhagic *E. coli* O157:H7 infection (Yi et al., 2017). In vitro, synthetic cramp can disrupt the murine enteric pathogen *Citrobacter rodentium*, which is normally able to adhere to the apical surface of IEC (Iimura et al., 2005). In cramp-deficient mice, the mucus layer in the colon is discontinuous and thin, making it far easier for *E. coli* O157:H7 to penetrate the intestinal epithelium, thus underscoring the important defensive role of this AMP (Chromek et al., 2012; Hing et al., 2013). When orally administered the pathogen *C. rodentium*, cramp^{-/-} mice exhibited markedly increased fecal *C. rodentium* counts relative to wild type mice (Iimura et al., 2005). Consistent with this, mice lacking cramp expression exhibit increased *E. coli* O157:H7 counts when infected with this pathogen, which was better able to penetrate the mucosal layer and to attach to the epithelium, thereby generating pathogenic lesions (Chromek et al., 2012). LL-37/cramp thus serves to protect the intestinal epithelium from colonization by adherent bacteria, thereby maintaining microbiome homeostasis. Singly-housed cramp^{-/-} mice have been shown to develop a distinct intestinal microbiota compared with wild type mice. Whereas when these mice were cohoused for 4 wk, the composition of the microbiome in wild type mice shifted to more closely resemble that of cramp^{-/-} mice. This suggests that pathogenic bacteria can grow more readily in the absence of cramp production and also suggests that pathogenic bacteria can be transferred to wild type mice upon cohousing (Yoshimura et al., 2018). These findings suggest that

endogenous cathelicidins are important regulators of homeostasis in the intestinal microbiome.

3.1.3. Reg III α / β / γ

Regenerating gene AMP are soluble lectins that interact with bacterial surface components. Paneth cells are major producers of Reg type-III γ (Reg III γ) and its human ortholog Reg III α (HIP/PAP) (Cash et al., 2006; Vaishnavi et al., 2008), and enterocytes can also produce these AMP (Brandl et al., 2007; Ogawa et al., 2003) upon infection, Toll-like receptor (TLR) activation, and inflammation (Behnsen et al., 2014; Brandl et al., 2008). In mice, Reg III β is generally co-regulated with Reg III γ (Zheng et al., 2008).

Recent work indicates that Reg III prevents pathogen colonization via a mechanism dependent upon the presence of a healthy gut microbiota. Ju et al. (2017) for example, found that metronidazole-treated mice exhibited decreased Turicibacteraceae abundance, excessive *E. coli* growth, and increased Reg III β and Reg III γ mRNA expression relative to mice not treated with antibiotics. Earle et al. (2015) similarly found through their analysis of fixed gut cross-sections that eliminating microbiota-accessible carbohydrates from the diet resulted in the thinning of distal colonic mucosa, leading to closer microbial proximity to the epithelium and increased expression of Reg III β . Deficient Reg III β expression can result in prolonged enteric *S. typhimurium* infection via impairing the recovery of normal gut microbiome homeostasis (Miki et al., 2017), indicating that the role of these AMP may differ in a pathogen-specific fashion. Using Reg III γ ^{-/-} mice, Vaishnavi et al. (2011) found that the loss of this AMP was associated with abnormal spatial relationships between mucosal surfaces and commensal microbes within the intestines, and these Reg III γ ^{-/-} mice exhibited a significantly increased mucosa-associated bacterial burden relative to cohoused wild type littermates. However, they did not observe any abnormalities in microbial localization within the colons of these Reg III γ ^{-/-} mice, consistent with the fact that lectin is expressed at lower levels in the colon as compared to the small intestine. This indicates that Reg III γ is a key innate immune peptide that mediates relationships between host tissues and the intestinal microbiome via controlling the spatial relationships between these entities.

3.2. Gut microbiota-derived AMP

Much like their hosts, many bacteria are capable of producing diverse AMP known as bacteriocins. Bacteriocins exhibit a range of functional activities, and some serve to disrupt protein, RNA, and DNA metabolism, whereas others can disrupt bacterial membranes (Cotter et al., 2013). The bactericidal properties of these bacteriocins enable them to effectively compete with other microbes for nutrients and environmental niches (Yang et al., 2014). Analyses of isolated microbes (Nakatsuji et al., 2017; Rodet et al., 2015) and metagenomic analyses (Donia et al., 2014) in mammals have revealed that AMP-producing microbes are highly prevalent. Unlike antibiotics, which are primarily secondary metabolites, bacteriocins are proteins and are thus sensitive to protease activity and thus cause minimal damage to host tissues in vivo.

The exact roles played by bacteriocins are often unclear, but in vivo evidence suggests that certain bacteriocins can aid in the establishment of microbes within the microbiota. For example, Hu et al. (2018) identified and validated *Lactobacillus gasseri* LA39 and *Lactobacillus frumenti* as 2 bacterial species that mediate diarrhea resistance by comparing the relative abundance of intestinal microbiota in saline and microbiota-transferred piglets. Zipperer et al. (2016) utilized wild type or mutant *Staphylococcus lugdunensis* that could or could not produce the AMP lugdunin in order to demonstrate that microbes capable of producing this bacteriocin

were capable of outcompeting *S. aureus* in the nasal epithelia, thereby impacting overall microbota composition. In a separate analysis, Roelofs et al. (2016) assessed AMP, which is termed Bacteroidales species secrete antimicrobial proteins (BSAP) produced by the ubiquitous gastrointestinal commensal species *Bacteroides uniformis*. By employing a gnotobiotic model of competitive colonization, they revealed that BSAP are important mediators of gut colonization for *B. uniformis*, and when mice were co-colonized with BSAP-producing bacteria and bacteria that were either resistant or sensitive to these BSAP, *B. uniformis* microbes were able to outcompete sensitive but not resistant strains. The metagenomic analyses of human samples further revealed that BSAP-producing microbes and BSAP-sensitive microbes are not present in a given host, suggesting that these AMP play a key role in governing competitive dynamics among microbes in the gastrointestinal tract. In the gastrointestinal tract of mammals, Abp118 is a *Lactobacillus salivarius* UCC118-derived bacteriocin that serves as one of the primary compounds capable of protecting against foodborne *Listeria monocytogenes* infection, as evidenced by the fact that mutant *L. salivarius* UCC118 strains that cannot produce this bacteriocin are unable to protect against *L. monocytogenes* infections in mice (Corr et al., 2007). The *Bacillus thuringiensis* DPC 6431 bacteriocin thuricin CD can similarly kill many *Clostridium difficile* isolates including those that cause human disease, despite having no significant adverse effect on commensal microbial species (Rea et al., 2010). Pheromone-responsive plasmids that encode bacteriocins are also frequently observed among enterococcal bacteria (Gilmore et al., 2013), suggesting that these AMP are able to influence the balance between these enterococci and other constituents in the gastrointestinal microbiome. Kommineni et al. (2015), for example, colonized the murine gastrointestinal tract with *Enterococcus faecalis* and then assessed the impact of the bacteriocin 21-encoding conjugative plasmid pPD1 on enterococcal colonization, revealing that colonization by *E. faecalis* strains that carried a conjugation-defective pPD1 mutant plasmid were able to clear vancomycin-resistant enterococci without undergoing plasmid transfer or disrupting the commensal microbiome. This indicates that the production of bacteriocins can control niche competition within the intestines, suggesting that bacteriocins produced by certain microbes may represent valuable therapeutic compounds capable of specifically targeting antibiotic-resistant pathogens within the intestines without adversely affecting the microbiome as a whole. However, it is important to note that certain bacteriocins serve as virulence factors, as in the case of listeriolysin S produced by certain strains of *L. monocytogenes*, which enables these bacteria to better colonize the intestine and to invade more deeply into host tissues and organs (Quereda et al., 2016). Other bacteriocins have also been shown to serve as mediators of bacterial quorum sensing in microbial communities such as biofilms (Gobbetti et al., 2007), and the exact roles of these AMP are likely to be highly context-dependent (Gillor et al., 2008).

Together, these prior studies offer clear evidence that AMP-producing microbes may have value as a probiotic alternative to traditional antibiotic treatment (Kommineni et al., 2015; Sassone-Corsi et al., 2016). At the epithelial surface, a diverse array of host- and microbiota-derived AMP are present, and both of them serve to regulate the dynamics and overall structure of the proximal microbiota.

4. Action modes of AMP and gut microbiota interaction

AMP can not only prevent pathogen infection, but also shape the overall composition of the microbiome. Shared features of host-derived AMP allow them to act broadly on target microbes to suppress cell division, to interfere with microbial metabolism, and/

or to disrupt ATP synthesis. In addition to these direct antimicrobial activities, certain AMP can modulate immune responses and related signaling activities, thereby shaping both innate and adaptive immunity. These activities suggest that certain AMP may be leveraged in a therapeutic context to treat diseases associated with changes in the composition of the microbiome, particularly given the increasing global prevalence of antibiotic-resistant pathogens.

4.1. Direct interactions between AMP and the gastrointestinal microbiome

There are 2 primary types of AMP — those that permeabilize target cell membranes, and those that do not disrupt membrane integrity and instead interact with intracellular targets (Toke, 2005). Electrostatic interactions lead these cationic AMP to interact with anionic LPS or lipoteichoic acid in the membranes of Gram-negative and -positive bacteria, respectively, thereby enabling these AMP to enter into bacterial cells.

Membrane-disrupting AMP typically form α -helical structures, with C-terminal positively charged residues interacting with one another (Powers and Hancock, 2003). This has been confirmed by 3D analyses of α - and β -defensins: their amphiphilic composition was thought to be necessary for their ability to interact with target cell membranes. AMP can disrupt microbial membranes either via directly disrupting it by forming barrel-stave and toroidal pores, or by thinning the lipid bilayer such that it dissolves (carpet model) (Jenssen et al., 2006). Multiple studies have evaluated the impact of human α -defensins on *E. coli* strain (Lehrer et al., 1989). In one of these studies, inner and outer bacterial membranes were sequentially permeabilized within 1 h by human neutrophil-derived α -defensins, after which DNA and RNA synthesis were disrupted, and cells died. *C. albicans* treatment with human α -defensin 1 also induced non-lytic release of ATP and other low-molecular-weight substances from cells, ultimately resulting in their death via mechanisms similar to those induced by salivary peptide histatin 5 (Edgerton et al., 2000). The θ -defensin (RTD-1) is able to interact with lipid bilayers such that it promotes their thinning, forming large cylindrical complexes with 1-palmitoyl-2-oleoyl-phosphatidylcholine–1-palmitoyl-2-oleoyl-phosphatidylglycerol bilayers (Buffy et al., 2004). Studies of RTD-1 underscore its ability to distort the phospholipid bilayer, underscoring the AMP of θ -defensins noted in vitro. AMP can also adversely impact pathogens via enzymatically degrading bacterial membranes. For example, lysozyme can hydrolyze bacterial peptidoglycan linkages, whereas secretory phospholipase A2 can break down bacterial membrane phospholipids (Ragland and Criss, 2017). Intestinal pathogen infection interferes with normal secretory activity (Keestra-Gounder et al., 2016), thereby interfering with luminal AMP production. To preserve normal immunity, host cells may therefore employ alternative compensatory mechanisms in this context. For example, Paneth cells can maintain antimicrobial activity in the context of *S. typhimurium* infections via rerouting lysozyme for release via secretory autophagy (Bel et al., 2017).

AMP that do not disrupt cell membranes can instead cross into cells wherein they can interact with polyanionic structures such as RNA or DNA, inhibiting the activity of particular intracellular targets (Jenssen et al., 2006). AMP-mediated inhibition of particular intracellular pathways can occur following penetration or endocytosis of these peptides (Madani et al., 2011; Nicolas, 2009). During endocytosis, the cellular membrane folds inward to form small vesicles that are coated with clathrin or caveolin prior to their internalization.

4.2. Indirect interactions between AMP and the gut microbiome

Defensins exert a number of activities in addition to their direct antimicrobial activities that enable them to influence innate immunity. Human β -defensin (HBD1) and HBD2, for example, can attract cells which express the CC-chemokine receptor 6 (CCR6) such as dendritic cells (Yang et al., 2002; Yang et al., 1999). In addition, certain murine intestinal α -defensins can drive epithelial cell ion flux (Yue et al., 2002), and HD5 is capable of neutralizing bacterially-derived exotoxins (Lehrer et al., 2009).

Cash et al. (2006) previously demonstrated that colonizing germ-free mice with *Bacteroides thetaiotaomicron* induces Paneth cells to express *Reg III γ* , whereas the expression of *Reg III γ* has been found to be absent in myeloid differentiation factor 88 (*MyD88*)-deficient mice indicating that it is induced by commensal microbes in a TLR-dependent fashion (Brandl et al., 2007). In another study building upon these results, Vaishnav et al. (2008) utilized transgenic *MyD88*-deficient mice that expressed *MyD88* only in Paneth cells and found that these cells were able to recognize and respond to bacterial signatures in *MyD88*-dependent fashion to induce *Reg III γ* , *Reg III β* , CRP-ductin (also known as DMTB1), and resistin-like molecule- β (RELM β) secretions. When Paneth cells and *MyD88* expression were absent in these mice, commensal and pathogenic microbes were also able to more readily disseminate to mesenteric lymph nodes (Brandl et al., 2007; Vaishnav et al., 2008). Deleting individual TLR in these mice failed to replicate the phenotype of *MyD88*-knockout mice, indicating that multiple TLR are likely necessary for bacterial recognition and AMP induction (Vaishnav et al., 2008). The upregulation of *Reg III β* , however, has been found to be specifically driven by TLR2 such that mice lacking this TLR are more susceptible to *Y. pseudotuberculosis* infection (Dessein et al., 2009). Broad-spectrum antibiotic treatment in mice impairs their production of *Reg III γ* , whereas oral LPS administration can reverse this phenotype (Brandl et al., 2008). Overall, this thus suggests that Paneth cells recognize commensal microbes via TLR, and that they respond by producing key AMP that help to prevent pathogen invasion and shape the overall gastrointestinal microbiota. Treatment with broad-spectrum antibiotics can thus have off-target effects by reducing AMP production and thereby increasing susceptibility to pathogen colonization within the gastrointestinal tract (Brandl et al., 2007).

Paneth cell-derived AMP are important regulators of the overall composition of the microbiome. Mice that did not express the pattern recognition receptor nucleotide-binding oligomerization domain-containing protein 2, which is expressed in Paneth cells, exhibited decreased α -defensin mRNA expression in these Paneth cells (Kobayashi et al., 2005), and dysregulated Th1-induced inflammation (Biswas et al., 2010) and abnormal microbiome composition in the small intestine relative to wild type mice (Petnicki-Ocwieja et al., 2009). Similarly, germ-free mice that do not express *CD1d1*, which is necessary for glycolipid antigen presentation to natural killer T (NKT) cells, exhibit altered Paneth cell-derived α -defensin secretion in response to *E. coli* challenge, and also exhibit an altered small intestine microbiome composition relative to that observed in wild type animals (Nieuwenhuis et al., 2009).

Mice that do not express matrix metalloproteinase-7 (*MMP7*) and that thus do not produce mature α -defensins or that produce higher levels of α -defensins because they express human *HD5* have been used to assess the role of these Paneth cell-derived AMP in the regulation of the gastrointestinal microbiota (Salzman et al., 2010). Reciprocal changes in the dominant bacterial groups within the gastrointestinal microbiome were also observed in these mice, and α -defensin deficiencies were associated with reduced Bacteroidetes abundance and increased Firmicutes abundance relative to wild

type mice, whereas *HD5* expression was associated with the opposite phenotype. However, the total bacterial burden in these 2 mouse models was comparable to that observed in wild type mice, suggesting that Paneth cell-derived α -defensins do not regulate total bacterial load in the small intestine. Mice lacking *MyD88* have also been found to exhibit comparable numbers of culturable bacteria within the lumen of the small intestine as compared to wild type mice (Vaishnava et al., 2008).

Transgenic *HD5* expression in mice has also been shown to eliminate SFB colonization in these animals, and these bacteria are gradually lost in hemizygous *HD5*-transgenic breeding pairs and thus not transmitted from mothers to their pups through coprophagy. This absence of SFB resulted in an absence of detectable Th17 cell differentiation within the small intestine lamina propria. Mice deficient for α -defensin production, in contrast, exhibited higher levels of SFB colonization than wild type mice (Nieuwenhuis et al., 2009). Paneth cell-derived AMP are therefore able to modulate lamina propria T cells responses via modulating the makeup of the intestinal microbiota, thus influencing the balance between pro- and anti-inflammatory immune responses.

These prior findings have significant implications, suggesting that Paneth cells and effectors derived therefrom can influence both intestinal homeostasis and systemic inflammation by controlling the microbiota composition. Given that Paneth cells constitutively produce many AMP, these molecules may be important regulators of baseline intestinal microbiota composition, thus setting a homeostatic baseline for local and systemic inflammatory responses. Paneth cell genetic or signaling abnormalities, in turn, may adversely affect the balance between hosts and the intestinal microbiome, resulting in potentially significant proximal and distal consequences.

5. Conclusions and future directions

This review aims to provide a comprehensive overview on the interplay of diverse antimicrobial responses with enteric pathogens and the gut microbiota, which should have therapeutic implications for different intestinal disorders. In mammals, microbial interactions at barrier tissues are complex and dynamic processes, and the microbial homeostasis at these sites helps to maintain anti-pathogen defenses. To constrain pathogenic microbial growth and infection, large quantities of diverse AMP are produced in the intestines. These peptides can not only play immunomodulatory roles but also directly kill certain microbes. In addition to these direct antimicrobial activities, AMP have also been found to play essential roles in shaping the composition of the local microbiome.

Future research will be required in order to fully understand the intricate mechanisms whereby host-derived AMP expression is controlled temporally and spatially at barrier tissue surfaces so as to support microbial homeostasis. Further studies on how individual AMP impact the composition of the intestinal microbiome are also necessary. There is also a clear need to better understand how AMP dysregulation influence the development of diseases including IBD, psoriasis, and atopic dermatitis.

Author contributions

Xin Zong: writing, original draft preparation. Jie Fu and Bocheng Xu: investigation and reviewing. Yizhen Wang: supervision, validation. Minliang Jin: reviewing and editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately

influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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