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Innate defenses of the intestinal epithelial barrier

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Abstract. The innate immune system plays a crucial role in maintaining the integrity of the intestine and protecting the host against a vast number of potential microbial pathogens from resident and transient gut microflora. Mucosal epithelial cells and Paneth cells produce a variety of antimicrobial peptides (defensins, cathelicidins, crytdin-related sequence peptides, bactericidal/permeability-increasing protein, chemokine CCL20) and bacteriolytic enzymes (lysozyme, group IIA phospholipase A2) that protect mucosal surfaces and crypts containing intestinal stem cells against invading microbes. Many of the intestinal antimicrobial molecules have additional roles of attracting leukocytes, alarming the adaptive immune system or neutralizing proinflammatory bacterial molecules. Dysfunction of components of the innate immune system has recently been implicated in chronic inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, illustrating the pivotal role of innate immunity in maintaining the delicate balance between immune tolerance and immune response in the gut.

Key words. Paneth cells; antimicrobial peptides; enteropathogens; pathogen-associated molecular patterns; pattern recognition receptors; inflammatory bowel disease; probiotics.

Introduction

The intestine is an extremely complex organ and represents the body's largest surface area – more than 300 m^2 in human adults. The intestinal epithelium does not just accomplish the digestion and uptake of nutrients. Its huge surface is constantly exposed to intestinal microflora consisting of more than 400 bacterial species and a total of 10^{14} microbial cells [1]. This requires an important role for intestinal tissues in host defense to ensure tolerance to commensal bacteria or efficient recognition and elimination of pathogens. The two major parts of the intestine, the small intestine and the colon, differ profoundly in their bacterial loads, and each section represents a dynamic ecosystem for several hundreds of bacterial species, including innocuous commensals and potential pathogens. Whereas the colon is heavily colonized with bacteria ($10^{11–12}$ bacteria per gram of feces), the small intestine contains numbers of severial magnitudes lower microorganisms ($10^{3–9}$ bacteria per gram of gut content) [2].

Commensal bacteria are usually found only in the intestinal lumen, while the dense mucus covering the mucosal surface and the crypts of Lieberkühn containing intestinal stem cells represent rather sterile environments. Bacteria of the commensal intestinal flora seem to be associated mainly with mucus components, such as mucins, while direct binding to epithelial cells is inhibited in noninflamed mucosa [3, 4]. There is still no clear picture of the mechanisms and factors protecting mucosal surfaces and crypts, but it is becoming more apparent that components of both the innate and the adaptive immune systems contribute to this phenomenon. This review focuses on recent advances in understanding the innate components of intestinal immunity.

Cells of the intestinal immune system

The intestine represents a primary immune organ with several specialized cell types. The components of the adaptive immune system, designated as gut-associated lymphoid tissue (GALT), comprise an afferent part that includes the Peyer's patches (PPs), which sample luminal

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antigens to ensure their uptake by antigen-presenting cells [5]. Specialized epithelial cells, called M cells accomplish the translocation of antigens to the dendritic cell(DC)-rich subepithelial areas of PPs. Upon activation and migration, cells of the GALT create an effector part consisting of immunoglobulin A(IgA)-producing plasma cells and mature T cells, which are diffusely scattered around the intestinal mucosa. About 5–15 g of secretory IgA is transported to the mucus of the intestinallumen and contributes to prevention of epithelial colonization by inhibiting binding of bacterial surface structures to the epithelial cells [6]. During infection by enteric pathogens such as *Salmonella enterica* or *Yersinia enterocolitica,* the GALT has a critical role in containing and curing the infection by stimulating the production of pathogen-specific immunoglobulins or the expansion of cytotoxic and helper T lymphocytes [5, 7]. Initiation of adaptive immune responses requires several days, however, and many infections are eliminated by rapidly acting innate immune functions before they become apparent.

Paneth cells, another type of specialized epithelial cell, reside at the base of the crypts and fulfill a crucial role in innate immunity [8]. They produce several antimicrobial peptides and enzymes [9], as described below. Some of these molecules are constitutively produced to protect the crypts against invading microbes. Paneth cells are located in the direct vicinity of the multipotent stem cells, which require particular protection to ensure their ability to replace epithelial cells, whose life span is only a few days. If Paneth cells get in direct contact with invading microbes or microbial molecules, production of further and higher amounts of antimicrobial factors is induced [10]. This response is fast and ensures prompt counteraction at the onset of infection. Paneth cells also produce proinflammatory cytokines [11], and some of the released antimicrobial peptides have the ability to attract antigen-presenting cells and lymphocytes [12]. Thus, Paneth cells play a role in warning the adaptive immune system of severe and persisting infection. Paneth cells express several receptors that recognize conserved microbial molecules (pathogen-associated molecular patterns, PAMPs), such as Toll-like receptors (TLRs) [13] and nucleotide-binding oligomerization domain (NOD) proteins [14], and enable them to react appropriately upon contact with invading microbes.

In healthy individuals, Paneth cells are found only in the small intestine. Under conditions of inflammatory bowel disease (IBD), such as Crohn's disease or ulcerative colitis, Paneth cells also develop in the colonic mucosa. These metaplastic Paneth cells have many features in common with regular Paneth cells and produce, for instance, α-defensins and lysozyme [15]. In addition to the Paneth cells, differentiated enterocytes also have the ability to produce β-defensins and cathelicidins in a constitutive or inducible fashion [16, 17]. However, the pattern of antimicrobial factors produced by enterocytes differs from that of Paneth cells. Mucosal epithelial cells are thus believed to contribute to intestinal innate immunity and to the relative sterility of the mucosal surface.

Table 1. Functions of human intestinal antimicrobial peptides and polypeptides.

Antimicrobial molecule	Producing intestinal cells	Basis of antimicrobial activity	Additional activities
α-Defensins HD-5, HD-6	Paneth cells	pore formation*	
β-Defensins HBD1, HBD2, HBD3, HBD4	intestinal epithelial cells	pore formation*	chemotactic for leukocytes
Cathelicidin LL-37/hCAP-18	intestinal epithelial cells	pore formation*	chemotactic for leukocytes; stimulates epithelial wound healing; angiogenic; neutralizes LPS
Bactericidal/ permeability-increasing protein (BPI)	intestinal epithelial cells	disruption of outer and inner membranes of Gram-negative bacteria	neutralizes LPS; opsonic
Chemokine CCL20 (MIP-3α)	intestinal epithelial cells	?	chemotactic for dendritic and T cell subsets
Group IIA phospholipase A2 (PLA2)	Paneth cells	degrades bacterial lipids	?
Lysozyme	Paneth cells	degrades bacterial peptidoglycan*	modulates the inflammatory ability of peptidoglycan

* Additional antimicrobial activities, such as activation of bacterial autolysins, are possible.

Antimicrobial peptides – innate effector molecules

Most of the host's antimicrobial molecules are cationic (cationic antimicrobial molecules, CAM) to ensure efficient binding to the anionic bacterial surface polymers [18]. They include bactericidal peptides or proteins such as defensins, cathelicidins, cryptdin-related sequence (CRS) peptides, certain chemokines and bactericidal/permeability-increasing protein (BPI) as well as the antimicrobial enzymes lysozyme and group IIA phospholipase A2 (PLA2) (table 1). Most of the nonenzymatically active CAMs are thought to damage the integrity of bacterial membranes by pore formation, although such an activity has been demonstrated only in some instances, and it is possible that the antimicrobial activities of CAMs involve further microbial targets [19, 20].

Many microbial species of the resident and transient gut flora are opportunistic pathogens with the ability to cause infections in immunocompromised or healthy individuals. Accordingly, there is strong selection pressure on both the bacterial and the host sides for improvement of host defense factors on the one and microbial evasion strategies on the other. Bacteria have found a number of ways to limit the effectiveness of CAMs that include modifications of the bacterial cell surface, secretion of CAM-specific proteases or expression of CAM-specific efflux pumps [18, 21, 22]. On the other hand, mammalian CAMs such as defensins, cathelicidins and CRS peptides are extraordinarily diverse in sequence, structure and abundance and exhibit profound differences in, for instance, rodents and humans.

Defensins represent a family of evolutionarily related small cationic peptides with a characteristic β-sheet-rich fold that is stabilized by three intramolecular disulfide bonds [20] (fig. 1). Two main defensin subfamilies, α- and β-defensins, have been identified in humans and mice, and differ in the length of peptide segments between the six conserved cysteines and their pairing pattern by disulfide bonds. α- and β-defensins have similar three-dimensional structures in solution [23–25] and are synthesized as inactive pre-pro-peptide forms. They are post-translationally processed by mostly unknown proteolytic factors to mature bioactive peptides of 28–44 aminoacids in length with a molecular weight of 3–5 kDa [26–28]. Both subfamilies of defensins are encoded by a cluster of at least eight genes on chromosome 8p23 in humans and mice [26, 29, 30].

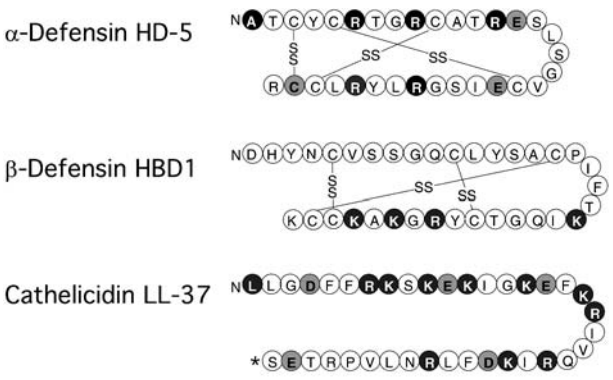

Figure 1. Structure of human antimicrobial peptides. Positively and negatively charged amino acid positions are highlighted in black and gray, respectively. The charges of amino acid side chains and the terminal amino (N) and carboxyl groups were considered. The C-terminal amidation of LL-37 is indicated with an asterisk.

Enteric α-defensins

To date, six members of α-defensins have been identified in humans. They include four neutrophil peptides, HNP1–4 [20], which are predominantly expressed in circulating neutrophils and natural killer (NK) cells. Two enteric α-defensins, HD-5 and HD-6, are expressed at 90–450 μg/cm^2 in Paneth cells of the small intestine [8, 9]. Steady-state storage quantities of 50–250 μg/ml of secreted HD-5 have been suggested to occur in the intestinal lumen. Mice lack α-defensins in leukocytes [31] but express at least 20 isoforms of α-defensins, termed cryptdins, in intestinal epithelial cells [8, 32, 33]. Murine α-defensin peptides have been found at concentrations of 25 μg/ml in single-crypt lumens [34].

Human and murine enteric α-defensins are produced and stored as inactive pre-pro-peptides in granules of the Paneth cells. Murine cryptdins are processed into mature, biologically active peptides within the Paneth cell secretory granules by the co-expressed matrix metalloproteinase matrilysin (MMP7) [35]. Cryptdin as well as MMP7 expression are constitutively present at basal levels in the small intestine of fetal and germ-free mice [34, 36, 37]. MMP7 activates the majority of pro-forms of cryptdins in the Paneth cell granules but appears to generate several precursor fragments due to more than one cleavage site [34]. Pro-forms and mature cryptdins are stored in the Paneth cell granules until they are discharged apically into the crypt lumen in response to Gram-positive or Gram-negative bacteria, bacterialPAMPs such as lipopolysaccharide (LPS), lipoteichoic acid, lipid A and muramyldipeptide, or to cholinergic substances [9, 10]. However, live fungi and protozoa do not stimulate degranulation. So far it is not known whether TLR/NOD signaling pathways are involved in degranulation and secretion of cryptdins by Paneth cells. In contrast to mice, human Paneth cells contain only pro-forms of HD-5, which are further processed into mature forms after secretion of the granule contents into the crypt lumen. In vitro experiments have shown that pro-forms of HD-5 can be processed in vitro by trypsin [38], which together with α1-antitrypsin and pancreatic secretory trypsin inhibitor is expressed in Paneth cells [39]. MMP7, how-

ever, is normally not detectable in human Paneth cells. Stimulation of isolated intact human ileal crypts with LPS clearly showed that an intermediate form of HD-5 is released into the crypt lumen [40], but also that the predicted mature form of HD-5 is produced [38]. Although it is likely that trypsin is also the processing enzyme in vivo, it is still unknown whether the mature peptides of HD-5 and HD-6 are generated extracellularly in a single or multistep process with the involvement of additional enzymes.

Detailed immunohistochemical analysis showed that the expression of cryptdins in mice, as well as HD-5 and HD-6 in humans, mostly correlates with the intestinal distribution of Paneth cells, which are normally restricted to the small intestine in the gastrointestinal tract [41]. Paneth cells are more numerous in the ileum than in other areas of the small intestine. Accordingly, α-defensins are usually absent from the stomach and colon but strongly expressed in the ileum and in smaller amounts in the duodenum and jejunum. Differential distribution of individual cryptdins was found, with cryptdin 4 being absent from the duodenum but highly abundant in the terminal ileum [42]. Since the ileum is an area of the small intestine where reflux of resident bacteria from the colon can occur, α-defensins may play an important role in restricting bacterial colonization and keeping the small intestine relatively sterile. This may ensure efficient uptake of nutrients and provide continuous protection of the enteric stem cells of the crypt. α-Defensins were also detected in Paneth cells of the fetal intestine [43] and that of germ-free mice [44]. These findings suggest that α-defensins are constitutively expressed and not produced only in response to bacterial stimuli. However, enteric α-defensin in correlation with MMP7 expression may also be further up-regulated under inflammatory alterations of the gastrointestinal tract. Infection with pathogenic bacteria or parasites such as *Trichinella spiralis* have been shown to induce changes in intestinal epithelial differentiation in mice, with an increase in Paneth and intermediate cells and a rise in the intestinal production of cryptdins [45]. In addition, activation of intraepithelial lymphocytes has been shown to increase the number of Paneth cells differentiating from the enteric stem cells in the crypt, probably via the secretion of pro-inflammatory cytokines, such as tumor necrosis factor α [42]. The promoter region of HD-5 contains binding sites for transcription factors, such as nuclear factor interleukin 6 (NF-IL6), which could mediate induction of HD-5 in response to inflammatory stimuli [43]. Recent studies showed that another rare epithelial cell type (termed intermediate cell with morphological characteristics of Paneth) and goblet cells in the small intestine may also express α-defensins in mice and humans, particularly during enteric infection [40, 45]. In addition to the enteric α-defensins, abnormal presence of neutrophil α-defensins has been observed in absorptive enterocytes, but not in Paneth cells, during active IBD [46]. So far, it is not clear whether the mature neutrophil α-defensins are produced by the enterocytes or taken up from neighboring granulocytes in the tissue. Production of neutrophil α-defensins has also been described in epithelial cells from human renal carcinomas [47].

The mature peptide of HD-5, as well as all detected proforms, was found to have higher and broader antibacterial activity than the neutrophil α-defensins. Six of the 20 murine cryptdins have been extensively studied in vitro for their antimicrobial activity. All are active, although with individually variable potency against *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Giardia lamblia* and a *phoP* mutant of *Salmonella enterica,* serovar Typhimurium [33, 48, 49]. The various α-defensins, which are present in Paneth cell secretions of the intestinal lumen and also above the crypt-villus boundary, most likely interact differentially with bacteria and thus influence the composition of the enteric microflora.

Cryptdins act by forming anion-conductive channels in microbial cell membranes that depolarize and kill the microbes [20, 50]. Certain cryptdins (e. g. cryptdins 2 and 3) can also form apical anion-conductive channels in eukaryotic cell membranes [51]. The formation of such channels in enterocytes leads to salt and water secretory responses, which may contribute to flushing the intestinal crypt of noxious agents and thus protect mitotically active crypt cells from colonization of potentially pathogenic microbes. In addition, paracrine binding of certain cryptdins, such as cryptdin 3, to absorptive enterocytes can provide proinflammatory stimuli via nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signal transduction and lead to the induction of proinflammatory cytokines and chemokines, such as IL-8 (CXCL8) [52]. IL-8 secretion initiates the first step in the recruitment of neutrophils and other immune cells. Absorptive crypt epithelial cells may thus contribute to innate intestinal host defense by orchestrating the recruitment of immune cells to the intestinal mucosa.

Until recently, direct evidence of the biological relevance of enteric α-defensins in the host defense has been scarce. In particular transgenic mouse strains having complete loss of the Paneth cell population, and thus lacking enteric α-defensins, showed no defects in host-microbial interactions [53]. However, knockout mice, which no longer express matrilysin and therefore secrete only inactive forms of cryptdins, were found to be more susceptible to lethal infections with *S. enterica* serovar Typhimurium and less effective in clearing infections with an enteropathogenic strain of *E. coli* than wild-type littermates [35]. In addition, transgenic mice that expressed the human enteric α-defensin HD-5 were demonstrated to be resistant to oral infections with *S. enterica* serovar Typhimurium at doses lethal to nontransgenic animal controls [54]. Thus, there is increasing in vivo

evidence of the importance of enteric α-defensins in normal innate gut defense mechanisms.

Enteric β-defensins

Whereas the epithelial α-defensins HD-5 and HD-6 are physiologically confined to Paneth cells of the small intestine, the second major family of defensins, the β-defensins, appears to be expressed in epithelial cells of the whole intestine [16]. Up to now, a total of 28 β-defensin genes have been identified in the human genome, located in five clusters. The cluster on chromosome 8p22–23 contains all eight β-defensins currently known to be expressed (HBD-1 to HBD-4, Def105 to DEF108) [55]. To date, HBD-1 and HBD-2 have been demonstrated to be present in different epithelial cells of the gastrointestinal tract at the peptide and messenger RNA (mRNA) levels [15, 56]. mRNA expression of HBD3 and HBD4 has been shown in epithelial cells of the normal small and large intestine only recently [57]. While HBD-1, HBD-3 and HBD-4 seem to be constitutively expressed in intestinal epithelial cells, HBD-2 is upregulated only during the course of inflammation [15, 27]. HBD-3 and HBD-4 mRNA, but not HBD-1, could also be increased by certain proinflammatory cytokines and bacteria [57]. In vitro experiments have shown that among different proinflammatory cytokines and flagellated bacteria only IL-1 clearly induces the expression of HBD-2 [16, 58]. HBD-2 expression is also increased in response to infection with *Helicobacter pylori* in gastric epithelial cells [59].

Consensus sequences for NF-κB, activator protein 1 (AP-1) and NF-IL-6 in the promoter region of HBD-2 indicate that different transcription pathways may be involved in the regulation of this gene [16, 29, 58]. Thus, it has been shown that expression of HBD-2 is regulated by TLR4- and TLR2-dependent pathways requiring NF-κB and AP-1 transactivation. Intestinal epithelial cells normally respond to PAMPs of Gram-negative and Gram-positive bacteria only poorly, since they express low levels of TLR2, TLR4 and TLR6. In IBD and during infection, however, expression of TLRs may be increased by proinflammatory cytokines. Recently it has been shown that, depending on the signaling pathway used, epithelial cells may differentiate between commensal and pathogenic bacteria. HBD-2 induction by pathogenic, but not commensal, bacteria appeared to involve NF-κB-mediated signaling and gene transcription [60], which typically are associated with the activation of innate immunity and inflammation. Differentiation of commensal from pathogenic bacteria might also occur at the stimulation level of TLR expression by proinflammatory cytokines, but the actual mechanisms allowing discrimination between commensal and pathogenic bacteria are still elusive.

Homologues for HBD-1 and HBD-2, termed MBD-1 and MBD-2, have been detected in mice. MBD-2 was shown to act as an adjuvant when it was linked to a specific tumor antigen and used for stimulation of specific antibodies and T cells [61]. The immunostimulatory ability MBD-2 was claimed to depend on its chemotactic activity via binding to the chemokine receptor CCR6 on T cells and DCs, as well as on its induction of DC maturation via binding to TLR4 [62]. Thus, it has been suggested that in addition to their direct antimicrobial effect, the production and secretion of β-defensin 2 may also be relevant in the recruitment of immature DCs and memory T cells to subepithelial areas in cases of microbial invasion [63].

Intestinal antimicrobial peptides other than defensins

The cathelicidin group of mammalian CAMs reveals characteristic interspecies differences. The various mammalian cathelicidins share a similar pro-domain, the cathelin-like domain, while the mature peptides are very diverse [64, 65]. Mice and humans produce the cathelicidins CRAMP and LL-37/hCAP-18, respectively (fig. 1). Both compounds are alpha-helical linear peptides with broad-spectrum activity against Gram-positive and Gram-negative bacteria and *Candida albicans*. LL-37 is expressed by neutrophils, keratinocytes, and epithelial cells such as colonic epithelial cells [66]. Expression of LL-37 in the colon is upregulated by butyrate and other short-chain fatty acids, which are produced by many fermenting bacteria [17]. During *Shigella* infections, LL-37 expression has been shown to be downregulated by an unknown mechanism, and this ability to interfere with LL-37 expression is assumed to be an important aspect of *Shigella* virulence [67].

Mice produce CRS peptides that share similar pro-regions with cryptdins. The mature parts of these cysteine-rich peptides, however, are not related to any other group of antimicrobial peptides [68]. CRS peptides have four intramolecular disulfide bridges and represent dimers connected by an additional disulfide bridge [69]. CRS peptides can form homodimers or heterodimers. Given the large number of at least 23 CRS peptide genes identified on the complemntary (cDNA) level, a great diversity of peptide combination is possible. In fact, several combinations have been shown to exist in vivo and in vitro, and some of the resulting dimers had different activity spectra against enteric pathogens [69]. The extraordinarily high number of CRS genes may thus reflect a strategy to increase the spectrum of antimicrobial activities. It is not yet clear whether CRS peptides are restricted to the mouse genome or represent a more widespread class of antimicrobial peptides. CRS peptides are produced by Paneth cells, and matrilysin is likely responsible for the

processing of CRS precursor molecules, since the processing are sites found in pro-cryptdins are also present in pro-CRS peptides. Regulation of CRS peptides has not yet been studied. It has been shown that mice bred in germ-free conditions produce lower amounts of these molecules [44], but it is unclear whether this difference is based on increased expression upon contact with microorganisms or on an increased number of Paneth cells in colonized mice.

BPI is a cationic 55-kDa protein with antimicrobial activity against Gram-negative bacteria. It is an abundant constituent of neutrophils and has recently been discovered to be a product of mucosal epithelial cells [70]. The antibacterial activity of BPI is based on its ability to bind the lipid A portion of LPS with high affinity, destabilize the outer membrane and subsequently damage the cytoplasmic membrane [71]. Defensins, cathelicidins and PLA2 potentiate BPI [72, 73]. Because of its high affinity to LPS, BPI has an LPS-neutralizing activity, which may contribute to the control of inflammation in the gut.

Intestinal epithelial cells produce a variety of chemokines in response to proinflammatory cytokines or direct contact with bacteria [74]. Many chemokines have cationic properties, and several of them, such as CCL20 (MIP-3α), have high concentrations of antimicrobial activity against Gram-positive and Gram-negative bacteria and against fungi such as *C. albicans* [75–77]. It remains to be determined, however, whether intestinal chemokine production contributes to local host defense by direct antimicrobial activity in addition to recruitment of lymphocytes and phagocytes.

Intestinal antimicrobial enzymes – group IIA PLA2 and lysozyme

Paneth cells of mice and humans produce the antimicrobial PLA2, an enzyme with strong activity against many Gram-positive and Gram-negative microorganisms such as *Salmonella*, *Escherichia* and *Listeria* [73, 78]. PLA2 is a small enzyme, 14 kDa, that rapidly degrades bacterial phospholipids, thereby destroying cell integrity [79]. It exerts major antimicrobial activity at a variety of sites, including tears and many inflammatory fluids, as well as within granules of neutrophils and platelets. The expression of PLA2 by Paneth cells implies that it has an important role in intestinal innate immunity [66, 80].

Lysozyme is another antimicrobial enzyme found in neutrophils, macrophages, epithelial secretions and intestinal Paneth cells [81]. Peptidoglycan, the invariant bacterial cell wall polymer, is cleaved by lysozyme, rendering bacteria susceptible to disruption by osmotic pressure. Lysozyme is mainly active against Gram-positive bacteria, whose peptidoglycan is easily accessible because of the absence of an outer membrane. The antimicrobial activity of lysozyme may also include activation of bacterial autolytic enzymes or membrane disruption [82]. Infection with *S. enterica* serovar Typhimurium decreases the expression of lysozyme and α-defensins in Paneth cells. This activity is dependent on an intact PhoP virulence regulator, indicating that downregulation of antimicrobial components represents an active immune evasion strategy of *S. enterica* [83].

Peptidoglycan is a key inflammatory component that activates the innate immune system via TLR2 and NOD receptors [84]. Certain mutations in NOD2 have been associated with a predisposition for Crohn's disease [85]. Lysozyme seems to have a second function of modulating the inflammatory potential of peptidoglycan by increasing its solubility, clearance and availability. In fact, lysozyme-deficient transgenic mice exhibit increased inflammation in Gram-positive infections [86], and it is likely that lysozyme production by intestinal Paneth cells also serves to control peptidoglycan-induced inflammatory responses.

Other roles of antimicrobial peptides: chemokine-like activities linking innate and adaptive immunity

In addition to their antimicrobial properties, many mammalian CAMs, including LL-37 and several defensins, have chemokine-like activities [12] (table 1) [87]. Representatives of α- and β-defensins are chemotactic for human monocytes and subsets of DCs and T cells [88–90], while LL-37 is capable of recruiting human neutrophils, monocytes and T cells [88, 91, 92], thereby providing a link between innate and adaptive immune responses. Human β-defensin hBD2 and the chemokine CCL20 share the CCR6 receptor for triggering chemotactic responses in DCs and T cells [90].

Defensins and certain chemokines share similar characteristics, including size, disulfide bonding, interferon γ (IFNγ) inducibility and cationic charge. Moreover, elucidation of nuclear magnetic resonance (NMR) solution structures of human β-defensin hBD2 and murine CCL20 has recently revealed a surprising similarity in the three-dimensional structures of the two molecules [93], which provides an explanation for the ability of both to trigger the CCR6 receptor.

Toxic activities toward microorganisms and chemotactic activity toward leukocytes require different concentrations of a particular peptide and may be relevant in different situations during infection. Paneth cells release large amounts of the various peptides sufficient to kill microorganisms. Once these peptides are diluted by diffusion, they lose their antimicrobial ability, but they may be responsible for recruiting leukocytes to infected or inflamed tissues.

Cytokine production by epithelial cells

Cytokine production in epithelial cells is accomplished by activation of the transcription factor NF-κB. Interestingly, the signaling events leading to activation of NF-κB are quite variable for different microorganisms and PAMPs. For some pathogens, for example, *Salmonella* spp., it was demonstrated that the engagement of TLRs or other pattern-recognition receptors is involved. TLR5 interaction with bacterial flagellin triggers NF-κB activation [94]. LPS, a very potent bacterial PAMP, is recognized by the TLR4 receptor. TLR4 has been identified in the Golgi apparatus of intestinal epithelial cells, and TLR4-triggered inflammatory responses may require internalization of LPS [95]. Several bacterial surface molecules, such as *Yersinia* invasin protein, bind with high affinity to β1 integrins, most likely as a result of convergent evolution. Clustering of β1 integrins causes a signaling cascade that leads to activation of Rac-1 and MAP kinases and subsequent production of IL-8 [96, 97]. Other pathogens can trigger this response via indirect β1 integrin binding, which involves, for example, the fibronectin-binding protein of *S. aureus* [98]. In addition, bacterial internalization triggers signaling events that may also lead to NF-κB activation [97].

Upon engagement by microorganisms, epithelial cells can produce a number of proinflammatory cytokines and chemokines, including IL-8, IL-1, GM-CSF, GRO and MCP-1 [99]. Interestingly, pathogenic bacteria, but not commensals, can usually trigger such a response [100, 101]. Cytokine and chemokine production by epithelial cells leads to recruitment of polymorphonuclear leukocytes, macrophages and lymphocytes and initiates inflammatory tissue responses, thereby affecting both innate and adaptive immune responses at mucosal sites [99, 102]. Sensing the presence of pathogenic bacteria by epithelial cells is one of the integral mechanisms of the fine-tuned host response to pathogenic microorganisms at mucosal sites.

Many of the studies dealing with cytokine production in epithelial cells are limited, however, by the fact that they involve only in vitro-grown epithelial cell lines. There is little available data on proinflammatory epithelial cell responses in vivo. In fact, many of the events described in the in vitro studies may be only partially relevant in vivo due, for example, to basal polarization of the host cell receptors involved in pathogen recognition. Furthermore, a recent report demonstrated that bacterial LPS can be neutralized in epithelial cells by intracellular dimeric IgA [103], which may prevent LPS-induced NF-κB translocation and subsequent proinflammatory epithelial cell responses.

Role of the innate immune system in IBD

The balance between induction of mucosal immune tolerance and immune response is perturbed in chronic inflammatory diseases such as IBD. Intestinal inflammation is accompanied by direct adherence of bacteria to the mucosal surface, while the protective function of the mucus layer seems to be disrupted [104]. Moreover, the composition of the bacterial flora exhibits profound differences under inflamed and noninflamed conditions [105]. IBD-like disease develops in mice lacking the Th1 cytokine IL-2, supporting the notion that the adaptive immune system plays a pivotal role in the development of IBD [106].

Several recent studies have demonstrated that components of the innate immune system are involved in IBD. Induction of human β-defensins 2 and 3 is impaired in Crohn's disease [56], suggesting that reduced antimicrobial activity in the intestinal mucus contributes to induction of IBD. The intracellular PAMP receptor NOD2, expressed abundantly in Paneth cells and inflamed intestinal tissues, is mutated in certain populations of patients with Crohn's disease [85, 107], suggesting that perturbed recognition of bacterial molecules plays a critical role in IBD. Both β-defensins and NOD2-induced cytokines have the ability to augment and shape adaptive immune responses, and there is increasing evidence that dysfunctions of the innate immune system initiate the processes leading to IBD.

Probiotic microorganisms as inducers of innate host defense

Probiotics are viable food supplements that exert actions beneficial to the host. For example, they are claimed to modulate the composition of the microflora's metabolic activity and exclude enteric pathogens by colonization resistance or production of bacteriocins; furthermore, they are believed to stimulate the immune system and increase production of antimicrobial metabolites [108]. The number of putative probiotic microorganisms has increased over the past few years and includes yeasts, *E. coli*, enterococci, bacilli, lactobacilli lactococci and streptococci. Apart from a direct or indirect action on other bacteria, particularly exogenous pathogenic bacteria, it is believed that probiotics act directly on the mucosal immune system [108, 109]. Thus, some probiotics may increaseproduction of anti-inflammatory cytokines.

In IBD, the probiotic *E. coli* Nissle 1917 strain has proved to be as effective as the standard therapy for keeping patients in remission [110]. The molecular basis of this effect is not yet clear. Recent work provided evidence that the protective effects of probiotic microorganisms in experimental IBDs are mediated by DNA, which is rec-

ognized by the mucosal TLR9 receptor [111]. TLR9 activation may stimulate increased production of β-defensins. Bacterial DNA was administered intragastrically to mice prior to the induction of experimental colitis via dextran sodium sulfate (DSS). Probiotic or *E. coli* DNA ameliorated the severity of DSS-induced colitis. However, the molecular basis of the various effects of the different probiotics and their impact on the innate immune system still needs to be established.

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