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REVIEW

Gut feelings of safety: tolerance to the microbiota mediated by innate immune receptors

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ABSTRACT

To enable microbial colonization of the gut mucosa, the intestinal immune system must not only react to danger signals but also recognize cues that indicate safety. Recognition of safety, paradoxically, is mediated by the same environmental sensors that are involved in signaling danger. Indeed, in addition to their well-established role in inducing inflammation in response to stress signals, pattern recognition receptors and a variety of metabolic sensors also promote gut-microbiota symbiosis by responding to "microbial symbiosis factors", "resolution-associated molecular patterns", markers of energy extraction and other signals indicating the absence of pathogenic infection and tissue damage. Here we focus on how the paradoxical roles of immune receptors and other environmental sensors define the microbiota signature of an individual.

Key words gut, immune tolerance, microbiota, pattern recognition receptors, symbiosis.

SYMBIOSIS DEPENDS ON IMMUNE TOLERANCE

The immune system is not just a defense system, but also a maintenance system—maintaining both the organism and the organism's healthy symbiotic relationship with particular gut bacteria. It not only fights against foreign pathogens, but also invites certain microbial communities to colonize the gut and thrive there (1). Because commensal bacteria are composed of foreign antigens not encoded by host genes, the host's immune system must be tolerant of these bacterial molecules. The term tolerance has been used in many ways; for our present purposes, we here define tolerance as a state in which the immune system of the mammalian host not only fails to attack commensal bacterial cells, but actually promotes their residence in the gut. Induced tolerance to commensals is not a passive state of unresponsiveness; rather, it involves activation of tolerogenic mechanisms similar to those that mediate suppression of IL-22 by retinoic acid receptor-related orphan nuclear receptor gamma^{+} cells to establish optimal conditions for host development, metabolism and defense (2).

The role of the immune system in the gut is paradoxical: on the one hand it has to manage symbiosis with non-self commensal agents; on the other hand, it has to protect the host against gut pathogens. To achieve this discrimination, the gut immune system is attuned to biomarkers that indicate the degree of tissue integrity (3). Molecules that are released during infection or tissue damage and induce expression of inflammatory mediators have been collectively referred to as *danger signals* (4). Here, we discuss the

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List of Abbreviations: AhR, aryl hydrocarbon receptor; AMP, antimicrobial peptide; ATP, adenosine 5′-triphosphate; eATP, extracellular adenosine 5′-triphosphate; FoxP3, forkhead box P3; HSP, heat shock protein; IEC, intestinal epithelial cell; IKK, IκB kinase; KO, knock out; LP, lamina propria; MUC, mucin; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor-κB; NLRP6, NOD-like receptor family pyrin domain containing protein 6; NOD, nucleotide-binding oligomerization domain; PGN, peptidoglycan; PRR, pattern recognition receptor; RAMP, resolution-associated molecular pattern; RegIII, regenerating islet-derived protein 3; SCFA, short chain fatty acid; SFB, segmented filamentous bacteria; Tfh, follicular helper T cell; Treg, regulatory T cell; WT, wild type.

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evidence that, in addition to danger signals, there are also *safety signals*, molecules and molecular patterns that are released by healthy tissues, dietary components and commensals to induce tolerance.

Remarkably, both danger and safety signals are recognized by the same environmental sensors, which, depending on the chemical identity of their agonists and a variety of conditioning factors, can induce either tolerance or intolerance to recognized agents. These receptors include PRRs such as TLRs, nucleotide-binding oligomerization domain-like receptors and C-type lectin-like receptors, as well as a variety of metabolic sensors like AhR, purinergic receptors, receptors for retinoic acid and the family of G protein-coupled receptors. All of these sensors react to microbial-derived molecules (structural patterns and metabolites) to activate signaling pathways that control the expression of genes coding for a variety of immune mediators (5–7). The focus of this paper is how the paradoxical role of receptors that act as both inflammatory activators and suppressors enables colonization of the host mucosa by defined populations of symbiotic microbes. In addition, we shall also cite evidence that adaptive lymphocytes also participate in the sensing of symbiotic bacterial signals.

Paradoxical functions of immune sensors in the gut

Cells of the mucosal immune system express a wide range of PRRs and metabolic sensors that act as inflammatory activators and suppressors. Consider PRRs on the luminal surface of the intestinal epithelium: the observation that IEC-specific deficiency of a single component of a TLR signaling cascade such as MyD88, IKKβ or IKKγ increases susceptibility of mice to intestinal inflammation indicates that PRRs on the apical surface of IECs help to prevent inflammation (8–14). On the other hand, it is known that PRRs like TLR2 and TLR5, despite being expressed on the apical epithelial surface, can induce local inflammatory responses by promoting the release of IL-8 and chemokine (C-C motif) ligand 20 (15–17).

Pattern recognition receptors in the submucosal tissues also play seemingly conflicting functions. On the one hand, they promote destructive responses, as indicated by the observation that bone marrow chimeras, the hematopoietic cells of which are deficient in MyD88, fail to develop systemic inflammation in response to *Helicobacter hepaticus* (18). On the other hand they promote tolerance, as indicated by the fact that deletion of a critical component of the TLR signaling pathway, such as TNF receptor-associated factor in DCs, leads to a decrease in the number of $FoxP3^+$ Tregs and provokes spontaneous inflammation in the small intestine that is driven by otherwise commensal bacteria (19).

The ambiguity of immune reactions towards conserved molecular motifs is also evident in intestinal lymphocytes (20). In the case of B cells, MyD88-dependent signaling helps to induce proinflammatory Th17- and Th1-mediated immune responses to *Salmonella typhimurium* (21). Conversely, the same type of signaling in intestinal B cells protects mice from commensal-driven dextran sodium sulfate-colitis by controlling secretion of IgM and promoting opsonization of luminal microbes by C3-derived complement components (22).

Similar paradoxes in innate immune signaling have been observed in intestinal T cells. Activation of receptors like TLR8 on Tregs abrogates the suppressive properties of these cells; however, other receptors like TLR4 enhance the anti-inflammatory activity of these cells as seen in the transfer of naive $IL\text{-}10^{-/-}TLR4^{-/-}CD4^+$ T cells to $Rag1^{-/-}$ recipient mice, which provokes more severe colitis than transfer of $IL\text{-}10^{-/-}CD4^+$ T cells (23, 24). Furthermore, unlike the human version of this molecule, bacterial homologues of HSP60, fail both to direct migration of human T cells and to activate murine B cells to proliferate and produce IL-10 (25, 26). Thus, self-HSP60 specifically triggers some innate T cell regulatory responses.

Receptors for microbial-derived metabolites also promote contrasting responses. For example, recognition of commensal-derived eATP by purinergic P2 receptors on a subset of $CD11c^+$ cells promotes intestinal inflammation by driving development of Th17 cells in the LP (27). On the other hand, recognition of the same molecule by P2X receptor subtype 7 on Tfhs in Payer's patches helps to protect mice from sepsis (28). Indeed, eATP reduces the population of Tfh cells, leading to low-affinity IgA responses to commensals and increased LPS-mediated priming of B cells towards the production of IgM, which targets sepsis-promoting pathogens. Similarly, despite their established anti-inflammatory role in the gut (29), receptors for SCFA, GPR41 or GPR43 on IECs, have been found to promote neutrophil and effector T cell recruitment, thereby potentiating inflammatory reactions to ethanol or 2,4,6-trinitrobenzenesulfonic acid (30).

Collectively, the above studies indicate that immune sensors act as both pro-inflammatory activators and anti-inflammatory suppressors: they serve both to eliminate microbes and to help the gut tolerate them (31).

Safety signal recognition

If innate receptors acted only as specialized sensors of danger, there would be perpetual inflammation in the

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gut. In fact, microbial products routinely access subepithelial tissues and endogenous PRR ligands like HSPs, DNA, RNA, ATP and high-mobility group protein B1 are constantly present in the LP (16, 32–34). Moreover, TLRs persistently stimulate CX3C chemokine receptor 1+ cells which, rather than provoking inflammation actually sample the luminal contents and prime tolerogenic $CD103^+$ DCs to migrate to the mesenteric lymph nodes (35–37). All of this can happen without any evidence of destructive inflammation.

How is it possible for the same receptors to remain alert to signals originating from pathogens and damaged tissues while simultaneously tolerating molecules coming from commensals and healthy tissues? The answer is that innate immune recognition is collectively more specific than might be inferred from the promiscuous character of individual receptors (38). In fact, PRRs engage in interactions with other receptors and their signaling pathways to recognize agonist signals with great specificity. For example, despite the fact that PGN and Pam_3Csk_4 are both recognized by TLR2, only the former contains muramyl dipeptide, activates intracellular NOD2 and decreases the production of IL-12 (39). Another form of cooperation between receptors that generates fine specificity is heterodimerization: indeed, the distinct effects of TLR2 agonists Pam_3Cys and synthetic diacylated lipoprotein-TLR2/6 on Tregs result from the fact that the former binds to the TLR2/TLR1 heterodimer, whereas the latter binds to the TLR2/TLR6 heterodimer (40). The capacity of heterodimers to ensure specific recognition of structural patterns is also illustrated by the observation that the TLR2/TLR1 receptor complex on DCs induces Th17-mediated proinflammatory responses to *Yersinia enterocolitica*, whereas the TLR2/TLR6 heterodimer promotes the development of IL-10-producing T cells in reaction to the same pathogen (41, 42). The specificity of innate immune reactions is further highlighted by the acuity of intestinal metabolic sensors, like AhR, which induces differentiation of Treg cells in response to the dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin while promoting development of Th17 cells in reaction to tryptophan photo-product, 6-formylindolo[3,2-b]carbazole (43). Again, the ligand-specificity of AhR seems to be influenced by crosstalk with other receptors and transcription factors.

The outcomes of signal recognition do not merely depend on cooperation between receptors, but also on the milieu of cytokines and other factors that condition an immune cell to react to PRR agonists in a particular way (44–46). PRRs themselves help to create a conditioning, chemical niche by activating non-hematopoietic cells to exert inhibitory effects on LP macrophages, suppressing their release of TNFα and inhibiting expression of their activation markers (47). Furthermore, TLRs induce IECs and CD103+ DCs to release a variety of factors, like thymic stromal lymphopoietin, that promote development of Tregs in the mesenteric lymph nodes (48, 49). The importance of the cytokine milieu in guiding responses to microbial-derived products is also evident in reactions to bacterial metabolites like SCFA which, depending on the polarizing conditions in the gut, induce differentiation of naive T cells into Th1, Th17 or Tregs (50).

Hence, the reactions of cells to immune receptor ligands are directed by the immediate chemical environment embodied in the structure of the stimulating ligand and by other conditioning factors. Signals that induce tolerance include the so called "microbial symbiosis factors", RAMPs, and a variety of microbial-derived metabolic products that serve as markers of energy extraction (33, 51–53). Here we here refer to these tolerogenic signals collectively as *safety signals* and distinguish them from the pro-inflammatory *danger signals* that are released when there is infection and damage.

Tolerization by safety signals

Recognition of safety signals by PRRs does not result merely in the absence of induced pro-inflammatory mediators, but also in activation of mechanisms that promote a lack of responsiveness to danger signals (Fig. 1). These safety signal-mediated mechanisms include blocking access of danger signals to their receptors, termination of danger-activated pathways and inhibition of danger-response gene transcription.

One of the strategies for blocking access of danger signals to their corresponding sensors is induction of degradation of these signals. For example, in type 1 Tregs, AhR induces expression of CD39, which catalyzes degradation of proinflammatory eATP and interacts with CD73 in responder T cells to convert eATP into adenosine (54). This, in turn, helps to promote differentiation of type 1 regulatory T cells and to protect the gut from T cell-induced colitis. Safety signals can also help to prevent danger signals from activating proinflammatory cascades by inducing release of antagonists to proinflammatory receptors as illustrated by TLR5 on IECs, which prevents activation of the IL-1-mediated pathway by inducing release of secretory IL-1 receptor antagonist (55). In turn, termination of danger-activated proinflammatory cascades is mediated by receptors, like TLR9 on IECs, which prevent degradation of inhibitor of NF-κB downstream of TLR2, TLR3 and TLR5 and thus protect

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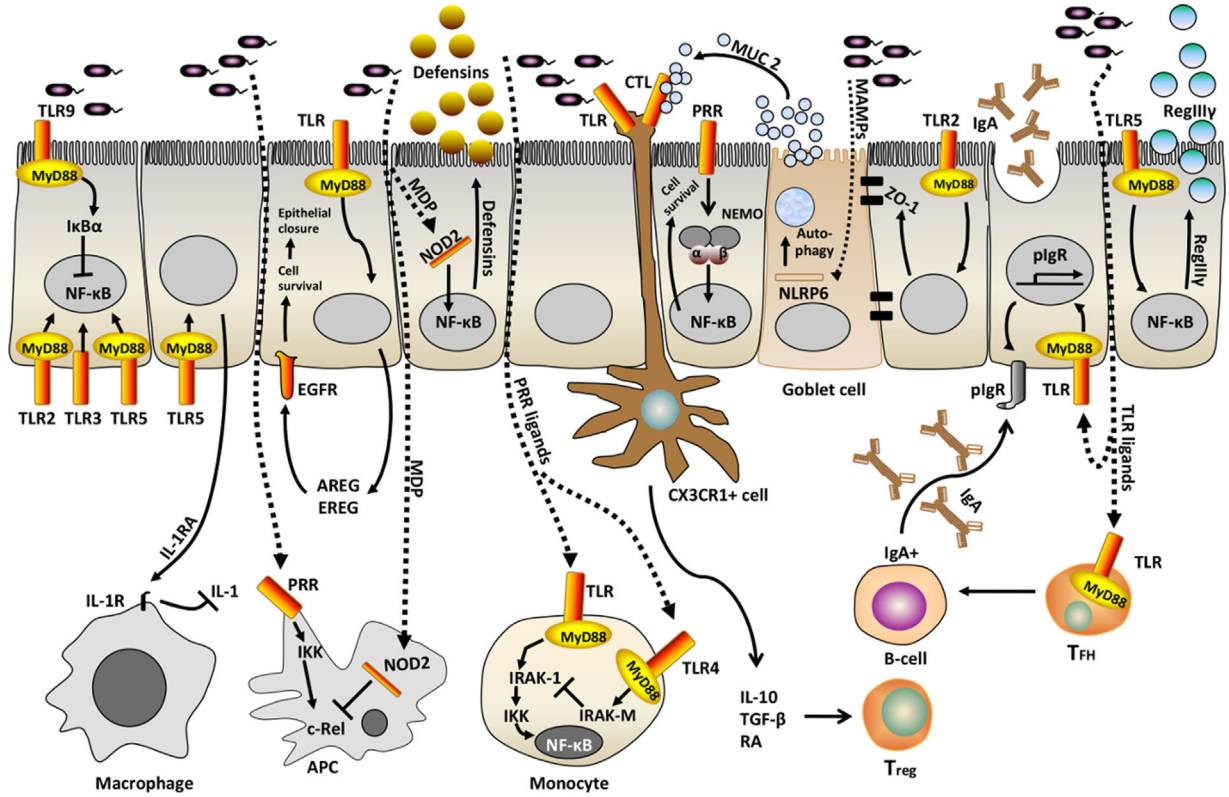

Fig. 1. PRR-dependent tolerogenic pathways in the gut. PRRs activated by safety signals tolerize the immune system to pro-inflammatory challenges in three ways: by directly blocking the activation of pro-inflammatory signaling cascades; by limiting the potential of luminal agents to over-stimulate PRR on the apical surface of IECs; and by preventing access of intestinal microbes to the sterile compartments of the LP. The outcome is fine-tuned by the collective complexity of these interactions. MAMPs, microbe-associated molecular patterns; RA, retinoic acid.

mice from intestinal inflammation (16). At the level of IL-1 receptor-associated kinase, pro-inflammatory cascades can be inhibited by TLR-mediated induction of IL-1 receptor-associated kinase-M (34). Finally, pro-inflammatory cascades can be blocked by PRRs at the level of NF-κB activation, as shown by the observation that NOD2 in antigen-presenting cells can prevent nuclear translocation of c-Rel and subsequent IL-12 release (56). Transcription of proinflammatory genes following PRR activation is prevented by signals of metabolic safety like n-butyrate, which inhibits histone deacetylase activity to down-regulate production of IL-6 and IL-12 in LPS-stimulated intestinal macrophages (57). Other metabolic signals interfere with LPS-dependent release of proinflammatory mediators by activating AhR, which interacts with signal transducer and activator of transcription 1 and NF-κB to prevent release of TNFα and IL-6 by macrophages (58). This mechanism likely explains the importance of AhR in protecting mice from endotoxin shock because initial exposure of innate immune cells to LPS upregulates production of the enzyme tryptophan 2,3-dioxygenase which, by mediating metabolism of tryptophan to an AhR agonist, L-kynurenine, attenuates expression of inflammatory genes (59).

Safety signals promote unresponsiveness to danger signals, not only by terminating proinflammatory pathways and inactivating their target genes, but also by inducing molecules that keep these signals away from the epithelium (60). AMPs, IgAs and MUCs which, when released into the lumen, restrict the contact of microbial molecules with receptors that can initiate pro-inflammatory cascades, are instrumental in this form of tolerization (61). The anti-inflammatory role of PRR-mediated AMP production has been demonstrated by studies showing that MyD88-dependent signaling is essential for production of RegIIIγ, which indirectly prevents accumulation of Th1 cells in the underlying tissues (62). Accumulation of these pro-inflammatory cells is also prevented by release of α-defensins by NOD2-stimulated Paneth cells (63). The anti-inflammatory activity of these AMPs is aided by IgAs, the release of which is also controlled by environmental and metabolic sensors. Some receptors induce expression of the polymeric immunoglobulin receptor, a molecule

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involved in the translocation of IgAs across the epithelial surface (11, 64). They also induce Tfh cells to activate IgA production by B cells in germinal centers (65). Finally, the action of AMPs and IgAs is supplemented by MUCs which, when induced by NLRP6, protect mice from *Citrobacter rodentium*–driven inflammation (66). PRR-dependent mucus production is a component of a feed-forward tolerogenic mechanism in which activation of C-type lectin on DCs by Muc2 complexed with galectin-3 induces expression of anti-inflammatory genes (67).

In addition to inducing termination of pro-inflammatory cascades and limiting the access of pro-inflammatory signals to the epithelium, environmental and metabolic sensors also promote unresponsiveness to danger signals by reinforcing compartmental boundaries between the lumen and LP. Maintenance of these boundaries depends on modulation of the growth, repair, survival and intercellular junctions of epithelial cells. Among other things, epithelial growth and repair are modulated by epidermal growth factor receptor ligands, amphiregulin and epiregulin which, when released by PRR-activated IECs, prevent intestinal inflammation following damage (68). The anti-inflammatory, pro-survival effect of PRRs on IECs is evidenced by studies of NF-κB essential modulator$^{IEC\text{-}KO}$ and IKKβ$^{IEC\text{-}KO}$ mice, the spontaneous intestinal inflammation of which is driven by increased epithelial cell death (13, 14). Tight junctions between IECs also help to prevent activation of submucosal pro-inflammatory cascades; TLR2 plays an important role in this process by stabilizing zonula occludens-1 and protecting mice from chronic intestinal inflammation (69). Sensors of metabolic safety also promote integrity of the epithelium, as illustrated by the SCFA receptors GPR43 and GPR109A, which activate the PRR-primed NLRP3 inflammasome in IECs to induce release of IL-18 by these cells; thus reinforcing the intestinal barrier and protecting these mice from colitis (70). All in all, the above studies demonstrate that stimulation of PRRs in the absence of infection or tissue damage promotes tolerance by limiting the ability of exogenous and endogenous danger signals to activate pro-inflammatory pathways in the gut.

Safety signals promote microbial colonization of the gut mucosa

In addition to limiting acute pro-inflammatory reactions to commensal bacteria, safety signals positively promote long-term gut colonization. Induction of hospitable conditions for microbes is particularly important at birth because the initial microbial inoculum confers protection against allergic, metabolic and autoimmune diseases later in life (71). To facilitate this significant colonization event, TLRs of neonates have a strong safety recognition bias, inducing release of anti-inflammatory cytokines like IL-10 in response to a wide range of microbial products (72). Thus, despite fully functional NF-κB and mitogen-activated protein kinase responses to PRR signals, infants are hypo-responsive to vaccines and have an increased risk of intestinal infection caused by *Escherichia coli.*

Even though PRRs later acquire the capacity to also recognize danger signals, the general microbial profile established soon after birth persists. Indeed, studies of the composition of microbes in the offspring of obese *ob*/+ mice reveal that maternal transmission plays a more important role in determining resident microbial populations than does the genetic makeup of the individual (73, 74). Intestinal microflora can persist because adult PRRs favor tolerance towards ligands that originate from established, rather than from newly arrived, microbial communities. Indeed, unlike the abrupt activation of basolateral TLR5 on IECs, repeated stimulation leads to inhibition of NF-κB and mitogen-activated protein kinase pathways and promotes internalization of TLR5; effects that establish a long-lasting lack of responsiveness to flagellin (75). Similarly, despite inducing pro-inflammatory IL-8 in response to liposomal immunostimulatory DNA sequence, TLR9 on the basolateral surface of IECs becomes unresponsive to the same molecule following repeated challenge (16). Induction of tolerance to persistently stimulated PRR signals has also been observed in intestinal macrophages which, when abruptly activated by LPS, upregulate TNF-α and IL-6 and, when repeatedly stimulated by the same TLR4 ligand, induce IL-10 and transforming growth factor-β (34). Similarly, acute activation of NOD2 in human macrophages triggers release of TNF-α, IL-8 and IL-1β, whereas prolonged stimulation of the same receptor leads to self-tolerization and cross-tolerization of innate immune receptors (76). Thus, PRRs tend to induce inflammatory responses in reaction to rapid alterations in the dynamics of receptor/ligand interactions; in contrast, the initial, chronically acquired microbial communities fail to induce these inflammatory responses and so can become established and persist (77).

In addition to the uniform tolerogenic treatment of initial commensal colonizers and established microbial communities, PRRs are also react in a highly selective manner towards certain newly arrived microbial antigens through specific innate recognition mechanisms. The ability of innate immune reactions to discriminate between different species of microbiota is illustrated by TLR2, which augments the suppressive activity of Tregs in reaction to polysaccharide A from *Bacteroides fragilis* and not to synthetic lipopeptide TLR2 agonists (78). Similarly, unlike another TLR2 agonist such as the low calcium response locus protein V-homolog protein

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hydrophilic translocator of type three secretion system, the TLR2/CD14 receptor ligand low calcium response locus protein V from *Yersinia* spp. can induce intestinal macrophages to up-regulate expression of IL-10 (79). Likewise, in contrast to LPS from other *Salmonella* species, LPS from *S. typhimurium* moderately enhances proliferation of Tregs (80). Furthermore, PGN originating from distinct strains of *E. coli* has been found to induce distinct transcriptional responses in trout macrophages (81). Thus, intestinal PRRs have the potential to specifically recognize microbe safety signals of certain species of bacteria.

Specificity is combined with a degree of non-specificity: innate immune specificity does not limit tolerance to particular members of microbial communities. Rather, specific recognition of microbes by the innate immune system also leads to nonspecific tolerance in the gut. This has been observed in lymphocyte antigen 6 complex^{hi} monocytes which, when stimulated by commensals through a variety of TLR agonists, produce prostaglandin E2, which helps inhibit activation of neutrophils and terminate inflammation against pathogenic *Toxoplasma gondii* (82). Some pathogens, such as mouse mammary tumor virus, take advantage of such generalized tolerogenic reactions by binding to commensal LPS and so activating TLR4-medited release of IL-10 (83). Overall, induction of regulatory cells and molecules by safety signal-stimulated PRRs has a nonspecific tolerogenic effect that is not only targeted towards the stimulating microbes but also towards other luminal microbes and food components.

However, these indiscriminate tolerogenic effects do not over-ride the power of innate environmental sensors to select for the host microbiota and thus define the microbial signature of an individual. The importance of PRRs in determining microbial populations is evidenced by the fact that a deficiency in these receptors leads to dysbiosis (84). Indeed, mice that are deficient in NOD2, NLRP6 or TLR5 have an increased susceptibility to colitis associated with altered composition of intestinal microbes which, when transferred to WT mice, maintain their colitogenic phenotype (85–87). However, there is also evidence to the contrary that suggests that the composition of microbiota depends mostly on maternal transmission, and that the activity of TLRs has no effect on the population of intestinal microbes in steady-state conditions and following challenge. Indeed, 16S ribosomal RNA sequencing has revealed that the composition of ileal and cecal microbes in mice deficient in TLR2, TLR4, TLR5, TLR9 or MyD88 is similar to that of their isolated littermate controls (88). The apparent stability of bacterial ecosystems has also been observed in MyD88-deficient *Hydra* (89). Nevertheless, in contrast to mice, following an antibiotic challenge or infection the capacity of these more primitive organisms to reestablish species-specific signatures of their microbiota is altered, confirming that PRRs develop as important determinants and stabilizers of symbiotic microbial communities (90).

The same molecules that limit access of danger signals to the epithelium are instrumental in PRR-mediated regulation of the composition of intestinal microbes (Fig. 1). In fact, dysbiosis in NOD2$^{-/-}$, NLRP6$^{-/-}$ and TLR5$^{-/-}$ mice has been found to be associated with alterations in their production of AMPs. The absent receptors control production of cytokines such as IL-18, IL-22 and IL-23, which act on IECs to promote the release of RegIIIγ, α-defensins, MUCs and other functional peptides (87, 91, 92). Indeed, like mice that are deficient in individual members of the TLR family, IL-22$^{-/-}$ mice exhibit alterations in microbial populations that can be transferred to WT mice together with their associated colitogenic phenotypes (93). The importance of IL-22 in shaping microbiota is further highlighted by the fact that this cytokine is induced by metabolic sensors, resulting in shifts in microbial composition. For example, it has been found that AhR in innate lymphoid cells limits the expansion of SFB by inducing expression of IL-22 by these cells (94). Another piece of evidence supporting IL-22-mediated regulation of microbial composition comes from studies that show that the AhR-dependent release of this cytokine by innate lymphoid cells in response to a tryptophan metabolite, indole-3-aldehyde derived from *Lactobacillus reuteri,* can limit expansion of *Candida albicans* (95). IL-22-mediated modulation of microbial composition may also account, at least partially, for the beneficial effects of 6-formylindolo[3,2-b]carbazole administration in experimental colitis (96).

Sensors of microbial structures and metabolites mold the intestinal ecosystem by excluding certain groups of microbes from the tolerogenic environment induced by these receptors. PRR-mediated exclusion of microbes from intestinal tolerance by means of AMPs is evidenced by the fact that IEC-specific deficiency of MyD88 leads to increased microbial diversity and overrepresentation of SFB in the lumen (97). These mice exhibit alterations in their associated microbial communities because they lack RegIIIγ, which is normally induced by MyD88-activating receptors such as TLR4 on Paneth cells and TLR5 on IECs; RegIIIγ binds to the PGN of SFB and of other gram-positive bacteria and limits their expansion (62, 91, 98). These mice also lack RegIIIβ, which is induced by MyD88 signaling pathways to recognize both PGN and LPS and thus to eliminate selected gram-positive and gram-negative bacteria (99). RegIIIβ preferentially targets *Clostridium butyricum*, *L. reuteri*

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and various strains of *E. coli*, but not other bacterial species such as *S. typhimurium*, giving the latter an advantage in inducing infection (100). Other examples of AMPs that are induced by PRRs to control microbial composition include α-defensins, such as human defensin 5, the PRR-dependent release of which is evidenced by a loss of function mutation in NOD2, which results in a deficiency of this AMP in humans (101). Despite having the same number of commensals as controls, mice that have been genetically modified to have Paneth cells that express human defensin 5 manifest a different ratio of *Firmicutes* and *Bacteroidetes* phyla (102).

Similar to AMPs, the roles of IgAs are not limited to preventing microbe-derived danger signals from accessing proinflammatory cascades, but also include active shaping of the microbiome. The involvement of TLRs in IgA-dependent sculpting of the microbial composition has been determined by the aforementioned studies of Tfh cells which, when activated by TLRs, promote class-switch recombination of B cells towards production of IgA (65). Deficiency of MyD88 in these T cells leads to a significant shift in the composition of the microflora that is characterized by a loss of microbial diversity and marked differences between the microbial populations of individual mice. Similarly, many endogenous and exogenous metabolites modulate composition of intestinal microbes by controlling the number and diversity of released IgAs (103). For example the vitamin A metabolite, retinoic acid, activates B cells directly to promote expansion of IgA-producing cells in PP; an effect that helps to prevent development of specific bacterial groups in the gut (104). Furthermore, intestinal metabolites, such as SCFA, and endogenous ligands of AhR, such as 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester, induce differentiation of FoxP3+ T cells in the intestine (105–107); this class of T cells has been recently found to play a critical role in shaping commensal composition. Indeed, FoxP3+ T cells can differentiate into follicular regulatory cells (Tfr), which control Tfh cells to modulate affinity maturation of IgAs in Peyer's patches the PP (108). Consequently, a deficiency of FoxP3+ T cells results in alterations in microbial communities that is characterized by reduced diversity of *Firmicutes* in general and nonpathogenic *Clostridia* in particular. As revealed by studies of activation-induced (cytidine) deaminase$^{-/-}$ mice, the targets of IgA include SFB and certain members of the *Clostridium* spp. (109). However, the exact mechanism(s) that allows IgAs to mold the microbiota is not known; these antibodies do not kill bacteria and many beneficial microbes are known to be coated with antigen-specific IgAs (110). Whatever the actual mechanisms at play, it is clear that the symbiotic relationship between individuals and their resident bacteria is maintained by integrated networks of innate and acquired receptors that are expressed on innate cells and lymphocytes (111–113).

Taken as a whole, the above evidence demonstrates that safety signals activate PRRs to establish a nonspecific tolerogenic tone in the gut. This, in turn, creates a background against which these same receptors induce antimicrobial molecules to focus on selected members of microbial communities (Fig. 2).

SUMMING UP

In 1994 Polly Matzinger formulated the Danger Model, which embodies the concept that the immune system responds to signals originating from damaged or stressed tissues to initiate inflammatory and adaptive responses (114). Later studies have supported the Matzinger hypothesis by identying PRRs and toxin receptors such as AhR as specialized sensors of stress and damage (4, 115). Recent experimental data indicate that, in addition to danger signals, there are also safety signals in the form of molecules released by healthy host tissues and commensal-derived molecules and structures. These safety signals generate two processes that fine tune gut inflammation: one inhibits production of pro-inflammatory cytokines and the other limits the capacity of danger signals to activate pro-inflammatory cascades. Moreover, safety signals actually sculpt the specific bacterial repertoire established in the gut. Thus, the immune system not only protects the individual against pathogenic invaders, but also functions to maintain a healthy symbiotic host–bacterial relationship (116).

Classical reductionist expectations would foster the notion that the protective and maintenance functions of immunity should be reducible to two separate signaling pathways—a pro-inflammatory danger pathway for protection and an anti-inflammatory safety pathway for symbiosis. Paradoxically, however, both safety and danger signals are recognized by the same receptors which, depending on their immediate chemical environment, can induce or oppose inflammatory responses. There is no neat subdivision of seemingly opposite effects into distinct systems of function-specific receptors and ligands; indeed, the immune system is an integrated whole that navigates an individual through a dynamic landscape of symbiosis and threat by sensing context and being mindful of the evolving history of the individual and the species. That similar receptors have roles in both danger and safety may seem paradoxical from the perspective of linear, human engineering; nonetheless, such signaling appears to be advantageous.

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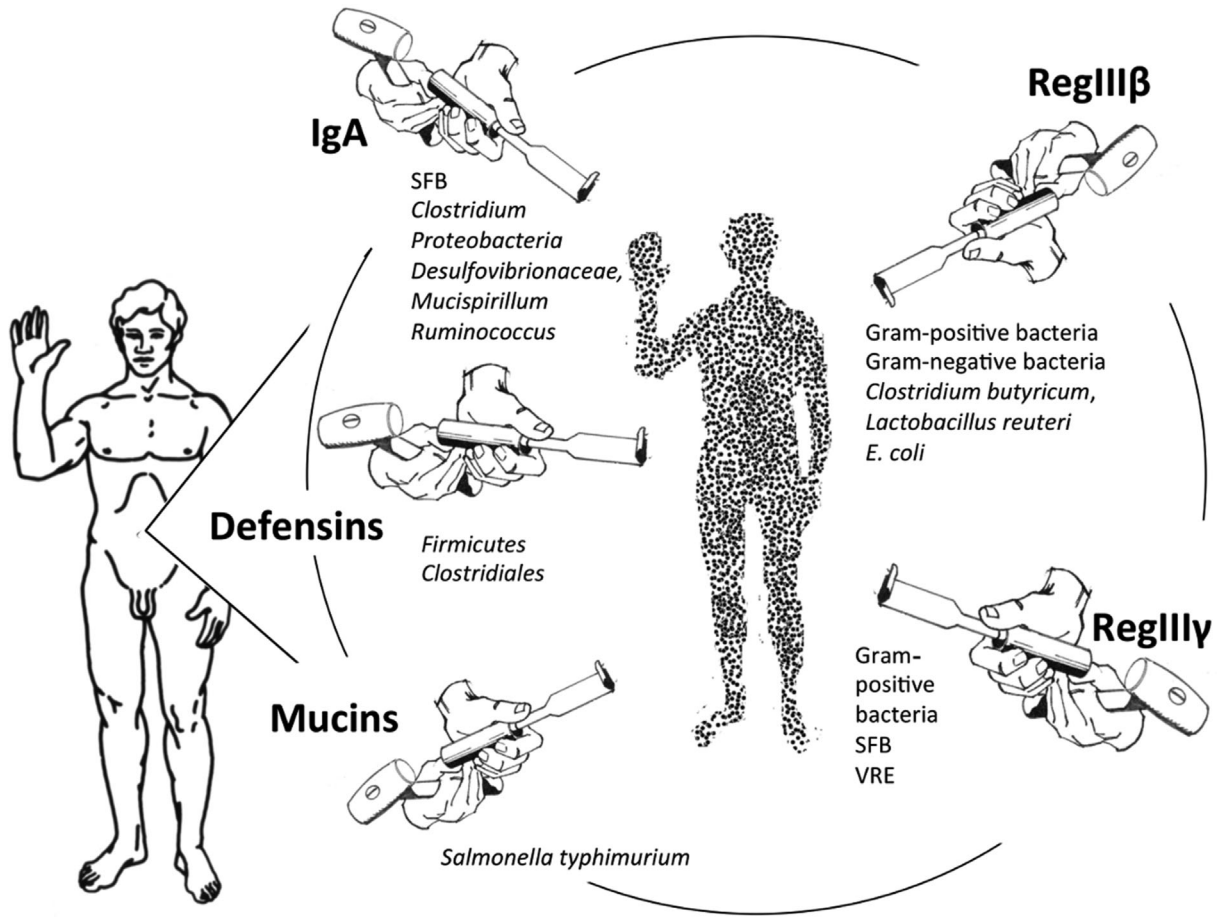

Fig. 2. PRRs define the microbiota signature of an individual. PRRs shape the microbiome by a process of negative selection that excludes certain members of microbial populations from the tolerance these receptors themselves establish. In the course of this dynamic process, the host imprints the microbiota with metabolic and immunologic characteristics that can be transferred by the modified microbial community to other hosts—signified by the dotted figure in the center. VRE, vancomycin-resistant *Enterococcus*.

Even more astoundingly, safety signals and danger signals are often mediated by the same molecules, which, are, in turn, have been found to be either identical to or homologous with self-molecules. Consider endogenous signals like HSPs, DNA and ATP, all of them which, depending on the context, can indicate danger or safety, and all of which have microbe-derived counterparts. This particular focus on self and self-like signals within the larger context of conditioning factors is a manifestation of the activity of a self-recognition network, which was defined by one of us (I.R.C) as the "immunological homunculus" (117, 118). The mimicry between endogenous and exogenous signals permits this regulatory network, this homunculus, to stretch its activity beyond the limits of a genetically defined individual to modulate interactions between gut microbes as if they were integral parts of the organism itself. No wonder that the mammalian host has co-evolved with its gut symbionts such an intricate and complex network of mutual signaling—resident microorganisms are an essential factor in the evolution of multi-cellular life general (119).

Paradoxical signaling has recently been addressed in the context of the role of IL-2, which regulates T cell population density by inducing both proliferation and apoptosis of these cells (120). By means of mathematical modelling and experimental data, these authors have demonstrated that systems in which one controller mediates conflicting functions are much more robust than are systems in which two separate controllers are each dedicated to a separate function. For example, if TLR4 only recognized danger and TLR2 only recognized safety, the intuitive result would be that TLR4 deficiency would inevitably lead to an excess of anti-inflammatory molecules and hypo-responsiveness to pathogens. However, because each of these receptors can support seemingly conflicting functions, the overall balance

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between pro- and anti-inflammatory mediators can be maintained in the face of a deficiency of either of them (88). This contention is confirmed by the fact that mice that are deficient in individual members of the TLR family rarely exhibit spontaneous inflammation or infection. Indeed, in the gut pro- and anti-inflammatory signals are summated to establish a neatly calibrated tolerogenic tone that readily reverts to pro-inflammatory reactivity when confronted by pathogenic invasion (121–123).

In closing, the diverse roles of immunity in both maintaining symbiosis and protecting against pathogens challenge the idea that immunity is the science of self/nonself discrimination (116) and call for an ecological view of life (124–127).

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DISCLOSURE

The authors have no conflicts of interest to declare.

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