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Microreview

Control of mucosal polymicrobial populations by innate immunity

Katie L. Mason[1,2] and Gary B. Huffnagle[1,2]*
[1]*Pulmonary Division, Department of Internal Medicine and* [2]*Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA.*

Summary

The gastrointestinal tract carries out the complex process of localizing the polymicrobial populations of the indigenous microbiota to the lumenal side of the GI mucosa while absorbing nutrients from the lumen and preventing damage to the mucosa. This process is accomplished through a combination of physical, innate and adaptive host defences and a 'strategic alliance' with members of the microbiota. To cope with the constant exposure to a diverse microbial community, the GI tract, through the actions of a number of specialized cells in the epithelium and lamina propria, has layers of humoral, physical and cellular defences that limit attachment, invasion and dissemination of the indigenous microbiota. However, the role of the microbiota in this dynamic balance is vital and serves as another level of 'innate' defence. We are just beginning to understand how bacterial metabolites aid in the control of potential pathogens within the microbiota and limit inflammatory responses to the microbiota, concepts that will impact our understanding of the biological effects of antibiotics, diet and probiotics on mucosal inflammatory responses.

Introduction

The gastrointestinal (GI) tract carries out the complex process of localizing the polymicrobial microbiota to the lumenal side of the GI mucosa while absorbing nutrients from the lumen and preventing damage to the mucosa. This process is accomplished through a combination of physical, innate and adaptive host defences, and a partnership with members of the microbiota. The GI mucosa is continuously exposed to ingested antigens and there are an estimated 10^{14} indigenous bacteria that are also in constant contact with the mucosa. The GI microbiota is composed of thousands of species that are a mix of potential pathogens and beneficial microbes. The host uses a variety of innate and adaptive immune mechanisms to limit microbial invasion and maintain a tolerogenic environment that is still able to mount an appropriate inflammatory response when needed.

The GI tract is the largest mucosal surface in the human body and is lined with a single layer of columnar intestinal epithelial cells (IECs), with an overlying glycocalyx, which form a physical barrier between the lumen and the host. Antimicrobial peptides and proteins, such as defensins, cathelicidins, C-type lectins and trefoil peptides, are used to limit microbial invasion and maintain intestinal homeostasis. Specialized cell types, like M cells and unique subsets of dendritic cells (DCs), play a role in sampling lumenal antigens. Macrophages also play a major role in the intestinal lamina propria (LP), where they phagocytize and kill bacteria that have escaped from the lumen, without the release of inflammatory mediators. While the adaptive immune system plays an important function in establishing gut homeostasis (Edelman and Kasper, 2008), the focus of this review is the innate immune system in the GI tract. This review will focus on innate effector mechanisms that the host utilizes to control polymicrobial populations on GI mucosal surfaces, beginning with the lumen and moving inwards to the LP.

Lumenal defences and mucus layer

On the lumenal side of the GI mucosa, there are a variety of antimicrobial proteins and peptides that target conserved structures of microorganisms and inhibit their growth (Fig. 1). The concentration, and therefore activity, of these molecules is greatest at the mucosa–lumen interface and they function largely to limit microbial attachment

Received 9 April, 2009; revised 8 June, 2009; accepted 8 June, 2009. *For correspondence. E-mail ghuff@umich.edu; Tel. (+1) 734 936 9369 (office), 734 936 7934 (secretary); Fax (+1) 734 764 2655.

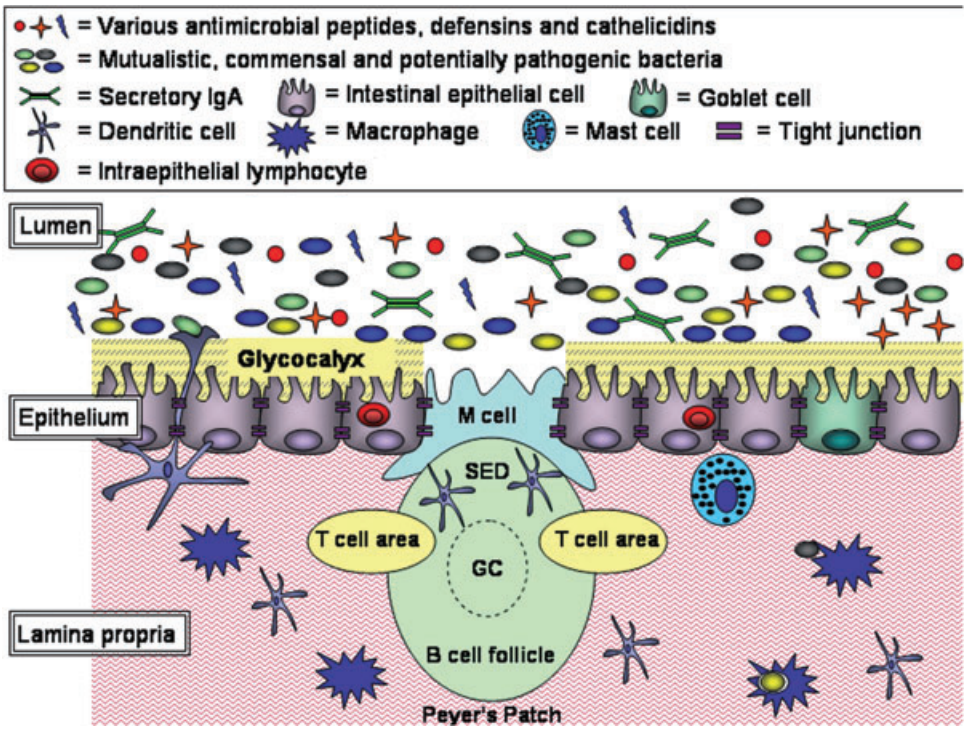

Fig. 1. Mechanisms of the innate immune response in the gastrointestinal tract. This diagram shows the location of different cell types and molecules that play a role in the innate immune response in the GI tract. The lumen of the GI tract is rich in microbes, but it also is dense with various antimicrobial peptides, defensins and cathelicidins that all play a role in maintaining host–microbe homeostasis. Beneath the lumen is the glycocalyx, which protects the intestinal epithelium. The epithelium forms a physical barrier to the lumen through the formation of tight junctions between epithelial cells. M cells are also connected to intestinal epithelial cells with tight junctions, and M cells overlie the subepithelial dome that is rich in dendritic cells. Underneath the SED is lymphoid tissue dense in B and T cells. In the lamina propria, many classes of specialized dendritic cells and macrophages. One proposed mechanism of antigen recognition within the GI tract is that antigen enters through the M cells to the dendritic cells in the SED. Dendritic cells are also capable of altering tight junctions to extend dendrites to directly sample the lumenal contents. All of these cell types and specialized molecules play an important role in maintaining the strategic alliance between the microbiota and the host.

and invasion into the mucosa. One such antimicrobial protein is lysozyme. Paneth cells in the small intestine produce lysozyme and secrete it into the lumen where it can target the lumenal microbiota. Lysozyme is very effective at inhibiting the growth of Gram-positive bacteria because it is a glycosidase that hydrolyses the 1,4-β-glycosidic linkages between *N*-acetylglucosamine and *N*-acetylmuramic acid that make up peptidoglycan (Ganz, 2004). Gram-negative bacteria are not as easily targeted by lysozyme due to the peptidoglycan being in the periplasmic space. With its glycosidase activity, lysozyme acts in a broad fashion throughout the small intestine to target the cell wall of many bacteria.

Secretory phospholipase A_2 ($sPLA_2$) is another enzyme that targets a conserved moiety in bacteria, through hydrolysis of the phospholipid component of the bacterial cell membrane. $sPLA_2$ is produced by Paneth cells and macrophages, so it is found ubiquitously throughout the tissue layers of the GI tract (Vadas *et al*., 1993; Harwig *et al*., 1995). This enzyme is basic, allowing it to penetrate the bacterial cell wall to reach the bacterial membrane where it hydrolyses the membrane phospholipids. In this way, sPLA2 damages bacterial cell surfaces in a non-specific manner.

Antibacterial proteins also play a critical role in maintaining microbe–host homeostasis in the GI tract, although the mechanisms of many of these proteins remain unknown. C-type lectins, calcium-dependent carbohydrate-binding proteins, are one group of antibacterial proteins and include RegIIIγ (and its corresponding human protein, hepatocarcinoma-intestine-pancreas/pancreatic-associated protein, HIP/PAP). They are expressed by Paneth cells and enterocytes in the small intestine. RegIIIγ targets Gram-positive bacteria specifically by binding to peptidoglycan to mediate bacterial killing (Cash *et al*., 2006). The bactericidal mechanism of RegIIIγ is still not understood, and may be occurring via enzymatic activity or through direct membrane disruption. The mechanisms of the other members of the Reg family of C-type lectins are also not understood, but many of these Reg family members are expressed in GI tissues (Dieckgraefe *et al*., 2002). The ubiquitous nature of these

and other C-type lectins points towards a general antibacterial defence mechanism in the GI tract.

Another class of broad spectrum antibacterial proteins is the ribonuclease family. Angiogenin-4 (Ang-4) is a member of this ribonuclease family, and like other RNases, has the ability to hydrolyse RNA, but this enzymatic activity has not been directly linked to its bactericidal function. Ang-4 is broadly bactericidal, having activity against Gram-positive and Gram-negative bacteria through an unknown mechanism (Hooper *et al*., 2003). Despite its broad bactericidal activity, Ang-4 is solely expressed by Paneth cells, thus limiting its activity to the small intestine. RNases undoubtedly play a role in maintaining homeostasis in the GI tract where diverse populations of bacteria come into contact with the mucosa.

Defensins are the major family of membrane-disrupting peptides and are highly expressed throughout the gut. They are expressed by diverse cell types, including IECs, neutrophils and macrophages. They are small peptides ranging in size from 2 to 6 kDa and have conserved cysteine residues that form disulfide bonds resulting in a three-dimensional structure. Defensins are classified into three groups, α, β and θ, based on their disulfide bond arrangements and the location of the cysteine residues (Selsted and Ouellette, 2005). Regardless of their classification, the defensins are broadly antimicrobial, and target both Gram-negative and Gram-positive bacteria. Defensins have also been shown to be active against some fungi, viruses, and even protozoa (Selsted and Ouellette, 2005). The highly basic nature of defensins permits electrostatic interactions with negatively charged phospholipid groups found on bacterial membranes. Defensins form pores to osmotically lyse the bacterium when a critical concentration has been reached (Kagan *et al*., 1990). The tissue-specific and heterogeneous expression of the specific defensins along the mucosa points towards a critical role in shaping the composition of the microbial communities found at these different locations along the mucosa.

α-Defensins are expressed solely by Paneth cells within the small intestine, most abundantly within the distal ileum, and expression corresponds with maximum bacterial exposure (Ouellette and Selsted, 1996). Murine α-defensins (cryptdins), and mice have many cryptdin-related sequence (CRS) peptides. These CRS peptides all share four intramolecular disulfide bridges that form covalent dimers through another intermolecular disulfide bridge (Mukherjee *et al*., 2008). The CRS peptides can form both heterodimers and homodimers and exhibit broad spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. In humans, there are two enteric α-defensins characterized so far, human defensins 5 and 6 (HD-5 and HD-6). HD-5 localizes to Paneth cells of the small intestine, but not of the stomach or colon in healthy patients. During chronic inflammatory bowel diseases the colonic mucosa is vulnerable to microbial penetration, and HD-5 is expressed by Paneth cells in the colonic epithelium. Another group of human α-defensins are the neutrophil α-defensins, HNP 1–3, which can be expressed by IECs, and not Paneth cells during inflammatory bowel disease (Mukherjee *et al*., 2008).

Unlike α-defensins, β-defensins are expressed in enterocytes in the small and large intestine. In the intestinal mucosa, multiple β-defensins are expressed, such as hBD-1, hBD-2 and hBD-3. Unlike α-defensins that are broadly bactericidal, each β-defensin has specific bactericidal activity. hBD-1 and hBD-2 are active against Gram-negative bacteria while hBD-3 is specific for Gram-positive bacteria. In addition to their role as antimicrobial peptides, both α- and β-defensins can be chemotactic for T cells and DCs (Mukherjee *et al*., 2008). Through their chemotactic ability, defensins play an important role in innate immunity, but also modulate the adaptive immune response within the GI tract.

Another class of membrane-disrupting antimicrobial peptides found in the GI tract is the cathelicidins. These peptides are expressed by neutrophils, epithelial cells in the colon and other mucosal surfaces such as the lung and urinary tract (Hase *et al*., 2002) and have bactericidal activity similar to defensins. Cathelicidins bind to bacterial membranes through electrostatic interactions to disrupt the membrane of Gram-negative and Gram-positive bacteria as well as some fungi (Bals and Wilson, 2003). They are cationic, α-helical peptides with a variable C-terminal region and a conserved cathepsin L inhibitor (cathelin)-like domain. LL-37 is one of these cathelicidins and has been shown to have additional mechanisms aside from bactericidial activity. LL-37 has been shown to induce Th1 cytokine secretion by DCs and to act chemotactically for macrophages and T cells (Koczulla *et al*., 2003; Davidson *et al*., 2004).

Another important lumenal effector protein is a product of adaptive immunity. IgA, which is the predominant immunoglobulin isotype secreted by plasma cells in the LP, is a non-inflammatory immunoglobin found in high concentrations in the lumen and mucosa as a dimer. The polymeric immunoglobulin receptor (pIgR) is found on enterocytes and attaches to the Fc of IgA, and remains bound as secretory component (SC). SC prevents proteolytic damage to secretory IgA (sIgA) in the harsh environment of the lumen. SC also transfers antigen out of the LP to the apical surface of the enterocytes using pIgR (Kerr, 1990). sIgA plays a vital role in maintaining immune homeostasis in the gut lumen.

The mucus layer forms the interface between the lumenal contents and the epithelium, acting as a physical barrier against the dense microbial population of the

lumen. This protective barrier is composed of mucus gel, bicarbonate and surfactant phospholipids, which results in a neutral pH that protects the underlying epithelial cells. Mucus gel is secreted by the gut epithelium and is a combination of water and mucin glycoproteins. Trefoil factor family (TFF) peptides are cosecreted with mucus. TFFs are low-molecular-weight peptides that play a role in assembly of mucins. TFF-2 stabilizes the mucus layer by increasing the viscosity of gastric mucin as it is secreted (Thim *et al*., 2002). Within the mucosal surface, the glycocalyx is the most concentrated glycoprotein layer that plays a role in regulating microparticle access to the apical surface of IECs. The interaction between mucins and TFFs results in a stable protective layer against acid, bacteria, and other potentially damaging molecules.

The outermost extracellular layer (the film of mucus and complex polysaccharides) on the lumenal side of the epithelium also acts as a nutrient source for specific (largely beneficial members of the microbiota). These bacteria produce metabolites, such as short-chain fatty acids (SCFA), bacteriocins and quorum sensing molecules that can dampen innate inflammatory responses, which aids in the alliance between the host and its indigenous microbiota. These molecules are discussed in more detail below in the 'microbial control of gut homeostasis' section.

Epithelium

The innate immune system constantly samples the diverse and numerous microbial products within the GI tract, and to deal with this, there are specialized cell types within the epithelium. This review will cover IECs, Goblet cells, Paneth cells, mast cells and M cells. The IECs play a dynamic role in GI immune homeostasis. IECs are themselves a barrier, but they also secrete many antimicrobial peptides and proteins, as discussed earlier. The presence of tight junctions (TJs) between IECs to prevent the passage of molecules between cells is another important characteristic and will be discussed below. They also express a wide range of pattern recognition receptors (PRRs) that recognize numerous commensal and pathogenic bacterial factors, including lipopolysaccharide (LPS), flagellin and unmethylated CpG-containing DNA (Harris *et al*., 2006). While potentially pathogenic bacteria tend to activate NF-κB through Toll-like receptors (TLRs) and/or NOD-like receptors (NLRs), some non-pathogenic bacteria can actively inhibit the NF-κB pathway (Harris *et al*., 2006). In this way, bacteria can modulate the innate immune system and the contribution of mutualistic bacteria to this process is key to mucosal homeostasis.

Intestinal epithelial cells can also activate the adaptive immune system through antigen presentation. IECs can be induced to express MHC class II molecules, the invariant chain and active cathepsins (intracellular proteases), which points towards their role in antigen processing and presentation to professional antigen presenting cells (APCs) (Hershberg and Mayer, 2000). Because IECs lack a costimulatory molecule, they are not able to prime naïve T cells and may promote local T-cell tolerance to the lumenal antigen.

One critical feature of the epithelium in the gut is the presence of TJs between the IECs. All IECs express TJ proteins and form TJ between each other, resulting in a physical barrier between the LP and the lumen that is vital for health. There are other intercellular junctions, but TJs are the most apical and consist of the transmembrane proteins claudin and junctional adhesion molecule and the cytoplasmic plaque proteins, ZO-1, ZO-2, ZO-3, cingulin and 7H6 (Qing-Hua and Qian, 2009). Butyrate, a bacterial metabolite in the GI tract, can increase TJ protein expression to strengthen the barrier between the LP and bacteria in the gut.

In the GI tract, specialized DCs play a pivotal role in connecting the adaptive and innate immune response. DCs in the gut are adept at acquiring antigens from the lumen. One group of specialized DCs express TJ proteins that allow the DCs to extend dendrites into the lumen to directly sample the microbiota without compromising mucosal barrier function. This process is dependent on CX3C-chemokine receptor 1 (CX3CR1), and mice deficient in CX3CR1 exhibit impaired lumenal sampling (Rescigno *et al*., 2001). Many DCs localize within the Peyer's patches and mesenteric lymph nodes, but the epithelium also plays a large role in DC localization and regulation. One mechanism is through the IEC secretion of thymic stromal lymphopoietin (TSLP), which is constitutively expressed by epithelial cells within the GI tract and other mucosal surfaces. During infection or tissue injury, elevated levels of TSLP are secreted by IECs, which is a potent activator of DCs (Rimoldi *et al*., 2005). Other factors secreted by IECs that regulate DC function are TGF-β and prostaglandin E_2. TGFβ produced by IECs inhibits the NF-κB pathway, which limits DC expression of pro-inflammatory cytokines. All of these studies point towards a dynamic relationship in the gut between IECs and DCs that links the adaptive and innate immune responses, lumenal and tissue responses, and epithelial barrier functions.

Intraepithelial lymphocytes (IELs) in the GI tract play an important role in regulating proliferation and differentiation of IECs. IELs localize to the basolateral side of the gut epithelium, but the mechanism of their interaction with IECs is still unknown. The physical interaction between IECs and IELs is aided by a hemophilic adhesion called epithelial cell adhesion molecule that is expressed on both cell types. In the small intestine, E-cadherin (epithelial cell adhesion molecule) and occludin (TJ specific plasma-membrane protein) are constitutively expressed

by IELs, and may play a role in IEC barrier function (Inagaki-Ohara *et al*., 2005). More studies are needed to understand the barrier formed by IECs and IELs, because in chronic inflammatory conditions in the GI tract, there is a decrease in epithelial barrier function, and a correlated increase in IELs (Nasrat *et al*., 2000).

γδ T cells are a specialized group of innate T cells found at epithelial and mucosal surfaces, and play a role in the response to stress and inflammation. γδ T cells can influence immune homeostasis through secretion of keratinocyte growth factor-1 (KGF-1), which induces proliferation of epithelial cells in the GI tract, possibly reducing disease severity. γδ T cells also recruit inflammatory cells in response to tissue damage in the GI tract. In this way, γδ T cells play a vital role in maintaining tissue integrity after injury. Mice lacking γδ T cells show elevated disease severity in models of inflammatory bowel disease. The transfer of γδ T cells to mice lacking γδ T cells is able to improve chemically induced colitis, supporting the idea that γδ T cells regulate intestinal inflammatory responses. This was associated with an increase in TGF-β and a decrease in IFN-γ (Inagaki-Ohara *et al*., 2004). Furthermore, γδ T cells have been shown to decrease IL-15 secretion by IECs and increase IL-8 and IFN-γ, all of which have been associated with restoring epithelial homeostasis (Shibahara *et al*., 2005). Through their role in maintaining tissue integrity and modulating inflammatory responses, γδ T cells act as a special type of innate T cell that is important in gut homeostasis.

Mast cells reside near and within the epithelium of the GI tract. Mast cells express FcεRI on their surface and become activated when antigen cross-links IgE bound to these receptors. Upon activation, via surface IgE cross-linking or other pathways, mast cell degranulation begins rapidly. The molecules released by this degranulation are a diverse group of biologically active proteins and chemical mediators. One such molecule is histamine, a vasoactive amine, which increases blood flow and vessel permeability. Other molecules released include prostaglandins, leucotrienes and cytokines such as IL-4 and IL-13. These mediators activate a variety of processes, including attracting leucocytes, increasing vascular permeability and increasing mucin secretion. Intestinal mast cells are typically rare in mice, but are recruited rapidly during gastrointestinal infections caused by *Trichinella spiralis* (Knight *et al*., 2008). Thus, mast cells are also a critical bridge between adaptive and innate host defences and play a key role in GI homeostasis.

Goblet cells are found throughout the epithelium of the GI tract and produce the thick mucus layer that protects the IECs. Goblet cells produce mucins and trefoil peptides (discussed earlier) that form the glycocalyx that is found throughout the entire GI tract. As discussed earlier, the mucus layer forms a physical barrier between the lumen and the GI epithelium. Goblet cells secrete elevated levels of mucins upon exposure to bacteria or toxins in the lumen (Kingdon *et al*., 1995). Goblet cells are also adept at forming TJs with adjacent cells in the epithelium, thereby maintaining the physical barrier between the host and lumen.

Paneth cells are specialized epithelial cells found at the base of small intestinal crypts. They produce several important components in gut homeostasis, including lysozyme, defensins and secretory phospholipase A2 (sPLA2) (as discussed previously). Studies in the murine small intestine have found that Paneth cells secrete predominantly cryptdins following exposure to Gram-negative or Gram-positive bacteria (Ayabe *et al*., 2000). However, fungi and protozoa did not stimulate Paneth cell degranulation. Paneth cells play an important role in responding to bacterial stimulation in the small intestine through their release of antimicrobial peptides, especially α-defensins.

Microfold cells (M cells) are specialized epithelial cells that act as a bridge between innate and adaptive immunity. M cells lack the microvilli of IECs, and instead have a microfold appearance. Compared with IECs, M cell have fewer lysosomes, more mitrochondria and lack the glycocalyx that is typical of the GI tract. Without a thick mucus layer, M cells are more readily accessible to the microbiota in the lumen. Although the mechanism is not fully understood, M cells make direct contact with the microbiota, and through transcytosis, deliver antigen to the subepithelial dome where professional antigen presenting cells are located. The M cells have modified apical and basolateral surfaces that aid in antigen sampling. Studies have shown that M cell transcytosis of bacteria and viruses is receptor dependent (Tyrer *et al*., 2002). While IECs express many PRRs on their surface, the receptors utilized by M cells for lumenal sampling is unknown. One difference in receptor expression between IECs and M cells is the distribution of α5β1 integrin. IECs express α5β1 integrin on their lateral and basolateral surfaces while M cells only express α5β1integrin on the apical surface (Tyrer *et al*., 2002). Studies inhibiting α5β1 integrin on M cells resulted in inhibition of transcytosis. Other receptors implicated in aiding M cell transcytosis are TLR-4 and PAF receptor (Tyrer *et al*., 2006). Although much remains to be understood about M cell function, their role as a bridge between innate and adaptive immunity may play a critical role in maintaining gut immune homeostasis.

Lamina propria

Macrophages that differentiate in the intestinal LP have distinct properties and function compared with macrophages at the site of microbial infections. Macrophages in

the LP need to eliminate microbes while preserving gut homeostasis, resulting in a specialized subset that is highly phagocytic but have reduced pro-inflammatory cytokine production and lessened costimulatory activities. Some distinct properties of these LP macrophages are diminished cell surface expression of CD40, CD80 and CD86, while maintaining phagocytic and bactericidal activity (Smythies *et al*., 2005). LP macrophages are also deficient in some innate immune response receptors, such as TLR-4. Due to this, intestinal macrophages do not respond to LPS or many other PAMPs. Other alterations in receptor expression in LP macrophages is the absence of the Fc receptor for IgA and for IgG, complement receptors CR3 and CR4, and integrin $\alpha 2\beta 1$. LP macrophages are also less responsive to pro-inflammatory cytokines resulting in a more tolerant GI environment. The lack of several receptors that typically induce the adaptive immune response allows LP macrophages to eliminate microbes without cytokine secretion and the activation of immune cells.

However, the leucocytes in the LP are also able to respond to microbes by developing strong inflammatory and adaptive responses. In models of intestinal inflammation, LP macrophages have an inflammatory phenotype and function. When inflammation is present in the mucosa, LP macrophages express CD40, CD80 and CD86 at appropriate levels, unlike during steady state in the gut (Rugtveit *et al*., 1997). LP macrophages have also been found to express TLR-2, TLR-4, CD14 and CD89 during inflammatory conditions (Rogler *et al*., 1997). In addition to inflammatory LP macrophages, neutrophils can also play a role in inflammation of the LP. The LP is also the predominant site of T- and B- cells in the GI tract, including specialized lymphoid tissue underlying the M cells and subepithelial dome that is rich in B- and T-cells (Peyer's Patches). These cells are localized to detect infiltrating bacteria and mount the appropriate adaptive immune response. In the GI tract, each organ has characteristic lymphocyte populations and characteristics that directly affect the adaptive immune function of the organ.

Microbial control of gut homeostasis

The microbiota has a significant impact on the innate defences of the GI tract, so it is not a surprise that antibiotics are beginning to be implicated in alterations of gut innate defences. Because antibiotics disrupt the bacterial community, this interrupts the strategic alliance between the microbiota and the host. Recently, an association between antibiotic use and an increase in colonization by pathogenic bacteria has been found. Brandl *et al.* (2008) found that eliminating commensal bacteria with antibiotics, led to an outgrowth of enterococci. Notably, antibiotic use downregulated the intestinal expression of RegIIIγ, which plays a role in Gram-positive bacterial killing. In this study, the use of a combination of metronidazole, neomycin and vancomycin resulted in a decrease in commensal bacteria and compromised part of the innate immune defence in the GI tract. However, different broad-spectrum antibiotic regimens also showed similar results.

Bacterial metabolites play a large role in influencing the innate defence system in the GI tract. One group of these metabolites is the bacteriocins, of which there are several classes (more may still be discovered) (Galvez *et al*., 2007). Almost all bacteria produce one or more bacteriocins (Riley, 1998) and these small antimicrobial peptides have varying spectra of activity against other bacteria. Thus, similar to antimicrobials produced by the host, bacteriocins can function in innate defence against potentially pathogenic bacteria in the gut. The real importance of bacteriocins in maintaining a balance between the microbiota and the host remains to be determined.

Quorum sensing molecules are another bacterial metabolite that can affect the innate defences in the gut. Bacteria in the GI tract use auto-inducers as a cell to cell signalling mechanism to sense bacterial concentration in the local environment (Reading and Sperandio, 2006). Gram-negative bacteria typically use acyl homoserine lactones as auto-inducers, while Gram-positive bacteria tend to use auto-inducing polypeptides as their auto-inducers. Bacteria rely on these chemicals to detect, and accordingly alter gene expression based on the density of the auto-inducer. It has been suggested that pathogenic bacteria may rely upon quorum sensing to aid in their virulence, by promoting the expression of virulence factors when the bacteria has reached a significant concentration in the gut. However, some studies have found that quorum sensing can aid in bacterial to host communication. The auto-inducer for *Pseudomonas aeruginosa* can downregulate TNFα and IL-12 production in leucocytes and upregulated IFNγ expression (Telford *et al*., 1998). Given that bacteria can alter the host innate immune response, the role of quorum sensing for commensal bacteria needs to be further studied to understand that function of bacterial–host cross-talk in maintaining homeostasis within the gut (Hsiao *et al*., 2008).

Short-chain fatty acids, such as butyric acid/butyrate, are by-products of anaerobic fermentation by the normal members of the microbiota and are found in high levels in the GI tract. Butyrate possesses potent anti-inflammatory activity in a variety of *in vitro* culture systems (Bohmig *et al*., 1997; Andoh *et al*., 1999; Säemann *et al*., 2000; 2002; Cavaglieri *et al*., 2003). In addition, butyrate is critical for intestinal epithelial integrity and health, which reduces GI 'leak' of antigens that could stimulate immune responses (Nagler-Anderson *et al*., 2001; Bach Knudsen *et al*., 2003). Increased mucosal permeability is a very early change in colitis induced by DSS, is accompanied

by decreased cell survival, and precedes detectable changes in histology. Butyrate has been used to treat colitis and can reverse the increased mucosal permeability in this disease (Okamoto *et al*., 2000; Venkatraman *et al*., 2000). Butyrate can also prevent LPS-induced maturation of bone marrow-derived DC in mice and monocyte-derived DC in humans, including preventing homotypic DC clustering (aggregations associated with increased functional and phenotypic maturation of DCs), inhibiting IL-12, decreasing costimulatory molecule expression and blocking NF-κB translocation (Millard *et al*., 2002; Säemann *et al*., 2002). While *Lactobacilli* are poor producers of butyrate, they can produce ample quantities of lactic acid, which can be rapidly converted to butyrate by other members of the normal microbiota, such as *Clostridiales*. (Tsukahara *et al*., 2002; Wullt *et al*., 2007). Within the gut, a variety of polysaccharides can be utilized by butyrate-producing firmicutes and some species have high metabolic versatility (Louis and Flint, 2009). The ability, of some bacteria in the gut, to utilize different polysaccharide sources to produce butyrate implies that these bacteria play an important role in both dietary components and immunomodulatory effects.

Summary/conclusions

The microbiota and innate immune system in the GI tract maintain a strategic alliance that allows for nutrition absorption while restricting pathogen access to the host. To cope with the constant exposure to a diverse microbial community, the GI tract, through the actions of a number of specialized cells in the epithelium and LP, has layers of humoral, physical and cellular defences that limit attachment, invasion and dissemination of the indigenous microbiota. However, the role of the microbiota in this dynamic balance is vital. Pathogens are further kept in check through the secretion of bacteriocins, quorum sensing molecule production to alter gene expression and SCFA production. All of these bacterial metabolites aid in the control of potential pathogens. The relationship between the indigenous microbiota and the host immune system emphasizes the need for a clearer understanding of the bacterial–host partnership in maintaining GI tract homeostasis.

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