Review

Gut feelings of safety:

Tolerance to the microbiota mediated by innate immune receptors

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SUBJECT SECTION: Immunology, Innate immunity, Tolerance and Immune regulation

RUNING TITLE: Tolerance to the microbiota

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, Typesetting, pagination and proofreading process, which may lead to differences etween this version and the Version of Record. Please cite this article as doi: 10.1111/1348-0421.12318.

ABSTRACT

To enable microbial colonisation of the gut mucosa, the intestinal immune system must not only react to danger signals but also recognize cues that indicate safety. Safety recognition, paradoxically, is mediated by the same environmental sensors that are involved in signalling danger. Indeed, in addition to their well established role in inducing inflammation in response to stress signals, pattern recognition receptors (PRRs) 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 role of immune receptors and other environmental sensors define the microbiota signature of the individual.

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

Symbiosis depends on immune tolerance

The immune system is not just a defence 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). Commensal bacteria are composed of foreign antigens not encoded by host genes, and so the immune system of the host 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 does not attack commensal bacterial cells, but actually promotes their residence in the gut. Induced tolerance to commensals is not a passive state of unresponsiveness, but involves activation of tolerogenic mechanisms similar to those that mediate suppression of IL-22 by RORγt^{+} cells to establish optimal conditions for host development, metabolism and defence (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 perform this discrimination, the gut immune system is attuned to biomarkers indicating the level of tissue integrity (3). Molecules released during infection or tissue damage, which induce expression of inflammatory mediators, have been collectively referred to as *danger signals* (4). Here we discuss the 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, danger signals 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 pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs) and C-type lectin-like receptors (CLRs) as well as a variety of metabolic sensors like aryl hydrocarbon receptor (AhR), purinergic receptors, receptors for retinoic acid (RA) and the family of G protein-coupled receptors (GPCRs). All of these sensors react to microbial-derived molecules (structural patterns and metabolites) to activate signalling pathways controlling the expression of genes coding for a variety of immune mediators (5-7). The focus of this paper is on how the paradoxical role of receptors acting as both inflammatory activators and suppressors enables colonisation of the host mucosa by defined populations of symbiotic microbes. In addition, we shall also cite evidence that the adaptive lymphocytes too 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 signalling cascade like 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 CCL20 (15-17).

PRRs 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 (BM) chimeras deficient in MyD88 in their hematopoietic cells 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 signalling pathway, such as TRAF in DCs, leads to a decrease in the number of $FoxP3^{+}$ Tregs and provokes spontaneous inflammation in the small intestine 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 signalling helps to induce proinflammatory Th17- and Th1-mediated immune responses to *S. typhimurium* (21). Conversely, the same type of signalling in intestinal B cells protects mice from commensal-driven DSS-colitis by controlling secretion of IgM and promoting opsonisation of luminal microbes by C3-derived complement components (22).

Similar paradoxes in innate immune signalling are observed in intestinal T cells. While activation of receptors like TLR8 on Tregs abrogates the suppressive properties of these cells, other receptors like TLR4 enhance the anti-inflammatory activity of these cells as seen in the transfer of naive IL-$10^{-/-}$TLR$4^{-/-}$$CD4^{+}$ T cells to Rag$1^{-/-}$ recipient mice, which provokes more severe colitis than transfer of IL-$10^{-/-}$$CD4^{+}$ T cells (23, 24). Furthermore, bacterial homologues of HSP60, unlike the human version of the molecule, fail to direct migration of human T cells and fail to activate murine B cells to proliferate and produce

IL-10 (25, 26); thus, there are innate T cell regulatory responses specifically triggered by self-HSP60 .

Receptors for microbial-derived metabolites also promote contrasting responses. For example, recognition of commensal-derived extracellular ATP (eATP) by purinergic P2 receptors on a subset of $CD11c^+$ cells promotes intestinal inflammation by driving development of Th17 cells in the lamina propria (LP) (27). On the other hand, recognition of the same molecule by P2X7 on T follicular helper cells (Tfh) in Payer's patches (PP) 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 short-chain fatty acids (SCFA), GPR41 or GPR43 on IECs have been found to promote neutrophil and effector T cell recruitment thereby potentiating inflammatory reactions to ethanol or TNBS (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 as specialized sensors of danger there would be never-ending inflammation in the gut. In fact, microbial products routinely access subepithelial tissues and endogenous PRR ligands like HSPs, DNA, RNA, ATP and HMGB1 are constantly present in the LP (16, 32-34). Moreover, TLRs persistently stimulate CX3CR1+ cells, which instead of

provoking inflammation actually sample the luminal content and prime tolerogenic $CD103^+$ DCs to migrate to the MLN (35-37). All of this can happen without a sign of destructive inflammation.

How is it possible for the same receptors to remain alert to signals originating from pathogens and damaged tissues while tolerating molecules coming from commensals and healthy tissues? The answer is that innate immune recognition is collectively more specific than what might be inferred from the promiscuous character of individual receptors (38). In fact, PRRs engage in interactions with other receptors and their signalling pathways to recognize agonist signals with great specificity. For example, despite the fact that peptidoglycan (PGN) and Pam_3Csk_4 are both recognized by TLR2, only the former contains muramyl dipeptide (MDP), and activates intracellular NOD2 and decreases the production of IL-12 (39). Another form of cooperation between receptors that generates fine specificity is heterodimerization: Indeed, distinct effects of TLR2 agonists, Pam_3Cys and FSL-1 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 TCDD while promoting development of Th17 cells in reaction to tryptophan photo-product, FICZ (43).

Again, the ligand-specificity of AhR seems to be influenced by crosstalk with other receptors and transcription factors.

The outcome of signal recognition does not merely depend on cooperation between receptors but also on the milieu of cytokines and other factors, which 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 an inhibitory effect 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 TLSP, that promote development of Tregs in the MLN (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 other conditioning factors. The signals, which induce tolerance, include the so called “microbial symbiosis factors”, “resolution-associated molecular patterns” (RAMPs) as well as a variety of microbial-derived metabolic products which serve as markers of energy extraction (33, 51-53). Here we refer to these tolerogenic signals collectively as *safety signa*ls and distinguish them from pro-inflammatory *danger signals* released by infection and damage.

Tolerization by safety signals

Recognition of safety signals by PRRs does not merely result in the absence of induced

pro-inflammatory mediators but also in the activation of mechanisms that promote unresponsiveness to danger signals (Figure 1). These safety signal-mediated mechanisms include blocking the access of danger signals to their receptors, the termination of danger-activated pathways and the inhibition of danger-response gene transcription.

One of the strategies to block the access of danger signals to their corresponding sensors is by inducing degradation of these signals. For example, in type 1 regulatory T cells (Tr1), 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 Tr1 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 the release of antagonists for proinflammatory receptors as illustrated by TLR5 on IECs, which prevents activation of the IL-1-mediated pathway by inducing the release of the secretory IL-1 receptor antagonist sIL-1Ra (55). Termination of danger-activated proinflammatory cascades, in turn, is mediated by receptors, like TLR9 on IECs, which prevent IκB degradation downstream of TLR2, TLR3 and TLR5 and thus protect mice from intestinal inflammation (16). At the level of IRAK-1, pro-inflammatory cascades can be inhibited by TLR-mediated induction of IRAK-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 APCs 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 can interfere with the LPS-dependent release of proinflammatory mediators by activating AhR, which interacts with STAT1 and NF-κB to prevent the release of TNFα and IL-6 by macrophages (58). This mechanism is likely to explain the importance of AhR in protecting mice from endotoxin shock, as initial exposure of innate immune cells to LPS upregulates production of the enzyme TDO2, which, by mediating metabolism of tryptophan to an AhR agonist, L-Kynurenine, attenuates the expression of inflammatory genes (59).

Safety signals promote unresponsiveness to danger signals not only by terminating proinflammatory pathways and their target genes but also by inducing molecules that keep these signals away from the epithelium (60). Instrumental in this form of tolerization are antimicrobial peptides (AMPs), IgAs and mucins (MUCs), which, when released into the lumen, restrict the contact of microbial molecules with receptors that can initiate pro-inflammatory cascades (61). The anti-inflammatory role of PRR-mediated AMP production is demonstrated by studies showing that MyD88-dependent signalling is essential for the production of RegIIIγ, which indirectly prevents accumulation of Th1 cells in the underlying tissues (62). The accumulation of these pro-inflammatory cells is also prevented by the release of α-defensins by NOD2-stimulated Paneth cells (63). The anti-inflammatory activity of these AMPs is aided by IgAs, whose release is also controlled by environmental and metabolic sensors. Some of receptors induce expression of the polymeric immunoglobulin receptor (pIgR), a molecule 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 centres (GCs) (65). Finally, the action of AMPs and IgAs is supplemented with

MUCs, which, when induced by NLRP6, protect mice from *C. rodentium*–driven inflammation (66). This PRR-dependent mucus production is part of a feed-forward tolerogenic mechanism in which activation of CTL on DCs by Muc2 complexed with galectin-3 induces expression of anti-inflammatory genes (67).

Apart from 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 the LP. This is mediated by the modulation of growth, repair, survival and inter-cellular junctions of the epithelial cells. Epithelial growth and repair depend among other things on epidermal growth factor receptor (EGFR) ligands, AREG and EREG, 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 the studies of $NEMO^{IEC\text{-}KO}$ and $IKK\beta^{IEC\text{-}KO}$ mice whose spontaneous intestinal inflammation 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 the zonula occludens-1 (ZO-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 and thus reinforcing the intestinal barrier and protecting 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.

… FIGURE 1…

Safety signals promote microbial colonisation of the gut mucosa at birth

In addition to limiting acute pro-inflammatory reactions to commensal bacteria, safety signals positively promote long-term gut colonization. The induction of hospitable conditions for microbes is particularly important at birth, as the initial microbial inoculum protects the individual from allergic, metabolic and autoimmune diseases later in life (71). To provide an opportunity for this significant colonisation 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 MAPK responses to PRR signals, infants are hypo-responsive to vaccines and have an increased risk of intestinal infection caused by *E. coli.*

Even though PRRs later acquire the capacity to also recognize danger signals, the general microbial profile of an individual 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 the genetic makeup of the individual (73, 74). The persistence of intestinal microflora is possible because adult PRRs favour tolerance towards ligands originating from established rather than from newly arrived microbial communities. Indeed, repeated stimulation, unlike the abrupt activation of basolateral TLR5 on IECs, leads to the inhibition of NF-κB and MAPK pathways and

promotes internalization of TLR5; an effect that establishes a long-lasting unresponsiveness to flagellin (75). Similarly, TLR9 on the basolateral surface of IECs, despite inducing pro-inflammatory IL-8 in response to ISS-ODN, becomes unresponsive to the same molecule following repeated challenge (16). The induction of tolerance to persistently stimulated PRR signals is also 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 TGF-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; the initial, chronically acquired microbial communities, in contrast, fail to induce these inflammatory responses and so can settle in and persist (77).

In addition to the uniform tolerogenic treatment of the initial commensal colonizers and established microbial communities, PRRs are also highly selective in reacting 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 PSA from *Bacteroides fragilis* and not to synthetic lipopeptide TLR2 agonists (78). Similarly, the TLR2/CD14 receptor ligand, LcrV from *Yersinia* spp. unlike another TLR2 agonist such as the LcrV-homologue protein PcrV, can induce intestinal macrophages to up-regulate expression of IL-10 (79). Likewise, LPS from *S. typhimurium,* in contrast to

LPS from other *Salmonella* species, can moderately enhance 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 manifest the potential to recognize microbe safety signals of certain species of bacteria specifically.

Specificity is combined with a degree of non-specificity; innate immune specificity does not limit tolerance to particular members of microbial communities. Instead, specific recognition of microbes by the innate immune system also leads to unspecific tolerance in the gut. This is observed in Ly6C^{hi} monocytes that, when stimulated by commensals through a variety of TLR agonists, produce PGE2, which helps inhibit activation of neutrophils and terminates inflammation against pathogenic *Toxoplasma gondii* (82). Some pathogens like mouse mammary tumor virus (MMTV) take advantage of such generalised tolerogenic reactions by binding to commensal LPS and so activating TLR4-medited release of IL-10 (83). Overall, the induction of regulatory cells and molecules by safety signal-stimulated PRRs has an unspecific tolerogenic effect, which is not only targeted towards the stimulating microbes but also towards other luminal microbes and food materials.

These indiscriminate tolerogenic effects, however, do not over-ride the power of innate environmental sensors to select for the host microbiota and thus to define the microbial signature of the 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, suggesting 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 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 was also observed in MyD88-deficient *Hydra* (89). Nevertheless, in contrast to mice, these more primitive organisms manifested an altered capacity to reestablish species-specific signatures of their microbiota following an antibiotic challenge or infection, confirming that PRRs developed 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 (Figure 1). In fact, dysbiosis in NOD2$^{-/-}$, NLRP6$^{-/-}$ and TLR5$^{-/-}$ mice was found to be associated with alterations in the production of AMPs in these animals. 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, IL-22$^{-/-}$ mice, like mice deficient in individual members of the TLR family, exhibit alterations in microbial populations that can be transferred to WT mice together with their associated colitogenic phenotype (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 (ILCs) limits the expansion of segmented filamentous bacteria (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 the studies showing that the AhR-dependent release of this cytokine by innate lymphoid cells (ILCs) in response to a tryptophan metabolite, IAId derived from *Lactobacillus reuteri,* can limit expansion of *Candida albicans* (95). The IL-22-mediated modulation of microbial composition may also account, at least partially, for the beneficial effects of FICZ 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 members of gram-positive and gram-negative bacteria (99). RegIIIβ preferentially targets *Clostridium butyricum*, *L. reuteri* and various strains of *E. coli,* but not other bacterial species like *S. typhimurium,* giving the latter an advantage during infection (100). Other examples of AMPs which are induced by PRRs to control microbial composition include α-defensins, such as HD5, whose PRR-dependent release 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, genetically modified mice, whose Paneth cells express human HD5, manifest a different ratio of *Firmicutes* and *Bacteroidetes* phyla (102).

Similar to AMPs, the role of IgAs is not limited to preventing microbe-derived danger signals from accessing proinflammatory cascades, but also includes active shaping of the microbiome. The involvement of TLRs in IgA-dependent sculpting of the microbial composition is confirmed by the aforementioned studies of Tfh cells, which, when activated by TLRs, promote class-switch recombination of B cells towards the production of IgA (65). Deficiency of MyD88 in these T cells leads to a significant shift in the composition of the microflora characterized by a loss of microbial diversity and marked differences in the microbial populations between 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, RA activates B cells directly, to promote expansion of IgA-producing cells in PP; an effect which helps to prevent development of specific bacterial groups in the gut (104). Furthermore, intestinal metabolites like SCFA and endogenous ligands of AhR, like ITE, 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 the PP (108). Consequently, a deficiency of FoxP3+ T cells results in alterations in microbial communities characterized by reduced diversity of Firmicutes in general and nonpathogenic Clostridia in particular. The targets of IgA include SFB and

certain members of the *Clostridium* spp. as revealed by studies of $AID^{-/-}$ mice (109). However, the exact mechanism that allows IgAs to mold the microbiota is not known; this antibody does 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 expressed on innate cells and lymphocytes (111-113)

Taken as a whole, the above evidence demonstrates that safety signals activate PRRs to establish an unspecific 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 (Figure 2).

…FIGURE 2…

Summing up

In 1994 Polly Matzinger formulated the Danger Model, the idea that the immune system responds to signals originating from damaged or stressed tissues to initiate inflammatory and adaptive responses (114). Later studies identified PRRs and toxin receptors like AhR as specialised sensors of stress and damage supporting the Matzinger hypothesis (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: they both inhibit the production of pro-inflammatory cytokines and they limit 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 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 separate signaling pathways – a pro-inflammatory danger pathway for protection and an anti-inflammatory safety pathway for symbiosis. Paradoxically, however, safety signals 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 the individual through a dynamic landscape of symbiosis and threat by sensing context and mindful of the evolving history of the individual and the species. The roles of similar receptors in both danger and safety may seem paradoxical from the perspective of linear, human engineering, but such signalling appears to be advantageous.

Even more astoundingly, safety signals and danger signals are often embodied by the same molecules, which are, in turn, found either identical to or homologous with self-molecules. Consider endogenous signals like HSPs, DNA and ATP. All of them can indicate danger or safety, depending on the context, and all of them have their 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. Cohen) 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 the 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 signalling – resident microorganisms are an essential factor in the evolution of multi-cellular life generally (119).

Paradoxical signalling was recently 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, the authors demonstrated that systems in which one controller mediates conflicting functions are much more robust than are systems in which two separate controllers are dedicated to each separate function. Intuitively, if, for example, TLR4 would only recognize danger and TLR2 would only recognize safety, TLR4 deficiency would inevitably lead to an excess of anti-inflammatory molecules and hypo-responsiveness to pathogens. However, since each of these receptors can support seemingly conflicting functions, the overall balance between pro-inflammatory and anti-inflammatory mediators can be maintained in the face of a deficiency of either of these receptors (88). This is confirmed by the fact that mice deficient in individual members of the TLR family rarely exhibit spontaneous inflammation or infection. Indeed, pro-inflammatory and anti-inflammatory signals are summated in the gut to establish a neatly calibrated tolerogenic tone, which is readily reverted into

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 the immunity is the science of self/nonself discrimination (116) and call for an ecological view of life (124-127).

ACKNOWLEDGMENTS

This work was supported by Chinese Academy of Science (CAS) Research Grant and the University of Science and Technology of China Youth Innovation Fund.

DISCLOSURE

The authors have no conflicts of interest to declare.

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FIGURE LEGENDS

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 signalling 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.

Fig. 2. PRRs define the microbiota signature of the individual. PRRs shape the microbiome by a process of negative selection, which 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, which can be transferred by the modified microbial community to other hosts – signified by the dotted figure in the center. VRE, Vancomycin-resistant Enterococcus.

List of Abbreviations: AhR, aryl hydrocarbon receptor; AID, activation-induced (cytidine) deaminase; AMP, antimicrobial peptide; APC, antigen presenting cell; AREG, amphiregulin; BM, bone marrow; eATP, extracellular adenosine 5'-triphosphate; CCL20, chemokine (C-C motif) ligand 20; CLR, C-type lectin-like receptor; CTL, C-type lectin; CX3CR1, CX3C chemokine receptor 1; DC, dendritic cell; DSS, dextran sodium sulphate; EGFR, epidermal growth factor receptor; EREG, epiregulin; FICZ, 6-formylindolo[3,2-b]carbazole; FoxP3, forkhead box P3; FSL-1, synthetic diacylated lipoprotein - TLR2/6; GC, germinal centre; GPCR, G protein-coupled receptor; HD5, human defensin 5; HDAC, histone deacetylase; HMGB1, High-mobility group protein B1; HSP, heat shock protein; IAId, indole-3-aldehyde; IEC, intestinal epithelial cell; IκB, inhibitor of NF-κB; IKK, IκB kinase; ILC, innate lymphoid cell; IRAK, interleukin-1 receptor-associated kinase; ISS-ODN, liposomal immunostimulatory DNA sequence; ITE, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester; KO, knock out; LcrV, Low calcium response locus protein V; LP, lamina propria; LPS, lipopolysaccharide; Ly6C, lymphocyte antigen 6 complex; MAPK, mitogen-activated protein kinase; MDP, muramyl dipeptide; MLN, mesenteric lymph node; MMTV, mammary tumor virus; MUC, mucin; MyD88, myeloid differentiation primary response gene 88; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; NLR, nucleotide-binding oligomerization domain-like receptor; NLRP6, NOD-like receptor family pyrin domain containing protein 6; NOD, nucleotide-binding oligomerization domain; P2X7, P2X receptor subtype 7; PcrV, hydrophilic translocator of type three secretion system; PGE2, prostaglandin E2; PGN, peptidoglycan; pIgR, polymeric immunoglobulin receptor; PRR, pattern recognition

receptor; PSA, polysaccharide A; RA, retinoic acid; RAMP, resolution-associated molecular pattern; RegIII, regenerating islet-derived protein 3; RORγt, Retinoic acid receptor-related orphan nuclear receptor gamma; SCFA, short chain fatty acid; SFB, segmented filamentous bacteria; sIL-1Ra, secretory IL-1 receptor antagonist; STAT1, signal transducer and activator of transcription 1; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; Tfh, follicular helper T cell; Tfr, T follicular regulatory cell; TGF, transforming growth factor; Tr1, type 1 regulatory T cell; TDO2, tryptophan 2,3-dioxygenase; TLR, toll-like receptor; TNBS, 2,4,6-Trinitrobenzenesulfonic acid; TNF, tumor necrosis factor; TRAF, TNF receptor associated factor ; Treg, regulatory T cell; WT, wild type; ZO-1, zonula occludens-1.

Fig. 1.

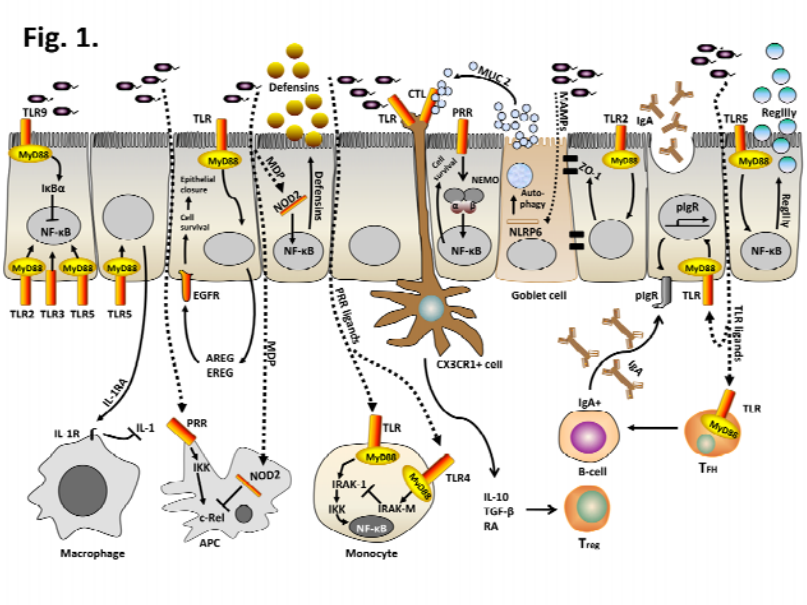

Fig. 2.