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Innate immune recognition of the microbiota promotes host-microbial symbiosis

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Pattern-recognition receptors (PRRs) are traditionally known to sense microbial molecules during infection to initiate inflammatory responses. However, ligands for PRRs are not exclusive to pathogens and are abundantly produced by the resident microbiota during normal colonization. Mechanism(s) that underlie this paradox have remained unclear. Recent studies reveal that gut bacterial ligands from the microbiota signal through PRRs to promote development of host tissue and the immune system, and protection from disease. Evidence from both invertebrate and vertebrate models reveals that innate immune receptors are required to promote long-term colonization by the microbiota. This emerging perspective challenges current models in immunology and suggests that PRRs may have evolved, in part, to mediate the bidirectional cross-talk between microbial symbionts and their hosts.

Conventional wisdom suggests that the immune system evolved to combat infection and that distinguishing between self and non-self molecules is a basic feature of innate immunity. As Charles Janeway proposed, the recognition of microbial molecules, termed pathogen-associated molecular patterns (PAMPs), is critical to selectively drive immune responses to infectious agents[1]. Studies identifying and characterizing host receptors that recognize specific PAMPs, called 'pattern-recognition receptors' (PRRs), have provided evidence that PRR signaling is critical in coordinating immune responses and protection against pathogens[2–5]. This view, however, has been challenged by the emerging appreciation that animals have a diverse and complex symbiotic microbiota[6–10], which normally does not trigger inflammation. PAMPs, by definition, are universally conserved, generally invariant and essential in all microorganisms. Thus, PAMPs are not limited to pathogens but are also common to the microbiota. As such, it has been proposed that these molecules be renamed microbe-associated molecular patterns (MAMPs)[11]. Furthermore, host PRRs are constantly exposed to MAMPs in the absence of infection. These MAMPs are largely provided by the commensal microbiota that colonize our skin and mucosal surfaces. Despite the continuous presence of many MAMPs, commensal microbes usually do not elicit inflammatory responses but rather may contribute to various aspects of host development and enhanced immune function[12]. To our surprise, this beneficial influence is mediated, in part, by commensal stimulation of host PRRs[13].

How these molecules and receptors can achieve such divergent and opposing responses between pathogens and symbiosis is a frontier in our understating of innate immunity. It has been proposed that the context in which the host receives MAMP stimulation dictates the quality of the immune response. During infection, MAMP signals are received in the presence of other cues, such as cell damage caused by infection[14] and/or cytosolic detection of MAMPs[15], resulting in inflammation. During symbiosis, not only does the microbiota generally not harm host cells and MAMPs are sensed in the absence of exposed self antigens, but it appears that some MAMPs directly promote beneficial outcomes. In this Review, we will focus on how recognition of MAMPs by PRRs under steady-state conditions promotes immune development, protection from disease and maintains homeostasis. The concepts presented here collectively demonstrate that PRRs may have evolved in both the invertebrate and vertebrate immune systems to communicate with commensals and maintain beneficial, symbiotic coexistence with the microbiota.

Pattern recognition in *Drosophila* promotes homeostasis

Extensive work using *D. melanogaster* as a model system has highlighted the important functions of PRRs in host defense as well as in homeostasis. Toll, one of the first PRRs to be identified, was initially discovered in *D. melanogaster*[16]. However, the realization that *D. melanogaster* Toll does not directly recognize MAMPs, unlike the PRRs in the mammalian Toll-like receptor (TLR) signaling pathway, left the open question of how bacterial ligands are recognized. *D. melanogaster* has 13 peptidoglycan recognition protein (PGRP) genes that are alternatively spliced into 19 different proteins, which is one of the largest repertoires of PGRPs currently known for any organism[17]. The role of *D. melanogaster* PGRPs as PRRs was discovered during the identification of upstream receptors that activate the signal-transduction pathways, Toll and Imd (immune deficiency)[18,19], which are highly similar to the mammalian interleukin 1 (IL-1)–TLR and tumor necrosis factor (TNF) pathways[20]. However, Toll does not function as a PRR because it does not directly recognize MAMPs[21].

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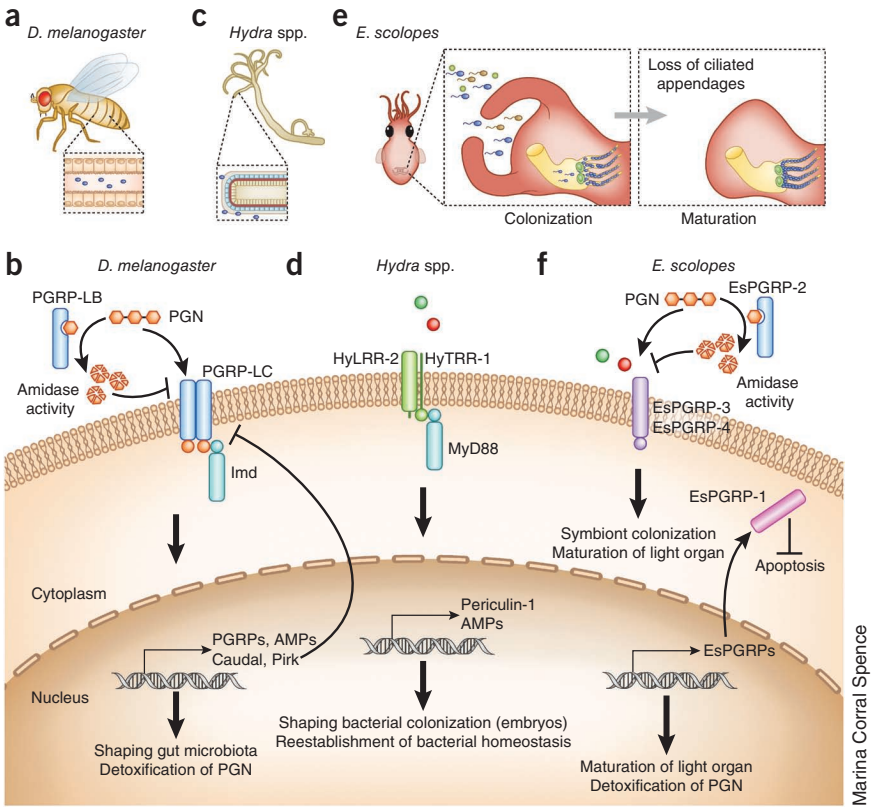

Figure 1 PRRs in invertebrate systems. (**a**) Macroscopic view of *D. melanogaster* and the midgut. (**b**) PGRP-LC expression on the cell surface senses meso-diaminopimelic acid and peptidoglycan (PGN) from Gram-negative bacteria, triggering the Imd pathway and the production of additional PGRPs, AMPs, Caudal and Pirk (negative regulators of the Imd pathway). The amidase PGRP-LB, induced upon Imd activation, cleaves microbiota-derived peptidoglycan, blocking additional activation of the Imd pathway. (**c**) Macroscopic view of the *Hydra* body plan consisting of two layers of epithelial cells, with the outermost layer exposed to bacterial symbionts. (**d**) PRR signaling in *Hydra* is mediated by the HyLRR-2–HyTRR-1 complex, where HyLRR-2 serves as the receptor and HyTRR-1 serves as the transmembrane domain. Upon MAMP stimulation, MyD88 is recruited, triggering the production of AMPs such as periculin-1. (**e**) Macroscopic view of the bobtail squid (*E. scolopes*) and the maturation of the light organ, induced upon colonization with *V. fisheri*. (**f**) *E. scolopes* expresses four PGRPs. EsPGRP-3 and EsPGRP-4 function as cell surface receptors that recognize MAMPs, resulting in signaling activation. EsPGRP-2 is secreted by epithelial cells and crypts of the light organ, and detoxifies peptidoglycan via amidase activity to dampen immune responses. Loss of EsPGRP-1 upon colonization by *V. fisheri* results in apoptosis and loss of appendages, marking the maturation of the light organ.

Instead, the soluble PGRPs PGRP-SA and PGRP-SD form a complex upstream of the Toll pathway[18,19]. Upon recognition of peptidoglycan (from Gram-positive bacteria), PGRP-SA triggers a protease cascade, resulting in the dimerization of Toll, leading to activation of the transcription factor NF-κB and production of antimicrobial peptides (AMPs)[18]. Additionally, fungi and Gram-negative bacteria trigger the Imd pathway via PGRP-LC, mediating innate immune responses[19,22,23]. Upon activation of the Imd pathway, NF-κB signaling results in the production of AMPs, targeting invasive pathogens during infection (**Fig. 1a**,**b**).

In addition to regulating the production of AMPs in response to infection, the Imd pathway also shapes the response to commensal microbiota. The Imd pathway regulator Caudal (**Fig. 1b**) is important for shaping the composition of gut microbiota[24]. Caudal suppresses Imd-dependent expression of AMPs by blocking promoters of genes encoding AMPs. Additional negative regulators of Imd, such as Pirk (**Fig. 1b**), sequester PGRP-LC in the cytoplasm to prevent exposure to the peptidoglycan and activation of the Imd pathway, which is critical in regulating commensal populations, maintaining intestinal homeostasis and restraining overactive immune responses[25,26].

In addition to sensing bacterial ligands and activating an inflammatory response, a subset of *Drosophila* PGRPs have amidase activity and participate in hydrolyzing proinflammatory peptidoglycan into nonimmunostimulatory fragments[27,28] (**Fig. 1b**). These catalytic PGRPs, such as PGRP-LB and PGRP-SC, limit the availability of peptidoglycan released from symbiotic gut bacteria, preventing overactivation of the Imd pathway and dampening the immune response. In fact, deletion of genes encoding PGRP-LB and PGRP-SC leads to a tenfold increase in the amount of AMPs compared to that in wild-type flies[28], revealing the importance of amidase PRGPs in detoxifying the biological activity of peptidoglycan and controlling an overactive systemic immune response. Although these studies highlight a role of PGRPs in maintaining gut homeostasis by controlling the immune responses to commensal bacteria, additional studies are necessary to reveal how this pathway discriminates between symbiotic and pathogenic bacteria. What is clear, however, is that sensing of bacterial ligands in *Drosophila* is critical for host-microbial symbiosis in the absence of infection.

Hydra TLR signaling promotes bacterial colonization

A member of the second oldest phylum, Cnidaria, *Hydra* spp. are a simple model system to study the evolutionary origins of commensalism (**Fig. 1c**). The intimate relationship between *Hydra* and specific bacterial species reveals that the primitive immune system of *Hydra* can recognize its symbionts, promoting a highly evolved partnership with its host[29]. *Hydra* lacks migratory phagocytic cells and hemolymph, instead relying on PRRs and the production of AMPs by epithelial cells for immune protection[30]. In the absence of conventional TLRs, analysis of the *Hydra magnipapillata* genome had identified two genes encoding proteins with a Toll–IL-1 receptor domain and a transmembrane domain (HyTRR-1 and HyTRR-2), each lacking leucine-rich repeats (LRR) in the extracellular region, which is typical of classical TLRs[31]. Two additional genes have been identified encoding transmembrane proteins with TLR-related LRRs in the extracellular region (HyLRR-1 and HyLRR-2). Signaling by the HyLRR-2 and HyTRR-1 complex after microbial pattern recognition recruits the adaptor protein MyD88 and triggers the production of AMPs, such as periculin-1 (ref. 30; **Fig. 1d**). In addition to bactericidial activity, maternal expression of periculin-1 is involved in shaping the microbiome of the embryo[32]. In transgenic polyps that overexpress periculin-1a, the structure of the bacterial community is dramatically different, with a decrease in β-proteobacteria and an

increase in α-proteobacteria. Additionally, these transgenic polyps have a significantly lower bacterial load compared to control polyps, indicating that periculin-1a has a role in both controlling and shaping bacterial colonization during *Hydra* development.

In addition to defense against pathogenic microbes, MyD88 signaling also contributes to host-mediated colonization by commensal bacteria. Knockdown of the transcript encoding MyD88 in *Hydra* showed that although the overall composition of the bacterial microbiota remained unchanged, MyD88-mediated PRR signaling promotes reestablishment of bacterial homeostasis[33]. Thus studies in *Hydra*, a primitive organism, reveal that recognition of commensal bacteria appears to be an ancient function of innate immune signaling, suggesting that PRRs have evolved to mediate host-microbe communication.

PRR signaling maintains squid-*Vibrio* symbiosis

The relationship between the Hawaiian bobtail squid, *Euprymna scolopes*, and the Gram-negative bacteria, *Vibrio fisheri*, is one of the most extensively studied examples of host-microbial symbiosis to date[34–36]. The bioluminescent bacterium, found in seawater, colonizes the crypts of the light organ in the juvenile host as a monospecific symbiont[37] (**Fig. 1e**). The light organ is continually exposed to many microbes in the surrounding seawater, similar to the mammalian intestinal tract. Yet the squid can selectively and exclusively harbor a single species of bacteria. This intimate symbiosis allows the nocturnal squid to use light produced by *V. fisheri* as a counterillumination camouflage strategy, a clever adaptation to avoid detection by predators swimming below.

Elegant studies have shown that shortly after hatching of the juvenile squid, the symbiont promotes colonization and beneficial coexistence with its host. MAMP signaling mediates this specificity and initiates the colonization process, promoting development of the light organ in the host[38,39] (**Fig. 1f**). During the initial interaction with the squid, the bacterial symbiont induces a sequence of events to promote colonization. The juvenile light organ has a pair of ciliated appendages, which secrete mucus upon exposure to the bacterial trigger, a peptidoglycan fragment known as tracheal cytotoxin[38]. This mucus not only serves as a chemoattractant for *V. fisheri*, but also a substrate for growth and aggregation to seed the founding microbial population of the light organ. Following the initial colonization, the symbiont then induces a second sequence of events driving the maturation of the squid light organ by directing a switch from a permissive to a restrictive state to ensure *V. fisheri* colonization dominance. The bacterial triggers that drive this switch, lipopolysaccharide and tracheal cytotoxin, are necessary to induce morphogenesis of the light organ via apoptosis and regression of epithelial cells, leading to a loss of ciliated appendages and preventing entry of other bacteria into the developed light organ[40] (**Fig. 1e**,**f**). Therefore, the squid uses MAMPs as morphogens that direct normal developmental programs.

How does *E. scolopes* sense the symbiotic bacterial cues? PGRPs have been implicated in mediating responses to MAMPs during symbiosis[41]. To date, five EsPGRP have been identified in the bobtail squid[39,42], although only EsPGRP-1 and EsPGRP-2 have been extensively characterized, as both are expressed in the juvenile light organ. EsPGRP-1 is expressed in the ciliated epithelium of the light organ, and upon colonization by the symbiont, the bacterial signal tracheal cytotoxin directs the loss of EsPGRP-1, inducing the initiation of the apoptotic pathway[43] (**Fig. 1f**). Apoptosis of the ciliated epithelium and the light organ appendage marks the completion of development and colonization of the squid light organ. Alternatively, EsPGRP-2 has N-acetyl-muramyl-L-alanine amidase activity, which allows hydrolysis of peptidoglycan for degradation, diminishing its toxicity and proinflammatory properties[44] (**Fig. 1f**). This activity is particularly important as *V. fisheri* colonization occurs in high numbers and peptidoglycan is continually shed in the light organ, posing the risk of inflammatory distress. Similar to *Drosophila*, the EsPGRP-2 amidase that degrades peptidoglycan into non-immunostimulatory fragments implicates it in the attenuation of inflammatory responses, a prerequisite for symbiosis. Thus *V. fisheri* has evolved to co-opt innate immunity to promote colonization of the squid, conferring benefits to both partners during mutualism. The collective data from the invertebrate models reveal that PRRs are instrumental in host defense against infection; the recent discovery of the role of PRRs during symbiosis reveals the dual function of innate immunity in responding to both pathogens and the commensal microbiota.

Maintaining tolerance in zebrafish

The zebrafish (**Fig. 2a**) has gained considerable attention as a model vertebrate system[45] because of the ease of genetic manipulation[46,47], the ability to study it in germ-free conditions[48] and the resemblance of its physiology to that of mammals. Specifically, the conserved PRR system is highly similar to that in other vertebrates, and the zebrafish model has proven to be a useful tool examining host-microbe interactions[49]. A recent study revealed a role of the zebrafish intestinal alkaline phosphatase in dephosphorylation of the immunostimulatory lipopolysaccharide, which results in modulation of intestinal inflammation in response to commensal microbes, the primary source of lipopolysaccharide in the gastrointestinal tract[50] (**Fig. 2b**). Lipopolysaccharide is a major component of the outer membrane of Gram-negative bacteria, found in pathogenic and commensal bacteria alike. However, recognition of lipopolysaccharide by TLR4 results in induction of signaling cascades that lead to activation of NF-κB and the production of proinflammatory cytokines. Upon bacterial colonization (or administration of exogenous lipopolysaccharide), expression and activity of intestinal alkaline phosphatase is induced in a MyD88-dependent fashion[50]. TLR orthologs have been identified in the zebrafish genome, with several TLR4 orthologs reported[51] and hypothesized to recognize lipopolysaccharide (several studies, however, have reported a lack of lipopolysaccharide responsiveness in some zebrafish TLR4 paralogs[52,53]). Deficiency in intestinal alkaline phosphatase results in excessive intestinal neutrophil infiltration, a process involving functional MyD88 and TNF. Thus, commensal-derived lipopolysaccharide signaling via TLR4-MyD88 leads to upregulation of intestinal alkaline phosphatase and detoxification of lipopolysaccharide, and prevents inflammatory responses to the resident microbiota. Additionally, after its initial discovery in zebrafish, intestinal alkaline phosphatase has been shown to be involved in the maintenance of homeostasis in the bobtail squid[54] and the mouse[55,56]. The identification of intestinal alkaline phosphatase revealed yet another mechanism in which PRR signaling is crucial in preventing uncontrolled inflammation in response to commensal microbes, thus promoting colonization by the microbiota.

PRR signaling promotes intestinal homeostasis in mice

The mammalian intestine is home to an abundant and complex consortium of bacteria that orchestrate important immune and metabolic functions in the host. Several studies in the mouse (**Fig. 2c**) have shown that the composition of the microbiome as well as the spatial location of gut bacteria in the intestine dictates the balance of tolerogenic (**Fig. 2d**) versus proinflammatory (**Fig. 2e**) immune responses in the gut. PRR signaling in mice appears to have a key role both in

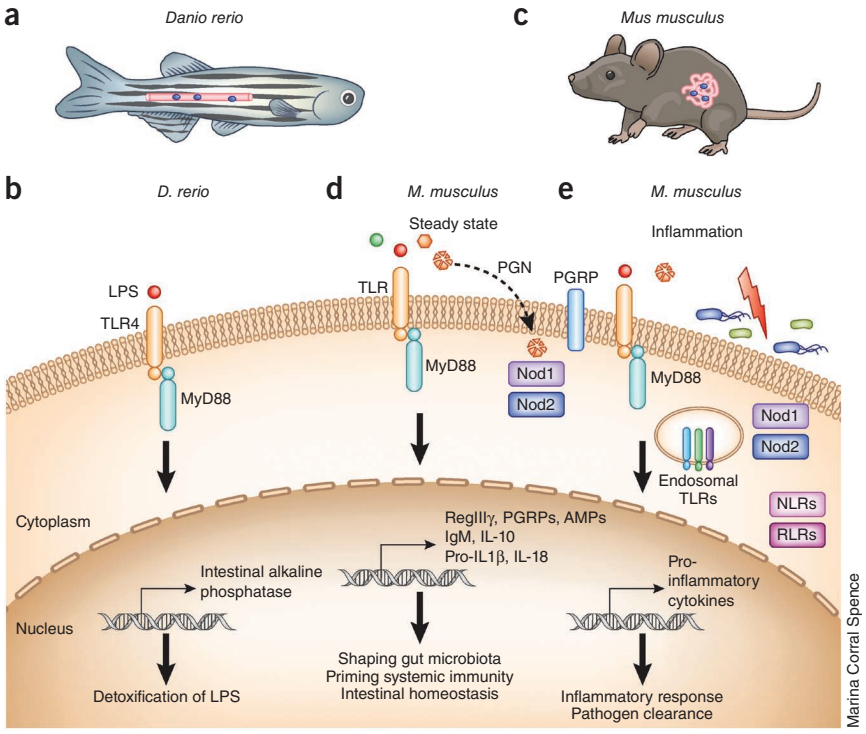

Figure 2 PRRs in vertebrate systems. (**a**) Macroscopic view of zebrafish and its intestinal tract. (**b**) Lipopolysaccharide (LPS) stimulation of TLR4 in zebrafish results in MyD88 recruitment and triggers the production of intestinal alkaline phosphatase (IAP) in epithelial cells. Induction of IAP is critical in maintaining gut homeostasis through dephosphorylation of microbiota-derived LPS. (**c**) Macroscopic view of mouse and its gastrointestinal tract. (**d**) Under steady-state conditions, signaling via TLRs and the receptors Nod1 and Nod2 by microbiota-derived MAMPs results in the recruitment of MyD88 and production of AMPs (such as PGRPs, RegIIIγ and defensins) and other mediators of intestinal homeostasis. (**e**) Under inflammatory conditions, pathogen-derived MAMPs trigger various TLRs, Nod-like receptors (NLRs) and RIG-I-like receptors (RLRs). Invasive microbes can stimulate cytosolic and endosomal PRRs. This infectious process results in a pro-inflammatory response, leading to clearance of pathogens.

directing the spatial segregation of the microbiota and in shaping the composition of the commensal microbiota.

The gastrointestinal surface is coated with a layer of mucus[57], largely limiting bacterial access to the epithelium that separates the trillions of microorganisms in the gut lumen from host tissues[58] (**Fig. 3a**). Additionally, secretory immunoglobulin A (IgA) maintains barrier functions of the epithelium[59,60]. This physical separation of microbiota and intestinal gut mucosa is also mediated by MyD88 signaling in intestinal epithelial cells[61]. In the absence of MyD88, commensal bacteria gain proximity to the intestinal surface, resulting in a 100-fold increase in bacteria associated with the mucosa relative to that in wild-type mice. Similar to invertebrate models, in vertebrate systems MyD88 signaling results in production of AMPs as well[62]. MyD88 signaling controls the production of several AMPs by specialized intestinal epithelial cells, termed Paneth cells, such as RegIIIγ, a C-type lectin that targets Gram-positive bacteria (**Fig. 3a**)[61–63]. RegIIIγ-deficient mice exhibit a defect in the spatial segregation of mucosa-associated bacteria with the microbiota penetrating the mucus layer and making intimate contact with host tissue, a phenotype similar to that of intestinal epithelial cell–specific MyD88-deficient mice[61]. The increased bacterial burden at the intestinal surface of the small intestine results in immune activation, with elevated IgA response and $CD4^+$ helper T cells that produce interferon-γ (T helper type 1 response). Upon depletion of the microbiota with antibiotic treatment, the elevated IgA and T helper type 1 responses in Myd88-deficeint mice were diminished. This suggests that commensal microbiota drives signaling of MyD88 via stimulation of TLR under steady-state conditions, inducing the expression of AMPs, such as RegIIIγ, to restrict bacterial colonization on intestinal surfaces and limit an immune response to resident bacteria.

In addition to controlling the spatial segregation of intestinal bacteria, PRRs have a key role in shaping the composition of the microbiota. The impact of this process is apparent in studies of inflammatory bowel disease. Crohn's disease, a form of inflammatory bowel disease, involves an overactive immune response and impaired barrier function in the gut. Genome-wide association studies have connected polymorphisms in the gene encoding the Nod2 innate immune receptor to increased susceptibility to Crohn's disease[64,65]. Nod2 is a member of the NLR family of cytoplasmic proteins. It recognizes a derivative of peptidoglycan, muramyl dipeptide, found in both Gram-positive and negative bacteria. Signaling of Nod2 requires the adaptor RIP2, which activates downstream signaling cascades involving NF-κB. Nod2 deficiency has been linked to impaired expression of Paneth cell α-defensins, a family of AMPs[66,67]. Accordingly, Nod2 is required for the regulation of commensal microbiota in the terminal ileum, where Nod2 is mainly expressed[68]. An increase in *Bacteroides* spp. and *Firmicutes* spp. is observed in Nod2-deficient mice compared to wild-type littermates, revealing that Nod2 is involved not only in shaping the composition of the microbiota, but also in restricting bacterial numbers in the ileal crypts. Notably, Nod2-deficient mice are impaired in the ability to clear the pathobiont *Helicobacter hepaticus*. Similarly, RIP2-deficient mice exhibit the same phenotype, and *Rip2* has also been identified as a Crohn's susceptibility gene[69], corroborating the role of this innate immune signaling pathway in shaping the commensal microbiota.

Consistent with the role of Nod2 in promoting host-microbial symbiosis, the PGRP family is involved in the regulation of commensal microbiota in mice. Mammals have four PGRPs (PGRP-1–4), where PGRP-1, PGRP-3 and PGRP-4 are directly bactericidal and PGRP-2 is an amidase that hydrolyzes peptidoglycan—all of which are expressed in the colon[70]. Mice deficient in any one of the PGRPs harbor a microbiota that promote increased sensitivity to dextran sulfate sodium (DSS)-induced colitis, a mouse model of inflammatory bowel disease[71]. Indeed, germ-free mice inoculated with stool from PGRP-deficient donor mice are more sensitive to DSS-induced colitis compared to mice that received stool from wild-type mice and exhibit greater mortality, weight loss and colitis scores. Thus, mammalian PGRPs are important in shaping a homeostatic commensal microbiota and preventing intestinal inflammation[72].

The studies we outline involving MyD88-mediated induction of RegIIIγ, Nod2 and PGRPs highlight the role of innate immune system signaling in shaping the composition and localization of the mammalian microbiota. It is important to emphasize that although this

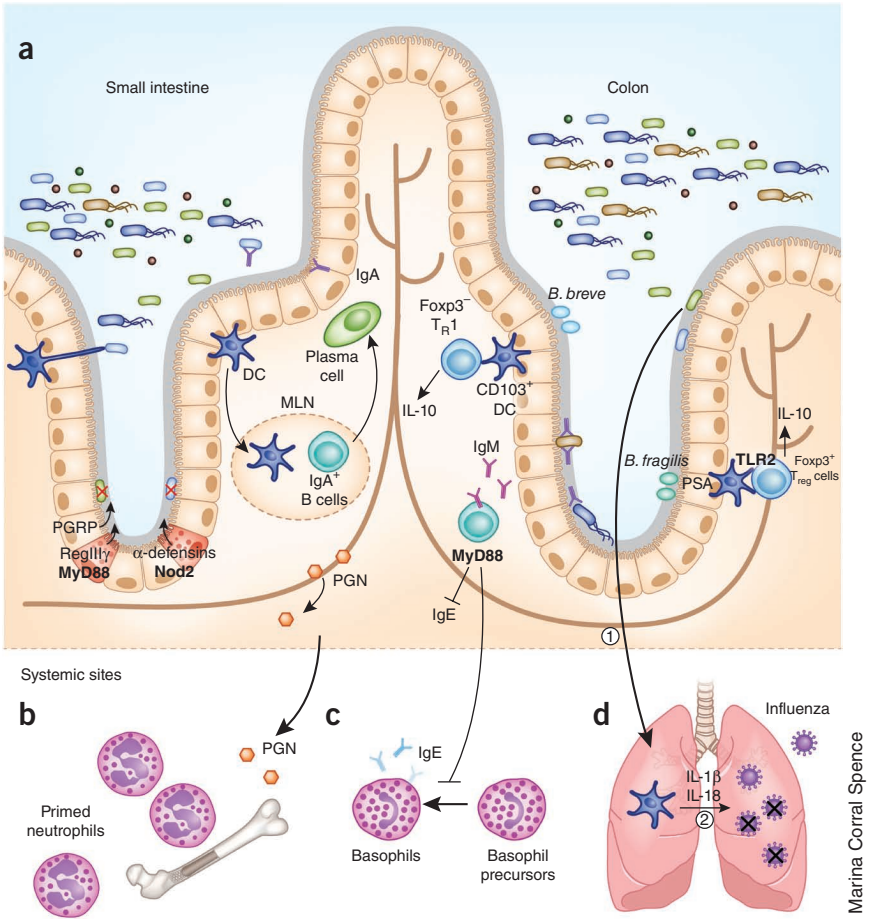

Figure 3 PRR signaling promotes immune homeostasis. (**a**) The small intestine and colon comprise a single layer of intestinal epithelial cells separating the abundant microbiota from host tissues. A complex mucus layer coats the epithelium, protecting the gastrointestinal tract from potential invasion. Additional protective mechanisms maintain intestinal homeostasis; many of these mechanisms are controlled by PRR signaling by commensal microbes. In the small intestine, AMPs such as PGRPs, RegIIIγ and defensins are induced upon PRR stimulation by commensals. In the colon, commensal microbes *B. fragilis* and *B. breve* signal through TLR2 to induce T_{reg} cells. The MAMP PSA, expressed by *B. fragilis*, drives the development of IL-10–producing Foxp3$^+$ T_{reg} cells via TLR2. *B. breve*, however, induces IL-10–producing Foxp3$^-$ T_R1 cells via CD103$^+$ dendritic cells in a TLR2-dependent manner. Additionally, B cell–intrinsic MyD88 signaling promotes secretion of IgM, which is important in controlling systemic spread of bacteria after intestinal injury. (**b**) Peptidoglycan derived from the gut microbiota is necessary to prime neutrophils in bone marrow stores in a Nod1-dependent manner. (**c**) MyD88 signaling in B cells suppresses serum IgE and inhibits the differentiation of basophils in systemic sites. (**d**) Commensal gut microbiota induces the production of pro-IL-1β and pro-IL-18 during steady state (signal (1)). During an influenza infection in the lungs, activation of IL-1β and IL-18 mediated by caspase-1 (signal (2)) is critical for clearance of influenza. DC, dendritic cell; MLN, mesenteric lymph node.

may be the result of bidirectional cross-talk between symbiotic gut bacteria and the host, there is clearly a role for detection of pathogens by these immune regulators. Future studies aimed at understanding how innate immunity shapes the microbiome, and whether gut bacteria actively signal through PRRs to coordinate the immune response, will be critical. As such, the identification of Nod1 as a receptor used by the microbiota to induce isolated lymphoid follicles in the gut may provide a glimpse into a process where specific gut bacteria educate the development of the immune system via PRRs[73].

TLRs mediate host-protective immune responses

Several studies have identified a role for TLRs in mediating non-inflammatory immune responses to the microbiota, challenging the paradigm that PRRs have evolved solely to recognize and respond to pathogens. MyD88-deficient mice are more susceptible to DSS-induced colitis, suggesting that commensal bacteria may be directly recognized by TLRs under steady-state conditions to mediate host-protective responses[74]. To corroborate this notion, depletion of gut bacteria with antibiotics results in increased susceptibility to DSS; remarkably, oral feeding of lipopolysaccharide and lipoteichoic acid corrects this predisposition to colitis, revealing that TLR ligands have beneficial effects on the host[74]. As DSS induces intestinal injury, these findings suggest that TLR signaling by the microbiota leads to maintenance of intestinal epithelial homeostasis in the absence of enteric pathogens.

Peptidoglycan, lipopolysaccharide and other widely conserved bacterial patterns mediate host-microbial interactions that span from *Hydra* to mice. It appears, however, that species-specific TLR ligands have evolved in various components of the microbiota. The human commensal *Bacteroides fragilis* produces polysaccharide A (PSA) that directs the maturation of the mammalian immune system, specifically promoting the development and function of CD4$^+$ T cells[75]. One outcome of this process is the activation of Foxp3$^+$ regulatory T (T_{reg}) cells to produce IL-10, which shapes a tolerogenic immune response that is protective in mouse models of inflammatory bowel disease and multiple sclerosis[76–78] (**Fig. 3**). PSA is a unique TLR2 ligand found to our knowledge only in the human microbiome, which orchestrates anti-inflammatory immune responses that ameliorate diseases mediated by the immune system. TLR2-deficient mice are not protected by PSA against colitis[79]. PSA is delivered by outer membrane vesicles that bud from the surface of *B. fragilis* and are internalized by intestinal dendritic cells[80]. TLR2-deficient dendritic cells do not promote responses of Foxp3$^+$ T_{reg} cells and production of IL-10 *in vitro*, demonstrating that specific gut bacterial molecules have evolved to promote benefits to the host via PRR signaling. The concept of interkingdom communication via innate immune receptors has been recently extended to *Bifidobacterium breve*, a probiotic that signals through TLR2 to induce another subset of regulatory T cells, termed Tr1 cells[81]. Although the bacterial ligand remains unknown, *B. breve* prevents intestinal inflammation by activating IL-10–producing Tr1 cells in the gut. The demonstration that *B. fragilis* and *B. breve* mediate beneficial adaptive immune responses via TLR signaling reveals a deep co-evolutionary symbiosis founded on a 'molecular dialog' using the PRR system.

PRRs on lymphocytes promote intestinal homeostasis

The PRRs discussed up to this point are expressed among epithelial cells and myeloid cells, which comes to no surprise, as PRRs are

classic innate immune signaling receptors (and invertebrates lack an adaptive immune system). However, several studies have highlighted the importance of TLR-MyD88 signaling among lymphocytes. In B cell–specific MyD88-deficient mice, bacteria disseminate to systemic sites, such as liver or lung, after DSS-induced damage of the colon, but not in epithelial cell–specific or dendritic cell–specific MyD88-deficient mice[82]. Further, it has recently been appreciated that subsets of T cells express functional TLRs[83]. Transfer of MyD88-deficient T cells into RAG-deficient mice results in less intestinal inflammation[84]. Conversely, whereas TLR signaling by T cells was classically thought to promote immunity, it now appears that this process can restrain inflammatory responses. For example, treatment of $CD4^+$ T cell subsets with a TLR4 agonist increases suppressive activity and enhances protection from colitis[85]. Therefore, TLRs represent a dynamic signaling system that triggers various immune outcomes, and TLR signaling directly by adaptive immune cells mediates reactions in the absence of innate immune cells[86–88].

Did gut bacteria evolve to induce anti-inflammatory responses through TLR signaling solely to improve host health or are there direct benefits to the microbe? PSA from *B. fragilis* enhances the anti-inflammatory function of T_{reg} cells by signaling directly through TLR2 on $CD4^+$ T cells[79]. Modulation of T_{reg} activity achieves long-term colonization of the gut by *B. fragilis* by suppressing responses of IL-17-producing cells directed toward the microbe. Therefore, PSA is distinct from TLR2 ligands of pathogens, which elicit inflammation, and sensing of gut bacterial molecules to mediate colonization supports a role for TLR signaling in promoting host-microbe symbiosis. These findings imply that the host is not 'hard-wired' to distinguish symbionts from enteric pathogens, and specific microbial ligands have evolved to actively allow mutualism between mammals and beneficial bacteria of the microbiota.

Commensal recognition promotes extra-intestinal immunity

Although the beneficial effects of the resident microbes have been extensively studied in the gut, a more diverse role for the microbiota in modulating systemic immunity is emerging. With abundant numbers of microbes inhabiting the mammalian gut, one can imagine the high concentrations of MAMPs that may circulate to systemic sites. Peptidoglycan from the gut microbiota is translocated into sera and bone marrow, priming systemic innate immunity by enhancing neutrophil function[89] (**Fig. 3b**). Specifically, peptidoglycan recognition is mediated via Nod1, which recognizes fragments of the cell wall that contain meso-diaminopimelic acid, found on Gram-negative bacteria. Treatment of mice with broad-spectrum antibiotics to deplete the microbiota, and thus circulating peptodoglycan, leads to a considerable decrease in neutrophil killing of both *Streptococcus pneumoniae* and *Staphylococcus aureus*[89]. This defect in neutrophil function is rescued by administration of meso-diaminopimelic acid, which restores the innate immune response after depletion of the microbiota. Bone marrow–derived neutrophils from Nod1-deficient mice are also defective in killing *S. pneumoniae* and *S. aureus*[89]. Hence, microbial products derived from the gut microbiota serve to prime systemic immune responses via Nod1 signaling to rapidly respond to pathogenic microorganisms.

The gut microbiota can have systemic protective influences, such as beneficial effects against allergic diseases. Pattern recognition of commensals by B cells results in less allergic inflammation[90]. Antibiotic-treated and germ-free mice have higher serum concentrations of IgE and more basophils, which indicates a role for the microbiota in regulating T helper type 2 responses and allergic inflammation[90]. B cell–intrinsic MyD88 signaling is critical in controlling steady-state amounts of IgE in the serum as well as populations of circulating basophils[90] (**Fig. 3c**). MyD88 deficiency in B cells results in more serum IgE and more IgE bound to basophil surfaces[90]. MAMPs derived from commensal bacteria limit basophil proliferation and development in the bone marrow[90]. Thus, commensal-derived signals can direct basophil development from bone marrow precursors via MyD88 signaling, linking the gut microbiota to the regulation of hematopoiesis.

Finally, PRR signaling in the gut is able to modulate immune responses at distant mucosal surfaces. Treating mice with antibiotics diminishes adaptive immune responses to intranasal infection with influenza virus[91]. Both $CD4^+$ T cell and cytotoxic $CD8^+$ T cell responses to influenza are significantly lower in antibiotic-treated mice as are virus-specific antibody titers, resulting in increased viral load in the respiratory tract[91]. Administration of TLR ligands intrarectally restores responses of T cells and antibodies to influenza in the lungs of these antibiotic-treated mice, suggesting that PRR stimulation in the gastrointestinal tract is important in priming immunity at other mucosal surfaces[91]. The intestinal microbiota was critical in providing signals necessary for inflammasome-dependent cytokine secretion in response to influenza infection[91]. Two signals are necessary for the production (signal 1) and processing (signal 2) of IL-1β and IL-18. Signal 1 is provided through TLR stimulation, resulting in the expression of pro-IL-1β and pro-IL-18. Signal 2 is mediated through inflammasome activation, leading to cleavage of caspase-1 to mature IL-1β and IL-18. The commensal microbiota provides signal 1 during colonization, initially priming the immune response whereupon influenza infection (signal 2) subsequently activates the inflammasome to help clear virus from the lungs[91] (**Fig. 3d**). These seminal examples of recognition of molecular patterns from commensal bacteria elegantly illustrate that the microbiota co-opts PRRs to mediate beneficial outcomes.

Do hosts distinguish beneficial from harmful bacteria via PRRs?

We have discussed several examples for how the innate immune system responds to the microbiota. However, a fundamental feature of the immune system is the critical distinction between the resident microbiota and invading pathogens, and recent data are beginning to suggest PRRs have a role on this process. The discovery of an intimate symbiosis with specific bacterial species indicates that the immune system of organisms ranging from *Hydra* to mammals can recognize its microbial partners or alternatively, that symbiotic bacteria actively promote a highly evolved associations with their hosts. In the case of *B. fragilis*, PSA signals through TLR2 to promote its symbiotic association with mucosal tissue[79], a finding that implies the host is not 'hard-wired' to distinguish symbionts from enteric pathogens, and that specific microbial ligands have evolved to actively allow mutualism between mammals and beneficial bacteria of the microbiota. Examples revealing that the microbiota protects the host from infectious agents via PRR signaling supports the notion that innate immunity is a mechanism of host-microbial communication. As PRRs have historically been studied in the context of infectious agents, these findings suggest that PRRs serve the dual function of sensing both pathogens and symbionts, with very different consequences both for microbes (clearance versus symbiosis) and for hosts (inflammation versus immune homeostasis).

Conclusions

Microbes dominate as the most abundant life form on our planet, occupying almost every terrestrial, aquatic and biological ecosystem. Throughout their lives, all metazoans continuously

encounter microorganisms that are essential for health or cause disease. The immune system is charged with the task of distinguishing beneficial microbes from pathogens, much like it distinguishes self from non-self antigens, to coordinate appropriate immune responses. As symbiotic microbes have molecular patterns similar to those of pathogens, why do hosts not immunologically reject the microbiota during lifelong colonization? It has been initially suggested that hosts simply ignored symbiotic bacteria[92]. Emerging evidence, however, reveals that certain microbes directly engage the immune system and in some cases actively shape beneficial host immune responses. Symbiosis is often achieved through microbial molecules that are sensed by PRRs, the same innate immune system that has been studied for years in responding to microbial infections. As the first eukaryotes evolved in a world inhabited by bacteria, PRRs seem to facilitate a wide range of interactions with this diverse microbial world. This new perspective suggests that simply distinguishing self from non-self is insufficient to explain the basic functions of the innate immune system, and future studies should consider how and why we tolerate our 'microbial self'.

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