Immunity
Review

Immune Responses to the Microbiota at the Intestinal Mucosal Surface

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The mammalian intestinal mucosal surface is continuously exposed to a complex and dynamic community of microorganisms. These microbes establish symbiotic relationships with their hosts, making important contributions to metabolism and digestive efficiency. The intestinal epithelial surface is the primary interface between the vast microbiota and internal host tissues. Given the enormous numbers of enteric bacteria and the persistent threat of opportunistic invasion, it is crucial that mammalian hosts monitor and regulate microbial interactions with intestinal epithelial surfaces. Here we discuss recent insights into how the innate and adaptive arms of the immune system collaborate to maintain homeostasis at the luminal surface of the intestinal host-microbial interface. These findings are also yielding a better understanding of how symbiotic host-microbial relationships can break down in inflammatory bowel disease.

Introduction

Mammalian intestinal mucosal surfaces interface with a dense and diverse community of microorganisms. The human gut harbors an estimated 10–100 trillion organisms (Xu and Gordon, 2003). The vast majority of these microbes are bacteria, although eukaryotes, viruses (Zhang et al., 2006), and even archaea (Eckburg et al., 2005) are also represented. Intestinal bacterial communities are comprised of 500–1000 different species (Eckburg et al., 2005) and constitute an exceptionally diverse and dynamic microbial ecosystem.

These resident bacterial populations make a number of key contributions to host health, including enhancing digestive efficiency, promoting proper immune system development, and limiting pathogen colonization. In return, resident microorganisms derive benefit from association with their hosts by inhabiting a protected, nutrient-rich environment. Thus, these host-microbial associations constitute a mutually beneficial symbiosis.

The symbiotic nature of the intestinal host-microbial relationship is dependent on limiting bacterial penetration of host tissues. This is a monumental challenge given the fact that intestinal microbial communities are complex, with individual members existing at different points on the continuum between mutualism and pathogenicity. The composition of intestinal communities is furthermore dynamic and can vary as a function of host geographic location, nutrition, and immunologic status.

Intestinal mucosal surfaces directly interface with these vast, diverse, and dynamic bacterial populations, and thus present the first line of defense against microorganism penetration. Monitoring and controlling bacterial interactions with the apical surface of the intestinal epithelium is an important strategy for minimizing bacterial invasion into deeper host tissues. A number of unique immunological responses have evolved specifically to defend the intestinal mucosal interface by limiting direct bacterial contact with the epithelial surface. Recent findings indicate that both the innate and adaptive immune systems actively monitor bacterial interactions with the mucosal interface and activate immune responses that function to limit bacterial contact with the epithelial surface.

In this article we discuss the composition of intestinal microbial communities, their contributions to the physiology and health of their hosts, and the unique immune strategies that have evolved in mammals to detect and regulate bacterial interactions with intestinal surfaces. Several other articles in this *Immunity* review issue on mucosal immunology will focus in detail on subepithelial immune responses to the microbiota, so our primary focus will be on immune responses that impact bacterial-mucosal interactions on the luminal side of the epithelial barrier. Understanding how host-microbial interactions are controlled at the luminal surface of the gut epithelium is crucial for understanding how the mammalian intestine establishes symbiotic relationships with complex microbiota while avoiding overactivation of immune responses by the vast bacterial loads.

Intestinal Microbiota Composition

For many years, our understanding of the composition of intestinal microbial communities was based strictly on culturing and identifying resident organisms. However, this approach left substantial gaps in the catalog of intestinal bacterial species, because the majority of gut organisms resist culturing by currently available methods. The development of molecular profiling methods such as 16S rRNA gene sequence analysis has led to a revolution in the understanding of the intestinal microbiota by allowing culture-independent analyses of microbial community composition.

The results of these microbial profiling or ''microbiome'' analyses are just beginning to reveal the complexity of intestinal microbial communities and indicate that the variability between individuals at the bacterial species level is quite high. However, common patterns emerge when microbial communities are compared at the phylum level. Across all vertebrates, Firmicutes and Bacteroidetes are the most common intestinal phyla (Ley et al., 2008b). The intestinal Firmicutes consist primarily of

Table 1. Taxonomic Examples of Bacteria from the Intestine

Phylum	Class	Species[a]	Contributions to Host Physiology
Bacteroidetes	Bacteroidales	*Bacteroides thetaiotaomicron*	complex polysaccharide hydrolysis (Martens et al., 2008; Sonnenburg et al., 2005)
		Bacteroides fragilis	immune modulation by capsular polysaccharide biosynthesis (Coyne et al., 2005; Liu et al., 2008; Mazmanian et al., 2005, 2008)
		Bacteroides ovatus	plant polysaccharide hydrolysis (Hespell and Whitehead, 1990)
Firmicutes	Bacilli	*Lactobacillus plantarum*	inhibition of intestinal inflammation, probiotic (Petrof et al., 2009)
		Lactobacillus brevis	attachment to the Intestinal epithelium, probiotic (Avall-Jaaskelainen et al., 2003)
		Lactobacillus acidophilus	immune modulation, induction of intraepithelial lymphocyte expansion (Roselli et al., 2009)
		Lactococcus lactis	potential probiotic (Avall-Jaaskelainen et al., 2003)
		Enterococcus faecalis	immune modulation, interleukin-10 stimulation, biogenic amine synthesis, horizontal gene transfer (Are et al., 2008; Ladero et al., 2009; Salyers et al., 2004)
		Enterococcus faecium	biogenic amine synthesis, horizontal gene transfer (Ladero et al., 2009; Salyers et al., 2004)
	Clostridia	*Clostridium spp.*	butyrate metabolism, associated with inflammatory bowel disease (Gophna et al., 2006; Manichanh et al., 2006)
Actinobacteria	Actinobacteria	*Bifidobacterium longum*	immune modulation, intraepithelial lymphocyte expansion (Roselli et al., 2009)
Proteobacteria	γ-Proteobacteria	*Enterobacter cloacae*	immune modulation (Macpherson et al., 2000)

Each phylum is divided into class and species groupings. Bacterial contributions to host physiology were compiled from in vitro and in vivo studies.
[a] Indicates examples of species from the various classes of bacteria found in the mammalian intestine and is not meant to be an exhaustive list.

bacteria belonging to the Clostridia class and a subset of Mollicutes and Bacilli including the Enterococci, Lactobacilli, and Lactococci, all of which are capable of oxidizing organic sugars via fermentation to produce large amounts of lactic acid and carbon dioxide (Eckburg et al., 2005; Hold et al., 2002; Vaughan et al., 2002). Those members of the intestinal community belonging to the Bacteroidetes are represented by several *Bacteroides* species including *B. thetaiotaomicron*, *B. fragilis*, *B. ovatus*, and *B. caccae*. The remaining intestinal bacteria, accounting for less than 10% of the total population, belong to the Proteobacteria, Fusobacteria, Actinobacteria, Verrucomicrobia, Spirochaetes, and a group of bacteria closely related to Cyanobacteria (Backhed et al., 2005; Eckburg et al., 2005; Ley et al., 2008a). A number of these organisms are facultative aerobes that use molecular oxygen as a terminal electron acceptor during respiration. The inability of these bacteria to successfully compete with members of the Bacteroidetes and Firmicutes in a strictly anaerobic environment like the gut may account for their low abundance.

Microbiota Contributions to Host Physiology and Health

The mammalian gut microbiota is thought to have evolved in response to alterations in host diet (Ley et al., 2008a). Plant polysaccharides exhibit enormous structural diversity generated by various simple sugars and glycosidic linkages. The limited repertoire of glycosylhydrolases in the mammalian genome is thus insufficient to fully harvest the energy content of most plant-rich diets. Mammals harbor a relatively stable, slowly evolving genome, so natural selection alone has not yielded the range of saccharolytic enzymes required to fully extract the energy content of a varied plant-based diet. By recruiting a complex community of bacteria, mammals acquired an adaptable, rapidly evolving "metagenome" that harbors a diversity of saccharolytic enzymes. Thus, intestinal microbes can hydrolyze a variety of dietary polysaccharides that would be otherwise indigestible, further allowing flexible adaptation to dietary changes. The profound contributions of the microbiota to mammalian digestive efficiency are highlighted by studies in germ-free animals, which are microbiologically sterile and thus lack an intestinal microbiota. Germ-free rodents require approximately 30% more calories to maintain their body weight than do conventionally colonized animals (Wostmann et al., 1983), emphasizing how gut microorganisms aid their hosts in maximizing extraction of dietary energy.

The importance of the intestinal microbiota to host metabolic health is highlighted by alterations in community composition in metabolic diseases such as obesity and diabetes. For example, in obese individuals, the microbiota are dominated by members of the Firmicutes, whereas lean people harbor a higher number of Bacteroidetes (Ley et al., 2006). Alterations in the microbiota are also observed in mouse models of type 1 diabetes (T1D), corresponding to the fact that microbiota confer protection against the onset of T1D in genetically susceptible mice (Wen et al., 2008).

Although the primary driving force behind the association between mammals and their microbiota appears to be enhanced host digestive efficiency, millions of years of coevolution have led to a fundamental intertwining of mammalian and microbial physiology. As a result, intestinal microbes make key contributions to a number of other aspects of host physiology and development (Table 1). For example, symbiotic bacteria provide instructive signals for the development of key lymphocyte

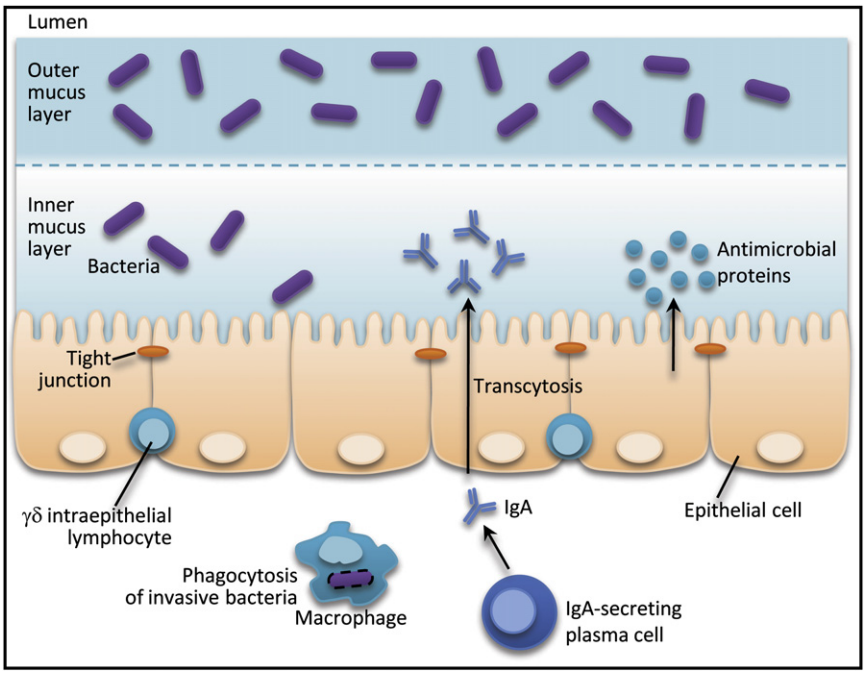

Figure 1. Cells and Molecules that Defend the Intestinal Mucosal Surface

The intestinal mucosal surface interfaces with a dense community of microbes. A thick mucus layer overlies the intestinal epithelium. Bacteria are abundant in the outer mucus layer, whereas the inner layer is resistant to bacterial penetration. Epithelial cells (enterocytes, Paneth cells, and goblet cells) form a further physical barrier against bacterial invasion and secrete antimicrobial proteins that target the bacterial cell wall, helping to eliminate bacteria that penetrate the mucus layer. Plasma cells (differentiated from B cells) secrete immunoglobulin A (IgA) that is transcytosed across the epithelial layer and secreted from the apical surface of epithelial cells, limiting numbers of mucosa-associated bacteria (Suzuki et al., 2004) and preventing bacterial penetration of host tissues (Macpherson et al., 2000; Macpherson and Uhr, 2004). γδ intraepithelial lymphocytes intercalate between intestinal epithelial cells on the basolateral side of epithelial tight junctions. γδ IELs respond to epithelial injury by secreting growth factors that promote epithelial repair (Chen et al., 2002) and by producing proinflammatory and antimicrobial factors that protect against bacterial penetration across damaged epithelia (Ismail et al., 2009). Lamina propria macrophages engulf and kill invading bacteria that have breached the intestinal barrier.

subsets. Intestinal bacteria direct class switching in human intestinal B cells (He et al., 2007), they govern the development of intestinal Th17 effector T cells (Ivanov et al., 2008), and they suppress production of T regulatory (Treg) cells (Hall et al., 2008). Additionally, intestinal bacteria impact the outcome of systemic immune responses by determining the ratio of Th1 and Th2 effector cells (Mazmanian et al., 2005). Intestinal symbionts also contribute to intestinal epithelial cell maturation and impact the host's ability to acquire essential nutrients (Hooper et al., 2001).

Intestinal microbes also play an important role in protecting their hosts against invasion by pathogenic bacteria. Two distinct factors contribute to this protective effect. First, intestinal pathogens, such as *Salmonella* and *Shigella* species, have a limited repertoire of saccharolytic enzymes in comparison to symbionts such as *Bacteroides thetaiotaomicron* (Xu et al., 2003; Xu and Gordon, 2003). Consequently, these pathogens are poorly adapted to compete with symbionts for nutrients from the host diet, restricting their luminal colonization (Stecher et al., 2005, 2007). Symbiotic intestinal microbes also stimulate immune responses that are cross-protective against pathogens. For example, invasion and dissemination of *Salmonella typhimurium* are limited by stimulation of epithelial Toll-like receptors (TLRs) by symbiotic bacteria. This stimulation triggers expression of a variety of antimicrobial proteins, which likely play a role in limiting *Salmonella* penetration of the epithelial barrier (Vaishnava et al., 2008).

Mucosal Tolerance and Ignorance

Despite their crucial contributions to mammalian metabolic health, intestinal microbes pose serious health challenges to their hosts. The intestine is frequently described as being "tolerant" to the high numbers of bacteria that reside in the lumen. However, the term "tolerance" has varied meanings depending on context, and it is important to define in what sense intestinal mucosal tissues are tolerant to the microbiota. When used in a general sense, tolerance refers to a diminished degree of responsiveness to the enormous intestinal microbial burden, which is a characteristic of mucosal surface tissues relative to other internal tissues (Sansonetti, 2004). There is also a more specific, immunological definition of tolerance, which is somewhat less applicable to understanding the intestinal host-microbial relationship. It has been known for nearly a century that feeding soluble proteins to rodents dampens the subsequent response to systemic challenge with the same protein. This phenomenon is termed "oral tolerance." This systemic hyporesponsiveness to soluble proteins is induced in the mesenteric lymph nodes (MLNs) after migration of antigen-loaded dendritic cells (DCs) from the intestinal mucosa and is a result of direct inactivation of antigen-specific T cells (Chen et al., 1995). Although this systemic hyporesponsiveness is elicited against soluble protein antigens, symbiotic bacteria do not elicit oral tolerance. This was demonstrated experimentally by showing that although mice lack specific immunoglobulin G (IgG) against the intestinal symbiont *Enterobacter cloacae*, a systemic IgG response is generated after intravenous inoculation of the organism (Macpherson et al., 2000). The systemic immune system is therefore ignorant of, rather than tolerant to, intestinal symbiotic bacteria (Macpherson et al., 2005).

The Intestinal Host-Microbial Interface

The intestinal mucosal surface is unique among tissues in that it is in continuous contact with a vast, diverse, and dynamic microbial community. Far from being a homogeneous cell population, mucosal surfaces are composed of several distinct cell types, each of which contributes in a unique way to limiting bacterial penetration across the epithelial barrier and thus maintaining immunological ignorance toward intestinal symbionts (Figure 1).

The most abundant intestinal surface cell is the enterocyte, an epithelial cell lineage. Enterocyte membranes, together with the tight junctions that they form with their neighboring cells, are essential for preventing bacterial penetration while allowing nutrient flux into host tissues. Besides providing an important physical barrier, enterocytes play a more active role in promoting luminal compartmentalization of symbiotic bacteria by secreting a variety of antimicrobial proteins. These natural antibiotics are members of several distinct protein families such as defensins, cathelicidins, and C-type lectins, and they promote bacterial killing by targeting the integrity of bacterial cell walls (Mukherjee et al., 2008). Antimicrobial proteins are produced either constitutively or are inducible by bacteria (Cash et al., 2006; Hooper et al., 2003; Putsep et al., 2000).

Gut surfaces harbor other less-abundant epithelial lineages that also help to limit bacterial penetration of host tissues. Goblet cells, found in both the small and large intestines, secrete large quantities of mucin, which is composed of highly glycosylated proteins that form a protective layer of gel-like mucus over the surface epithelium (Figure 1). Mucin glycoproteins can assemble into a protective gel-like layer that extends up to 150 μm from the epithelial surface (Gum et al., 1994). The mucus layer is composed of two distinct strata (Johansson et al., 2008). The outer layer is colonized with bacteria, whereas the inner layer is resistant to bacterial penetration, forming a protected zone adjacent to the epithelial surface (Johansson et al., 2008). The low bacterial numbers in the inner mucus layer likely also result from the fact that antibacterial factors secreted by epithelial cells are retained by the mucus layer and are prevented from diffusing into the lumen (Meyer-Hoffert et al., 2008). Mice lacking the mucin MUC2 are unable to maintain this relatively bacteria-free zone and suffer from intestinal inflammation (Johansson et al., 2008).

The Paneth cell is another intestinal epithelial lineage that plays an important role in limiting bacterial penetration into host tissues. Paneth cells secrete the majority of antimicrobial proteins produced by the small intestine. These specialized epithelial cells are situated at the base of small intestinal crypts and harbor secretory granules containing a number of microbicidal proteins including α-defensins, lysozyme, and RegIIIγ. When Paneth cells sense bacterial signals, they react by discharging their microbicidal granule contents into the gut lumen (Ayabe et al., 2000). In vivo genetic studies of this epithelial lineage indicate that Paneth cells are essential for controlling mucosal penetration of both symbiotic and pathogenic bacteria (Vaishnava et al., 2008). Mice with a genetic ablation of Paneth cells exhibit increased translocation of bacteria into the host tissues, indicating that Paneth cells contribute to maintaining luminal compartmentalization of intestinal bacteria (Vaishnava et al., 2008).

In addition to the various epithelial cell lineages that defend mucosal surfaces from bacterial invasion, subepithelial adaptive immune cells play an essential role in sequestering enteric bacteria in the gut. IgA-producing B cells are among the most abundant and best-characterized of the adaptive immune cell populations in the intestinal mucosa. These cells populate the intestinal lamina propria and secrete bacteria-specific IgA, which is transcytosed across the epithelium and deposited on the apical surface of epithelial cells (Figure 1; Macpherson et al., 2005). IgA is essential in maintaining luminal compartmentalization of intestinal bacteria, as shown by the fact that IgA deficiency leads to increased penetration of symbiotic bacteria into the host tissues (Macpherson et al., 2000; Macpherson and Uhr, 2004). Studies of mice that lack activation-induced cytidine deaminase (AID), which results in defective class-switch recombination and therefore a lack of IgA-producing plasma cells in the intestines, suggest that secreted IgA also regulates the composition and density of bacterial communities (Suzuki et al., 2004). Lack of IgA in AID-deficient (*Aicda*$^{-/-}$) mice leads to expansion of mucosa-associated bacteria such as segmented filamentous bacteria (SFB) (Suzuki et al., 2004). The exact mechanisms by which IgA confines symbiotic bacteria to the intestinal lumen remain unclear but may involve trapping bacteria in the mucus layer (Fagarasan and Honjo, 2003), recruitment of complement with subsequent bacterial lysis (Andoh et al., 1993), or promotion of phagocytic clearance of bacteria that have invaded mucosal tissues (Pasquier et al., 2005).

Another adaptive immune cell type that plays an important role in defending mucosal surfaces is the γδ T cell receptor bearing intraepithelial lymphocyte (IEL) (Figure 1). γδ IELs intercalate between intestinal epithelial cells on the basolateral side of epithelial tight junctions and contribute in several ways to restoring mucosal homeostasis after epithelial injury. First, they contribute to epithelial repair by secreting epithelial growth factors (Chen et al., 2002). Second, they express a number of proinflammatory and antimicrobial factors in response to signals from the microbiota (Ismail et al., 2009). Consistent with both of these functions, γδ T cells have been shown to play an essential role in limiting bacterial penetration across injured mucosal surfaces (Ismail et al., 2009).

Finally, a critical factor in maintaining systemic ignorance to the intestinal microbiota is the rapid elimination of symbionts that penetrate across the mucosal barrier. Symbiotic bacteria that breach the mucosal surface are quickly phagocytosed and killed by macrophages in the lamina propria (Figure 1; Macpherson and Uhr, 2004). This is in contrast to pathogens that actively interfere with macrophage microbicidal mechanisms, allowing survival and replication of these bacteria in host tissues (Sansonetti, 2004). The susceptibility of symbionts to the biocidal mechanisms of macrophages likely represents an evolutionary coadaptation with their hosts, because suppression or evasion of phagocytic killing would compromise host health and perhaps destroy the microorganisms' own niche (Macpherson et al., 2005).

Immune Mechanisms that Regulate Bacterial Interactions with Mucosal Surfaces

The cells of the intestinal mucosal surface are clearly essential for limiting bacterial invasion of intestinal tissues and preserving the ignorance of the systemic immune system toward intestinal symbionts. A key function of mucosal surface cells is to defend the luminal side of the epithelial barrier by limiting microbial interactions with mucosal surfaces. Controlling microbial-epithelial contact represents a crucial first line of host defense that is essential for maintaining the symbiotic nature of the intestinal host-microbial relationship. Recent studies have revealed the existence of regulatory feedback loops that actively sense mucosal surface bacteria and titrate appropriate immune

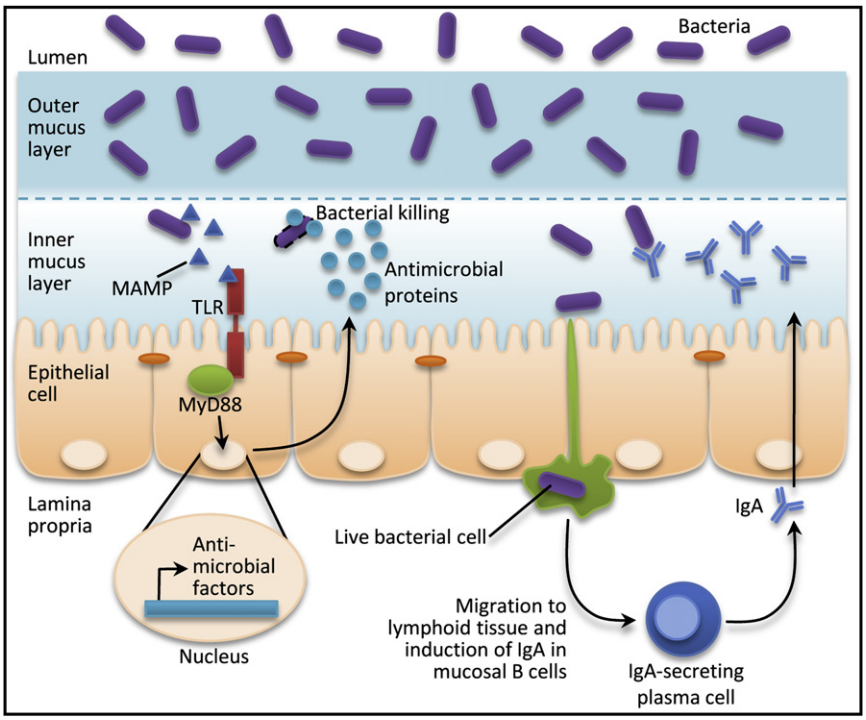

Figure 2. Innate and Adaptive Immune Feedback Loops Cooperate to Regulate Bacterial Interactions with Mucosal Surfaces

Intestinal epithelial cells sense mucosal surface bacteria through cell-autonomous Toll-like receptor (TLR) activation, activating expression of antimicrobial factors and limiting bacterial penetration of the epithelial barrier (Vaishnava et al., 2008). This suggests that epithelial cells monitor densities of mucosa-associated bacterial populations on the basis of MAMP concentration, allowing bacterial density-dependent activation of epithelial antimicrobial responses. The adaptive immune system also detects and regulates bacterial interactions with mucosal surfaces through a feedback mechanism. Dendritic cells sample live bacteria at the mucosal surface, traffic to mucosal lymphoid tissue, and induce B cells to produce bacteria-specific IgAs (Macpherson and Uhr, 2004; Rescigno et al., 2001). IgA^+ B cells differentiate to plasma cells that home to the lamina propria and secrete IgA, which limits bacterial penetration across the epithelium (Macpherson et al., 2008; Macpherson and Uhr, 2004). The bacterial census at the epithelial surface thus appears to be monitored by both epithelial TLRs and dendritic cells, triggering production of antimicrobial proteins and secretory IgA, which are retained at the epithelial surface by the mucus barrier (Meyer-Hoffert et al., 2008).

responses against these bacteria (Figure 2). These mechanisms work in concert to limit bacterial associations with mucosal surfaces, thus reducing opportunistic penetration by symbiotic bacteria and invasion by pathogens.

The mucosal adaptive immune system has evolved mechanisms for precisely monitoring and controlling bacterial interactions with mucosal surfaces. Secreted IgA functions to reduce the densities of surface-associated bacteria (Suzuki et al., 2004) and restricts penetration of symbiotic bacteria across the gut epithelium (Macpherson et al., 2000). IgA against intestinal bacteria is produced with the aid of dendritic cells that sample bacteria at various mucosal sites. Dendritic cells located beneath the epithelial dome of specialized intestinal lymphoid structures called Peyer's patches sample bacteria that penetrate the overlying epithelium. Lamina propria dendritic cells also actively sample the small numbers of bacteria that are present at the apical surfaces of epithelial cells, allowing them to monitor bacteria that have penetrated the inner mucus layer and are in close association with the mucosal surface (Figure 2; Rescigno et al., 2001). The bacteria-laden dendritic cells interact with B and T cells in lymphoid tissues including mesenteric lymph nodes and Peyer's patches. These interactions induce B cells to differentiate into plasma cells that produce IgA directed against intestinal bacteria (Macpherson and Uhr, 2004). IgA^+ plasma cells home from lymphoid sites to the intestinal lamina propria and secrete IgA that is then taken up by intestinal epithelial cells and transcytosed to the apical surface. The transcytosed IgA binds to luminal bacteria and prevents their penetration of host tissues through mechanisms that are not entirely clear.

Production of bacteria-specific IgA may also be a strategy by which the host controls the composition of luminal microbial communities. Expression of monoclonal IgA against a specific *B. thetaiotaomicron* capsular polysaccharide epitope leads to immunoselection against *B. thetaiotaomicron* expressing that epitope (Peterson et al., 2007). This indicates that IgA plays an important role in shaping intestinal microbial community composition. Modulation of capsular polysaccharide structure by *Bacteroides* species may thus be critical for allowing this genus to stably colonize the intestine (Comstock and Coyne, 2003; Coyne et al., 2005).

By coupling sampling of mucosal surface bacteria to production of bacteria-specific IgA, the adaptive immune system can precisely control the density and perhaps the composition of surface-associated bacterial populations. This system thus appears to function as a negative-feedback mechanism that maintains compartmentalization of the microbiota by limiting bacterial access to and penetration of the epithelial surface.

Studies of Paneth cells have disclosed the existence of an innate immune negative regulatory feedback loop in epithelial cells (Figure 2). Paneth cells are able to sense enteric bacteria directly through cell-intrinsic activation of TLRs (Vaishnava et al., 2008). Bacterial detection activates expression of a number of antimicrobial factors, including the antibacterial lectin RegIIIγ. Challenge experiments with both symbiotic and pathogenic bacteria reveal that epithelial cell-intrinsic sensing by TLRs functions to limit bacterial penetration of the mucosal surface. In the case of symbionts, this is seen as an effect on bacterial translocation to MLNs, whereas pathogens such as *Salmonella typhimurium* are prevented from disseminating to nonmucosal tissues such as spleen. Importantly, detection of bacteria by Paneth cell TLRs does not result in alteration of bacterial colonization densities in the intestinal lumen. This suggests that Paneth cells and their abundant antimicrobial factors function specifically to regulate bacterial interactions with the mucosal surface, without impacting the numbers of luminal bacteria. Conversely, Paneth cell TLRs sense bacteria that closely associate with the

mucosal surface and penetrate mucosal tissues, but are insensitive to overall luminal bacterial loads (Vaishnava et al., 2008).

A plausible model to explain these observations is that Paneth cell antimicrobial factors regulate the numbers of bacteria that are closely associated with the mucosal surface (Figure 2). Bacteria must become mucosa associated before uptake by DCs for translocation to MLN or before invasion into the lamina propria, so Paneth cells could limit bacterial penetration of host tissues by controlling the numbers of mucosa-associated bacteria. This idea is consistent with the fact that secreted Paneth cell antibacterial factors are retained by the mucus layer that overlies the intestinal epithelium, but are virtually absent from luminal content (Meyer-Hoffert et al., 2008). The mucus layer is also resistant to penetration by luminal bacteria (Johansson et al., 2008), so the mucus barrier may thus define a confined space that allows the host to specifically monitor and regulate a relatively limited population of bacteria that is in close contact with the intestinal surface (Figure 2). Activation of TLRs could provide information about the bacterial census in this confined space and activate expression of secreted antimicrobial proteins in order to maintain surface-associated bacterial populations at homeostatic levels. However, it is important to note that it is not yet clear whether the epithelial cell TLRs that trigger antimicrobial protein expression are localized on the apical or basolateral surfaces of epithelial cells.

The innate and adaptive immune systems thus collaborate to detect and regulate bacterial populations at intestinal mucosal surfaces. The bacterial census within the confined space created between the mucus layer and the intestinal epithelial surface appears to be monitored both by dendritic cells and by epithelial TLRs. Bacterial detection by these mechanisms triggers production of immune effectors, including secretory IgA and antimicrobial proteins, which are secreted from the apical surfaces of epithelial cells and are retained at the epithelial surface by the mucus barrier (Meyer-Hoffert et al., 2008). Innate antimicrobial proteins may be particularly important immediately after new microbial challenges, such as shifts in the composition of the symbiotic microbiota or a pathogenic infection. The specificity of the IgA response is probably of key importance for maintaining long-term homeostasis with established members of the intestinal microbiota. In support of this model, the antimicrobial protein RegIIIγ, whose expression is governed by epithelial cell-intrinsic TLR signaling (Brandl et al., 2007; Vaishnava et al., 2008), increases expression by ~3000-fold during weaning in mice (Cash et al., 2006). This suggests that RegIIIγ may function in part to maintain mucosal homeostasis in the face of the changing microbial ecology and withdrawal of passive immunity that is associated with weaning. Once the mucosal adaptive immune system has developed, IgA may become more important for maintaining homeostasis with a relatively stable adult microflora.

Limiting Immune Activation at Mucosal Surfaces

It has long been assumed that intestinal epithelial surfaces are in direct contact with the vast microbial communities present in the intestinal lumen. As a result, a number of models have been proposed to explain why mucosal tissues are not continuously inflamed. One possibility is that pattern-recognition receptors (PRRs) such as TLRs exhibit restricted expression or localization on epithelial cells. For example, it has been proposed that epithelial cells are minimally responsive to LPS because of negligible expression of TLR4 and CD14 (Abreu et al., 2001; Melmed et al., 2003). TLR5, which detects bacterial flagellin, is restricted to the epithelial cell basolateral surface, thus ensuring activation only when bacteria invade the mucosal surface (Gewirtz et al., 2001). These findings suggest that epithelial PRRs do not encounter microbe-associated molecular patterns (MAMPs) from symbiotic bacteria on the epithelial apical surface, but are positioned to trigger a response in the event of bacterial penetration of the epithelial barrier.

Additional models have been proposed in which symbiotic bacteria actively suppress or evade epithelial innate immune responses. The active suppression model is supported by studies in cultured epithelial cells demonstrating that nonpathogenic *Salmonella* strains suppress inflammatory responses by interfering with activation of NF-κB, a master proinflammatory transcription factor (Neish et al., 2000). In contrast, pathogenic *Salmonella* are not able to interfere with NF-κB activation, and thus elicit a robust proinflammatory response. The symbiont *B. thetaiotaomicron* also inhibits NF-κB function in model epithelia, but through a mechanism that is distinct from nonpathogenic *Salmonella* (Kelly et al., 2004). Other in vitro studies suggest that symbionts evade detection by the innate immune system by modifying molecular patterns that trigger PRR signaling (Munford and Varley, 2006).

Although PRR compartmentalization may play a role in limiting immune activation at mucosal surfaces, there are compelling evolutionary arguments to be made against active suppression and evasion of innate immunity as the pivotal mechanism promoting host tolerance to symbiotic bacteria in vivo. A dependence on specific bacterial characteristics to determine immune activation levels would pose a serious risk to the host for at least two reasons. First, if symbiotic bacteria actively repress innate immune signaling, the epithelium would be refractory to mounting immune responses when challenged by pathogens and would furthermore be vulnerable to opportunistic invasion by symbionts. Second, symbionts would have to harbor specific genetic determinants that confer the ability to actively suppress or evade epithelial immune responses. Given the frequency of genetic exchange among intestinal bacteria (Salyers et al., 2004), pathogens could acquire these genetic elements, allowing them to subvert or evade host immune responses.

Studies that visualize the normal spatial relationships between the microbiota and the epithelial surface suggest an alternative explanation for the lack of chronic mucosal inflammation. Johansson et al. (2008) have shown that the luminal surfaces of epithelial cells are protected from contact with large numbers of bacteria by the mucus layer. Because of the diffusion barrier provided by the mucus layer, PRRs on epithelial cells are likely to be shielded from the high densities of luminal bacteria and from their associated MAMPs. In this scenario, epithelial cells detect and respond only to bacteria that penetrate this protected zone. Apically oriented TLRs could monitor the total bacterial census in the apical protected zone, strictly on the basis of MAMP concentration, thus allowing bacterial density-dependent activation of epithelial antimicrobial responses that are governed by TLRs. Together with the negative-feedback loop that controls the IgA response to surface-associated bacteria, innate sensing

of bacteria in the protected zone could provide a sensitive mechanism for homeostatic control of surface-associated bacterial population densities. This model does not require that symbionts actively subvert innate immune signaling or that epithelial PRRs be able to distinguish between symbionts and pathogens, because neither should penetrate the protected zone at the mucosal surface under ideal conditions. Stated in simple terms, mucosal tissues may exhibit tolerance to the dense communities of intestinal bacteria largely because they are normally protected from direct bacterial contact.

Tissue-Specific Modulation of Epithelial Innate Immune Responses

The threshold at which epithelial PRR signaling is triggered may be modulated in a tissue-specific manner by specific epithelial factors. For example, studies in zebrafish demonstrate that host intestinal alkaline phosphatase (IAP) modifies bacterial LPS and dampens its proinflammatory potential (Bates et al., 2007). Because of its localization at the epithelial brush border, IAP likely modifies LPS specifically at the epithelial surface while leaving intact LPS that is encountered at subepithelial sites (Bates et al., 2007). In this way, IAP may control the concentrations of LPS required to activate epithelial cell innate immune signaling. This threshold concentration would be governed both by the affinity of LPS binding to its receptor(s) and the rate at which IAP dephosphorylates LPS (Vaishnava and Hooper, 2007).

Intestinal epithelial cells also express factors that inhibit PRR signaling. One such factor is A20, a zinc-finger protein whose expression is controlled by NF-κB (Krikos et al., 1992). A20 has a ubiquitin-editing activity (Wertz et al., 2004) that inhibits NF-κB activation by downregulating key polyubiquitination-dependent mediators of inflammatory signaling, including TNF-receptor-associated factor 6 (TRAF6) (Deng et al., 2000) and receptor-interacting protein kinase (Li et al., 2006). A20-deficient mice (*Tnfaip3*$^{-/-}$) mice develop severe intestinal inflammation, suggesting that A20 is critical for regulating the threshold of immune activation in the gut (Lee et al., 2000; Turer et al., 2008).

By expressing factors such as IAP and A20, intestinal epithelia could modulate the threshold bacterial density required to trigger an innate immune response. Such tissue-specific strategies may contribute to the relative tolerance of intestinal surfaces to the presence of high luminal bacterial loads.

Epithelial-Bacterial Interactions in Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is characterized by severe inflammation of the colon, rectum, and/or the distal small intestine. Although the exact causes of IBD remain poorly understood, several of its pathologic features suggest that the disease derives in part from dysregulated control of bacterial interactions with the mucosal surface. For example, IBD patients exhibit increased numbers of mucosal surface-associated bacteria (Swidsinski et al., 2005), suggesting a failure of mechanisms that normally sequester microbiota from direct contact with the surface epithelium. Moreover, several IBD risk alleles alter epithelial cell function, impairing production of antimicrobial peptides and/or mucus. First, polymorphisms in the cytoplasmic peptidoglycan receptor NOD2 are associated with ileal Crohn's disease, a specialized manifestation of IBD (Hugot et al., 2001; Ogura et al., 2001). Patients with NOD2 defects have reduced α-defensin antimicrobial peptide expression in Paneth cells, coincident with severe intestinal inflammation (Wehkamp et al., 2005). It is possible that reduced α-defensin production leads to increased numbers of surface-associated bacteria, which could contribute to uncontrolled inflammation, perhaps in conjunction with other genetic defects. Second, Atg16L1 is a Crohn's disease risk allele that contributes to intestinal inflammation by impairing exocytosis of Paneth cell secretory granules, thereby inhibiting antimicrobial protein release (Cadwell et al., 2008). Third, the transcription factor XBP1 is required for normal development of Paneth cells and goblet cells (Kaser et al., 2008). *Xbp1*$^{-/-}$ mice, which lack Paneth cells and show reduced numbers of goblet cells, exhibit spontaneous intestinal inflammation, and hypomorphic variants of XBP1 are linked to IBD (Kaser et al., 2008). Together, these studies suggest that defects leading to reduced antimicrobial protein and/or mucus production may increase the likelihood of bacterial invasion of the epithelial barrier with subsequent inflammation.

Summary and Future Prospects

Control of bacterial interactions with the intestinal mucosal surface is a critical first line of host defense that is key for maintaining a symbiotic relationship with the intestinal microbiota. By evolving innate and adaptive mechanisms for sensing bacteria at the mucosal surface, and by coupling bacterial sensing to production of secreted antimicrobial proteins and IgA, the host can flexibly adapt to new microbial challenges while maintaining homeostasis with relatively stable microbial communities. However, our understanding of host-bacterial interactions at the mucosal interface remains rudimentary. We still know relatively little about the spatial organization of microbial communities in the intestine, and how mucosa-associated bacterial species may differ from those that predominate in the lumen. Even less is known about the bacterial factors that regulate association with intestinal mucosal surfaces, or whether bacterial species that predominate at the mucosal surface exhibit unique genetic characteristics that differentiate them from bacteria that are found strictly in the lumen. Finally, it is not clear how antimicrobial proteins and IgA may alter the physiology of mucosa-associated bacteria and how these secreted immune effectors may impact microbial functions such as genetic exchange. Future studies of host-bacterial associations at the mucosal interface should reveal new insight into the factors that determine the outcome of interactions between symbionts and their mammalian hosts.

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