The interplay between the innate immune system and the microbiota
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The human gastrointestinal tract harbors one of the highest densities of microorganisms on earth, called the microbiota. In fact, the number of microbial cells in the intestine outnumbers the amount of human cells of the entire organism by a factor of 10. As such, a human being is more and more perceived as a super-organism consisting of a eukaryotic and a prokaryotic part. The compartment mediating the communication between both parts is the innate immune system and its various microbe-sensing pattern-recognition receptors. Co-evolution of the microbiota with the innate immune system has resulted in elaborate interdependency and feedback mechanisms by which both systems control mutual homeostasis. Here, we review the most important innate immune–microbiota interdependencies known to date. While microbial sensing by pattern-recognition receptors is required for stable microbial composition, the presence of the microbiota, in turn, is necessary for proper development and function of the immune system.

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Introduction: Innate immune recognition of the microbiota
The presence of a dense prokaryotic ecosystem within a eukaryotic host poses a conceptual paradox, since the host depends on the symbiotic presence of a massive microbial population, but at the same time has developed innate immune mechanisms to rapidly clear all non-mucosal tissue sites from pathogenic or invading prokaryotes [1,2]. For a long time, therefore, it has been assumed that the host has to circumvent the recognition of microorganisms at mucosal surfaces. A conceptual change came when it was observed that mice with deficiencies in innate immune receptor signaling harbor an altered composition of the microbiota, termed dysbiosis, and that this alteration in microbial ecology predisposes these mice to enhanced inflammatory disease [3**]. Innate immune deficiency leads to compensatory adaptive immune reactions against intestinal bacteria, indicating that homeostatic recognition of the bacterial microbiota takes place and is necessary for host–microbiota mutualism [4**].

Here, we summarize the most prominent examples of host innate immune components that are influencing or influenced by the microbiota. The microbiota is required for proper development and function of innate immune cells, while an intact innate immune system provides effector functions that maintain a stable microbial ecosystem. Disruptions of either side predispose to both local and systemic disease, demonstrating the necessity of functional host–microbiota mutualism for homeostasis and health.

Innate immune pathways in host–microbiota mutualism
The advent of next-generation sequencing methods coupled with metagenomic data analysis has enabled the study of intestinal microbial ecology at a population-wide level. Studies in mice with deficiencies in innate immune pathways have demonstrated that these pathways are not only necessary for elimination of pathogens, but also provide a stable microbial ecosystem composition (Table 1).

Toll-like receptors
Toll-like receptors (TLRs) were the first pattern recognition receptors (PRRs) implicated in the recognition of the commensal microbiota [3**,5,6]. TLR activation is based on the detection of a defined set of conserved molecular patterns that are unique to microorganisms [7,8]. Signaling through TLRs generally leads to either the production of pro-inflammatory cytokines, mediated by the adaptor molecule Myeloid differentiation primary response gene 88 (MyD88), or to production of type I interferons (IFNs) through engagement of TIR-domain-containing adapter-inducing interferon-β (TRIF) [9,10].

The role of TLR signaling in the control of intestinal microbial ecology remains controversial. The cecal microbiota of TLR5-deficient mice and MyD88-deficient mice was described to differ from that of WT mice [11**,12]. Vijay-Kumar et al. reported that altered gut microbial ecology in TLR5-deficient mice leads to manifestation...
of the metabolic syndrome (Figure 1b). By contrast, Ubeda et al. found that MyD88 or TLR signaling does not detectably alter the composition of the intestinal microbiota under steady state conditions or after antibiotic treatment and instead suggested a role for the parental lineage and husbandry of mice [13]. The degree to which the complex bacterial populations inhabiting the gut establish an equilibrium that is self-sustaining and independent of TLR recognition remains to be determined. Regardless of the effect of TLR deficiency on the community level of microbial composition, PRR signaling also influences the colonization of specific bacterial species (Figure 1a). The gut commensal Bacteroides fragilis requires TLR2 activation on CD4+ T cells to promote regulatory T cell-mediated immune tolerance and the restriction of T helper 17 responses [14]. Thus, co-evolution of PRRs and microbial colonization has probably led to an intricate network of inter-dependencies.

Nod-like receptors

Nod-like receptor (NLR) signaling is triggered by a wide array of both microbial ligands and host-derived signals of cell damage. Nod1 recognizes peptidoglycan from Gram-negative bacteria and induces the genesis of intestinal lymphoid tissues in a CCL20-CCR6 dependent manner (Figure 1c), which is in turn necessary for maintaining a stable microbial community in the intestine [15]. Microbial Nod1 ligands also stimulate the pathogen-killing ability of bone marrow-derived neutrophils [16]. Nod2-deficient mice were found to have increased amounts of commensals as well as reduced capability to clear newly colonizing bacteria [17]. Nod2 expression is dependent on the presence of commensal bacteria, creating a feedback loop in which commensal bacteria positively regulate Nod2 signaling, which in turn negatively regulates the commensal microbiota [17] (Figure 1d). Nod2 is highly expressed in Paneth cells, antimicrobial peptide-secreting cells found in small intestinal crypts, and loss-of-function mutations in Nod2 have been suggested to alter host-microbial interactions through altered antimicrobial activity [18]. Expression of a subgroup of α-defensins produced by Paneth cells is reduced in Nod2-deficient mice [19,20]. Interestingly, α-defensins have a key role in shaping the composition of the small intestinal microbiota, as demonstrated in a mouse model of defensin overexpression and deficiency [21]. Furthermore, RegIIIy, a secreted anti-bacterial lectin, is essential for maintaining a ‘clean’ zone that physically separates the microbiota from the small intestinal epithelial surface [22]. How microbial sensing by PRRs is linked to anti-microbial peptide secretion is in many cases unknown, but it is conceivable that yet elusive regulatory feedback loops exist that couple the recognition of microbial localization to a specific anti-microbial response.

Other NLR proteins are capable of forming a cytoplasmic complex called the inflammasome [23]. Inflammasome activation leads to autocatalytic cleavage of caspase-1, resulting in the production of mature IL-1β and IL-18. Deficiency of NLRP6 inflammasome in mouse colonic epithelial cells results in reduced IL-1β levels and altered...
fecal microbiota characterized by expanded representation of the bacterial phyla Bacteroidetes (Prevotellaceae) and TM7 (Figure 1e) [24*]. Dysbiosis development in the absence of the NLRP6 sensing system predisposes the host to inflammatory bowel disease [24*], colitis-associated colorectal cancer [25], and features of the metabolic syndrome, including non-alcoholic fatty liver disease, morbid obesity, and type II diabetes mellitus [26]. Some disease manifestations were found to be transmissible by microbiota transfer, and to be mediated by innate host responses involving the chemokine CCL5 (inflammation), the cytokine IL-6 (carcinogenesis) and integrative NLR-<abbr>TLR</abbr> signaling (metabolic syndrome) [20,24*,27].

Cooperative PRR signaling at mucosal surfaces might represent a common theme in the maintenance of host–microbiota mutualism, pointing to the evolution of ‘fail-safe’ mechanisms in which multiple innate sensors regulate the complex homeostatic conditions found at densely colonized surfaces. Cooperative NLR signaling has been documented in the host response against the
Regulatory feedback loops between the microbiota and the innate immune system. (a) Innate lymphoid cell (ILC) function is influenced by microbial colonization. The microbiota induces myeloid cell cytokine expression, which enhances ILC1 function. ILC1 cells are crucial for host defense against enteric infections with *Salmonella* and *Helicobacter*. Similarly, ILC3 cell function is influenced by microbiota-modulated myeloid cell function. IL-25 signaling through mononuclear phagocytes suppresses IL-22 secretion by ILC3 cells, while IL-23 coming from myeloid cells enhances IL-22 secretion. IL-22 production is necessary for host defense against *Citrobacter rodentium* and for local containment of *Alcaligenes* spp. ILC2 cell function is dependent on epithelial cell secretion of cytokines and is necessary for host defense against helminthes. (b) In the absence of microbial colonization, epithelial cell

intestinal pathogen *Salmonella* Typhimurium, where NLRP3 and NLRC4 both activate caspase-1 [28]. In addition, the NLRC4 inflammasome induces significant IL-1β production by intestinal mononuclear phagocytes after infection with pathogenic, but not with commensal, bacteria. NLRC4-mediated production of mature IL-1β in phagocytes thus represents an innate immune response that may discriminate pathogenic from commensal bacteria in the large intestine [29]. Thus, redundancy and compensation of inflammasome receptors *in vivo* during homeostasis and microbial infections allow the host to respond to complex microbial challenges [30].

C-type lectins

In addition to bacteria and viruses, the microbiota comprises numerous fungal species. Commensal fungi are recognized by the host via Dectin-1, a C-type lectin receptor that recognizes β-1,3-glucans found in the cell wall of nearly all fungi. Dectin-1 deficiency leads to increased susceptibility to colitis because of an inability of the host to cope with fungal colonization (Figure 1f) [31*]. Although many studies have shown that intestinal inflammation can lead to changes in commensal bacteria that affect the host [32,33], the effect of intestinal inflammation on fungal population ecology (the ‘mycobiome’) remains largely unknown [34].

Microbiota control of innate immune cell function

In addition to host regulation of the microbiota, functional characteristics of the microbiota have a profound effect on host mucosal and systemic immune system development. The generation of germ-free mice, which are devoid of any microbial colonization, has provided insight into microbiota control of immune cell development and function. In this section, we focus on innate immune populations directly influenced by the presence of intestinal microbiota.

Innate lymphoid cells

Innate lymphoid cells (ILCs) share a common origin and functional similarities with CD4⁺ T cells, but their differentiation occurs independently of somatic antigen receptor recombination. They can be classified into three subsets

DNA hypermethylation and enhanced CXCL16 release leads to the accumulation of CXCR6-expressing NKT cells, which promote mucosal inflammation through the release of IL-4 and IL-13. (c) Two compartments of myeloid cells regulate trafficking of microbial antigens to the mesenteric lymph node (mLN): During homeostasis, *CXCR1*⁺ cells sample microbial antigen, but are usually non-migratory. Dysbiosis or defective microbial recognition, however, induces their migration to the mLN. By contrast, *CD103*⁺*CD11c*⁺ cells are the major transporters of microbial antigen to the mLN where they induce adaptive immune responses. These cells are in some cases found in close proximity to intestinal epithelial cells. Microbial colonization also modulates myeloid cell epigenetic states, which is necessary for NK cell activation (compare to ILC1 in panel A), and proper antiviral immunity.

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*Current Opinion in Immunology* 2014, 26:41-48
that are developmentally dependent on the expression of the transcription factors T-bet (ILC1), GATA3 (ILC2), and RORγt (ILC3), respectively. Intestinal RORγt+ ILCs require intestinal microbial colonization for proper development or function [35*,36*,37*,38*]. In many cases, epithelial cells and myeloid cells serve as signaling hubs linking signals derived from the microbiota to ILCs function. In turn, ILCs exert crucial functions in regulating the composition and localization of microbial communities, thereby constituting bonafide mutualistic feedback loops that are likely the result of co-evolution between microbial species and ILC subsets (Figure 2a).

RORγt+ ILCs are the main source of intestinal IL-22 [38*], a cytokine regulating intestinal epithelial barrier function and anti-microbial peptide production. Mononuclear phagocytes induce IL-22 production through IL-18 or IL-23, and inhibit its secretion through commensal-dependent IL-25 release [38*,39,40]. ILC effectors are crucially important for host–microbiota mutualism. Specifically, RORγt-deficient mice feature elevated levels of commensal-specific serum IgG [41], and depletion of ILCs results in systemic dissemination of Alcaligenes spp. because of the absence of IL-22 [42**]. In addition, type III ILCs mediate the host response against enteric infection with Citrobacter rodentium [35*,43]. IL-22 production from RORγt+ ILCs is also required for weight gain in models of diet-induced obesity, presumably through changes to the microbiota, in particular reduction of segmented filamentous bacteria, that allows expansion of obesity-associated bacteria [44]. Similarly, ILC1 function depends on the presence of the microbiota [45], and absence of commensal bacteria impairs the antiviral response in myeloid cells [46]. T-bet deficiency in the innate immune system results in ulcerative colitis and Helicobacter typhlonius-induced inflammation [47,48]. Furthermore, ILC1 cells mediate the host response against Salmonella Typhimurium [49]. Likewise, microbial colonization induces epithelial expression of IL-7, IL-25, IL-33, and TSLP to induce the development and function of ILC2 cells that secrete IL-5 and IL-13 [50–52]. Type II ILCs are required for host responses against parasite infections. One might therefore speculate that the microbiota induces this host defense pathway to avoid parasite invasion of the intestinal microbial ecosystem and ensuing dysbiosis.

Natural killer T cells
Invariant natural killer T (iNKT) cells recognize lipids and glycolipids presented by CD1d molecules, and upon activation secrete pro-inflammatory cytokines (e.g. IL-4 and IL-13). As such, they have been proposed to play a significant role in the development of ulcerative colitis and asthma [53]. The levels of iNKT cells in non-mucosal tissues (spleen, liver and thymus) of germ-free mice are reduced compared to mice harboring a conventional microbiota, and are even further reduced in mice harboring a distinct, restricted microbiota [54]. This may be explained in part by antigenic drive, as glycosylceramides from the cell wall of Sphingomonas (not present in restricted microbiota) have been identified as CD1d ligands stimulating iNKT cells, but also by perforin-mediated depletion of iNKT cells [54].

By contrast, the levels of iNKT cells are increased in the colonic lamina propria and in the lungs of adult germ-free mice [55*], which are more susceptible to oxazolone-induced colitis and ovalbumin-driven allergic-asthma compared to bacteria-harboring mice. The increased susceptibility is CD1d-dependent and microbiota-dependent, as blockade of CD1d and microbial colonization of neonate, but not adult germ-free mice, reduced pathology in both models to specific pathogen-free (SPF) levels. Expansion of NKT cells in the colon and in the lung of germ-free mice is associated with hypermethylation of the Cxcl16 gene (encoding the ligand for the CXCR6 chemokine receptor found on NKT cells) and as a result increased expression of CXCL16 (Figure 2b). This hypermethylation is also microbiota-dependent, as colonization of neonate germ-free mice reduced hypermethylation to normal levels [55**]. Thus, exposure to commensal bacteria during early life has long-lasting regulatory effects on NKT cells, and in their absence later-life exposure to environmental stress may result in auto-inflammatory function of these cells.

Mononuclear phagocytes
A key feature of host–microbiota mutualism is the host’s ability to tolerate the presence of commensal bacteria while preserving the ability to rapidly react to invading bacteria. Distinct lamina propria-resident mononuclear phagocyte populations play a key role in sampling and trafficking of luminal antigens and subsequently invoking an adaptive immune response. CD11c+ CD103+ DCs express CCR7 and are considered as the major DC population that migrates to the mLN-LNs [56], where they imprint T cells for gut homing [57,58]. Nevertheless, they have been reported to be localized in the core of the villi, deep in the lamina propria [56] where access to luminal antigens is less likely, and display inferior ability to sample soluble luminal antigens in comparison to CX3CR1+ mononuclear phagocytes [59]. Those cells, in contrast, are able to form trans-epithelial dendrites and sample luminal antigens [60], and have been shown to be involved in clearance of enteroinvasive pathogens (Figure 2c). Nevertheless, CX3CR1+ DCs display poor T cell stimulatory capacity [56], and are not detected in the mLNs under steady state conditions.

However, in the case of dysbiosis, these concepts no longer apply. Migration of CX3CR1+ DCs to the mLN is apparent in DSS-induced colitis [61]. In antibiotic-treated or Myd88−/− mice, CX3CR1+ DCs transport noninvasive bacteria to the mLN, where they trigger specific immune response [62]. During enteric infection with
Moreover, DCs from germ-free mice fail to induce expression of various type I interferon genes, as well as inflammatory response genes including IL-6, TNF, IL-12 and IL-18 [45]. Consequently, NK cell priming and antiviral immunity are severely compromised. This may be explained by absence of activating histone marks (H3K4me3) on these genes in germ-free or antibiotic-treated mice. Thus, promotion of inflammatory responses in dendritic cells and subsequent priming of NK cells to combat infection is dependent on epigenetic cues from the microbiota.

**Perspective**

The examples provided above have established the concept of mutual interactions between innate immune pathways and the microbiota. Nonetheless, most questions that are being addressed by current research tend to view innate microbe--microbiota interactions from the perspective of a static super-organism. We believe that conceptual breakthroughs will come from an evolutionary viewpoint on this complex and dynamic ecosystem. The innate immune system is highly conserved between species and intestinal microbial colonization is an evolutionarily ancient feature of eukaryotes [65]. Remarkably, a recent study demonstrated that microbiota-dependent immune system maturation requires a species-specific microbiota composition, indicating that co-evolution has shaped the microbiota in a way that is ideally suited to a specific host [64**].

Such an approach will allow addressing the following intriguing questions: Which components of the microbiota are exerting a host-specific maturation function? Which innate immune effector mechanisms guarantee a stable microbial community composition, and which effector functions have been shaping this composition throughout evolution?

Finally, interactions between the microbiota and innate mechanisms of host defense might go beyond bonafide pattern recognition receptor signaling and innate immune cell function at mucosal tissues. Recent studies have shown that deficiencies in host metabolic pathways and changes in diet similarly lead to dysbiosis and ensuing pathologies [26,65]. Therefore, it is conceivable that, in addition to PRRs, the host has co-evolved a much broader, so far unexplored range of mechanisms which benefit from microbial colonization and in return ensure a stable microbial community composition. The examples of innate immune--microbiota mutualism might thus be merely exciting first insights into a perspective that will prospectively transform our understanding of the interface between the eukaryotic and prokaryotic parts of the mammalian super-organism.

**Acknowledgements**

We thank the members of the Elinav lab for fruitful discussions. We apologize to authors whose relevant work was not included in this review owing to space constraints. Eran Elinav is supported by grants provided by the Abish Frenkel foundation, Marie Curie Integration grant, Kenneth Rainin Foundation, The Leona and Harry B Helmsely charitable trust, Israel Science Foundation, and by the German-Israel Foundation. Christoph A. Thaiss receives a Boehringer Ingelheim Fonds PhD Fellowship.

**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This work underlines the importance of breeding and housing conditions in the study of dysbiosis in innate immune receptor deficient mice.


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This paper uncovers the impact of the microbiota on NKT cell function.


This study addresses the importance of host specificity in microbiota-mediated immune system development.


