REVIEW

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The microbiome and innate immunity

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The intestinal microbiome is a signalling hub that integrates environmental inputs, such as diet, with genetic and immune signals to affect the host's metabolism, immunity and response to infection. The haematopoietic and non-haematopoietic cells of the innate immune system are located strategically at the host–microbiome interface. These cells have the ability to sense microorganisms or their metabolic products and to translate the signals into host physiological responses and the regulation of microbial ecology. Aberrations in the communication between the innate immune system and the gut microbiota might contribute to complex diseases.

The past two decades witnessed a revolution in our understanding of host–microbial interactions that led to the concept of the mammalian holobiont — the result of co-evolution of the eukaryotic and prokaryotic parts of an organism. The revolution required two paradigm shifts that had a tremendous impact on their respective fields. The first occurred during the late 1990s with the discovery of pattern-recognition receptors (PRRs) in the innate immune system that sense microorganisms through conserved molecular structures. Several families of PRRs and their signalling pathways are now known, including the Toll-like receptors (TLRs), the nucleotide-binding oligomerization (NOD)-like receptors (NLRs), the RIG-I-like receptors, the C-type lectin receptors, the absent in melanoma 2 (AIM2)-like receptors and the OAS-like receptors[1]. These sensors are expressed by a variety of cellular compartments and constitute a continuous surveillance system for the presence of microorganisms in tissues.

The second shift occurred fewer than 10 years later and was driven by the culture-independent characterization of the microbiome[2] — the entirety of the microorganisms that colonize the human body and their genomes. Because of the enormous number of microorganisms that reside on the surface of the body — the skin and the gastrointestinal, respiratory and urogenital tracts — it seemed improbable that innate immune recognition of microorganisms could be coupled to the immediate initiation of immune responses against them without leading to overt, organism-wide inflammation and its damaging effects. It was therefore hypothesized that microbial sensing at the body surface needs to be tightly controlled to ensure a symbiotic relationship between the host and its indigenous commensal microorganisms[3], while allowing for the initiation of a rapid, sterilizing immune response on penetration of microorganisms into non-colonized sites. This idea was developed further after the realization that host–microbiota mutualism is lost in the absence of innate immune recognition of commensal microorganisms, with detrimental consequences for health[4,5]. The crosstalk between innate immunity and the microbiome is now known to extend far beyond the achievement of a careful balance between tolerance to commensal microorganisms and immunity to pathogens. The microbiota integrates into whole-organism physiology and influences multiple facets of organismal homeostasis through its effects on the innate immune system. Sensing by this system therefore serves as a rheostat for the metabolic activity of the microbiota and its exposure to diet and xenobiotics, as well as for the presence of mucosal infections. The information that is gathered is then processed at various levels of physiology to dynamically adjust the activity of the host to fit the state of the surrounding microbial ecosystem. Conversely, the innate immune system plays an important part in shaping the community and ecology of indigenous microorganisms into configurations that can be tolerated by the host and are beneficial for its metabolic activities. This complex, bilateral interaction between the host and its microbiota has a crucial role in human health. Many 'multifactorial' disorders, formerly considered to be idiopathic, might therefore be influenced or even driven by alteration of the intimate crosstalk that occurs between the innate immune system and the microbiota during homeostasis. In this Review, we highlight paradigms of interactions between the innate immune system and the microbiota, the mechanisms that are involved in this crosstalk and how aberrations in either of the partners of this communication network contribute to the molecular aetiology of common multifactorial disorders. Because the roles of viruses, fungi and parasites have been summarized elsewhere[6,7], we focus on the interplay between the innate immune system and the bacterial microbiome.

Physiological functions

A network of interactions characterizes the interdependence between the innate immune system and the microbiota[8]. The two systems affect one another to orchestrate whole-organism physiology.

Epithelial cells

Although not classically considered to be bona fide cells of the innate immune system, intestinal epithelial cells are equipped with an extensive repertoire of innate immune receptors[9] (Fig. 1). Expression of these receptors and active signal transduction on microbial recognition is pivotal for intestinal homeostasis because their epithelial-specific deletion leads to breaches in the epithelial barrier, which compromises the spatial separation between commensal bacteria and the lamina propria of the intestines, thereby predisposing the tissue to spontaneous inflammation. This has been demonstrated for components that are involved in TLR signalling, including myeloid differentiation primary response protein MyD88, TNF receptor-associated factor 6 (TRAF6), and NF-κB essential regulator (NEMO)[4,10–12], as well as for orchestrators of cell death such as receptor-interacting serine/threonine-protein kinase 1 (RIPK1), FAS-associated death domain protein (FADD) and caspase-8 (refs 13–16).

NOD-containing protein 2 (NOD2), which is highly expressed in the Paneth cells of the small intestine, is activated by microbial

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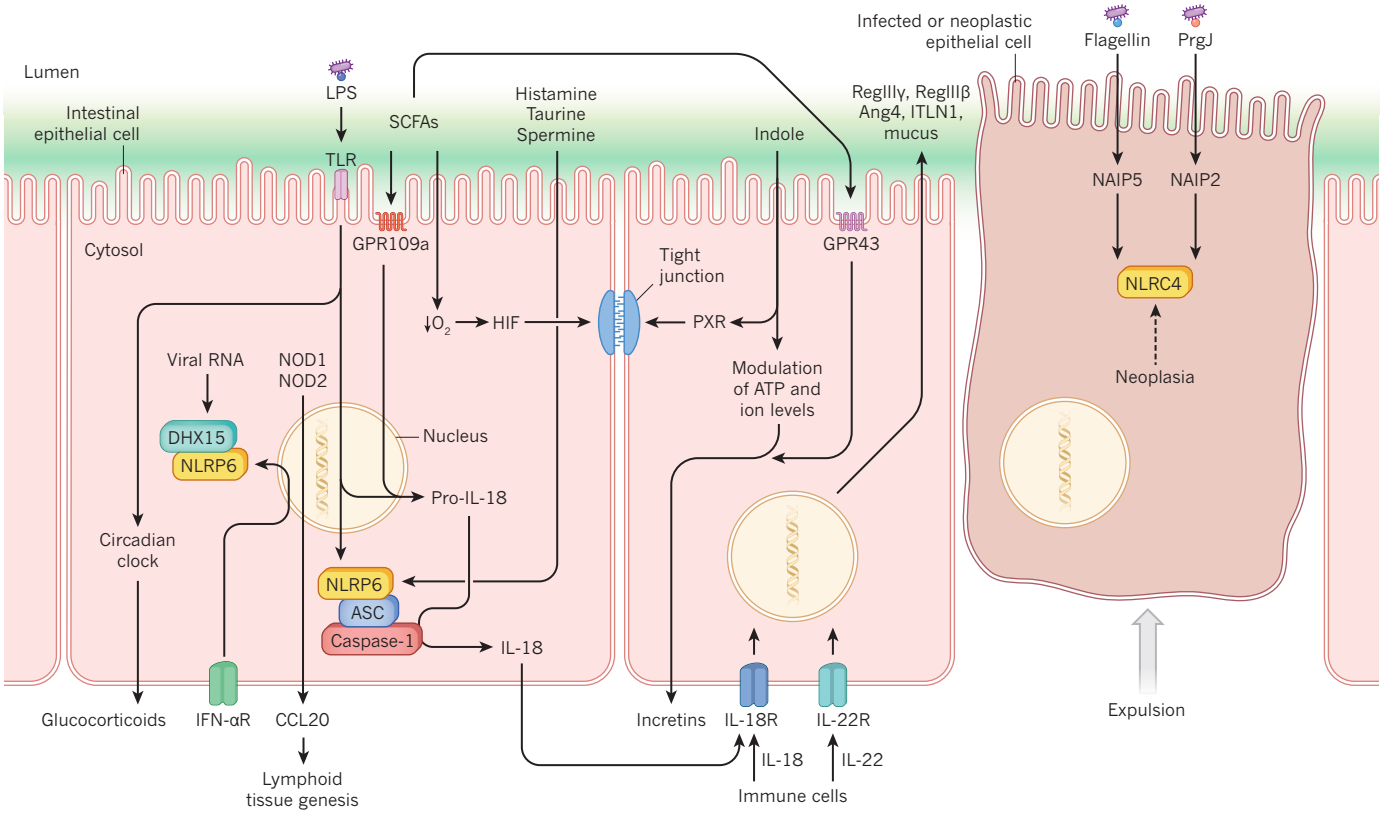

Figure 1 | Intestinal epithelial cells orchestrate the host–microbiota interface. Intestinal epithelial cells use the recognition of microbial-cell components and metabolites to adjust their antimicrobial programme and metabolic homeostasis. The activation of PRRs, such as TLRs and the NOD-like receptors NOD1 and NOD2, is directly coupled to the production of antimicrobial peptides (including RegIIIγ, RegIIIβ, Ang4 and Itln1) and of mucus. IL-18 plays an important part in this process through an autocrine loop. The secretion of epithelial IL-18 requires transcriptional activation through TLRs or the G-protein-coupled receptor GPR109a and posttranscriptional cleavage through the NLRP6 inflammasome. NLRP6 can also be induced by type I interferons and functions as a sensor of viral DNA with pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15. IL-18 and IL-22 derived from immune cells also help to regulate the antimicrobial responses of epithelial cells. CCL20, which is derived from epithelial cells downstream of NOD1 signalling, is involved in the genesis of lymphoid tissue. NLRC4 promotes the expulsion of neoplastic or infected cells from the intestinal epithelium. PRR signalling also orchestrates the circadian clock within intestinal epithelial cells and adjusts the secretion of epithelial-derived metabolic hormones, such as glucocorticoids. Epithelial cells also respond to the levels of microbiota-modulated metabolites, such as SCFAs (acetate, butyrate, propionate), polyamines (spermine), as well as amino acids and products that are derived from them (taurine, histamine, indole). Taurine, histamine and spermine modulate the activity of inflammasome component NLRP6. Indole modulates the levels of incretin section and promotes the barrier function of the epithelium through the PXR, which helps to fortify tight junctions between cells. SCFAs serve as energy sources for epithelial cells and also support barrier function through HIF. ASC, apoptosis-associated speck-like protein containing a CARD; R, receptor.

peptidoglycan and generates a cellular response that includes the secretion of cytokines, the induction of autophagy, intracellular vesicle trafficking, epithelial regeneration and the production of antimicrobial peptides, thereby influencing the composition of the microbiota[17–19]. Epithelial NOD1 is important for both the C–C motif chemokine 20 (CCL20)-mediated generation of isolated lymphoid follicles in the intestine and homeostatic bacterial colonization[20].

PRRs in the epithelium are also important for the elimination of pathogenic infection. Epithelial expression of the inflammasome-forming NLR family CARD-domain-containing protein 4 (NLRC4), a sensor of flagellin and bacterial secretion systems, promotes the expulsion of infected intestinal epithelial cells, thereby contributing to the elimination of enteric pathogens[21,22]. NLRC4 also protects the host from intestinal carcinogenesis[23,24], which provides evidence for a unified model in which epithelial NLRC4 protects the epithelial layer by identifying and dislodging cells that have undergone harmful insults.

Signalling by the NACHT-, LRR- and PYD-domain-containing protein NLRP6 in intestinal epithelial cells is modulated by levels of amino acids and polyamines in the lumen of the intestine. It regulates the interface between the host and microorganisms through the production of inflammasome-mediated interleukin (IL)-18 and the downstream expression of antimicrobial peptides[25], and it also controls the secretion of mucus by goblet cells[26]. Deficiency in NLRP6 leads to imbalances in the composition and function of the microbiota (dysbiosis), altered microbial biogeography and enhanced susceptibility to enteric infection[25–28]. Furthermore, NLRP6 has been described as a regulator of intestinal antiviral immunity[29], which suggests that it might function in the control of both bacterial and viral parts of the microbiome.

Other receptors also integrate microbial signals to adjust IL-18 levels, including hydroxycarboxylic acid receptor 2 (or G-protein-coupled receptor 109A), which is a receptor for butyrate and niacin[30,31], the DNA sensor interferon-inducible protein AIM2 (ref. 32) and the inflammasome component NLRP3. As a consequence, genetic deletion of these receptors leads to intestinal inflammation, tumorigenesis and susceptibility to enteric infection[33,34], which underlines the central role for epithelial IL-18 in orchestrating the intestinal host–microbial interface.

Intriguingly, the impact of microorganisms on intestinal epithelial cells extends far beyond the classical immunological functions of these cells. Commensal colonization probably has a major role in the metabolism of intestinal epithelial cells. Microbiota-derived short-chain fatty acids (SCFAs) serve as an energy source for the epithelium and they affect both oxygen consumption and hypoxia-inducible factor (HIF)-mediated fortification of the epithelial barrier[35]. The microbial metabolite indole promotes barrier function through the pregnane X receptor (PXR; also known as nuclear receptor subfamily 1 group I member

2 (NR1I2))[36] and increases the secretion of glucagon-like peptide-1 (GLP-1), an incretin with profound influences on host metabolism[37]. Microbiota-induced TLR signal transduction in intestinal epithelial cells also drives intestinal hormone production through the coordination of the circadian clock, a transcription-factor network that rhythmically controls the diurnal succession of cellular metabolic activity[38]. The microbiota itself undergoes rhythmic oscillations in composition and function[39,40], which suggests that the varying levels of microbial influence on the innate immune system might underlie marked fluctuations over the course of a day.

Taken together, intestinal epithelial cells integrate microbial signals into both the orchestration of the host–microbial interface, which consists of mucus and antimicrobial peptides, and the dynamic adjustment of cellular metabolism (Fig. 1).

Myeloid cells

Germ-free mice have a profoundly altered innate immune system. The microbiota influences the development and function of myeloid cells in multiple organs and at different time points during cellular development (Fig. 2). In the absence of the microbiota, myeloid-cell development in the bone marrow is reduced, which results in the delayed clearance of systemic bacterial infection[41]. The level of myelopoiesis correlates with the complexity of the intestinal microbiota and is adjusted in accordance with the level of TLR ligands that are present in blood serum[42]. Microbiota-derived SCFAs might similarly drive myelopoiesis in the bone marrow[41,43]. The influence of the microbiota on myelopoiesis begins before birth. The offspring of mice that are treated with antibiotics during pregnancy have lower numbers of blood neutrophils and their bone-marrow precursors[44], and gestational colonization with microorganisms increases the number of intestinal mononuclear cells in newborn mice[44].

The microbiome also influences the maturation of myeloid cells after haematopoiesis. The continuous presence of microbiota-derived TLR ligands drives the ageing of neutrophils[46]. The number of circulating basophils is likewise influenced by microbiome-derived TLR ligands[47].

In addition to affecting circulating myeloid cells, the microbiota strongly influences the biology of tissue-resident macrophages. Microglia, the macrophages of the central nervous system, display an altered morphology in germ-free mice — a phenotype that is, in part, due to a paucity of SCFAs[48]. In the skin, the microbiota influences the composition and inflammatory potential of resident myeloid cells[49]. In the lungs, treatment with antibiotics causes a shift in macrophage polarization that is mediated by prostaglandin E2, which enhances susceptibility to allergic airway inflammation[50]. In the intestine, microbial SCFAs serve as a signal to alter the gene-expression profile of local macrophages[31,51]. The microbiota also regulates the trafficking of myeloid cells in the gut. Intestinal microbial colonization drives the continuous replenishment of macrophages in the intestinal mucosa by monocytes that express C–C chemokine receptor type 2 (CCR2)[52].

The tissue-specific effects of the microbiome on resident myeloid cells go beyond bona fide immunological functions. Signals that are released by the microbiota might influence the interactions between neurons of the enteric nervous system and intestinal muscularis macrophages to facilitate gastrointestinal motility[53]. Commensal microorganisms regulate both the expression of bone morphogenetic protein 2 (BMP2) by muscularis macrophages and the production of colony-stimulating factor 1 (CSF1; also known as macrophage colony-stimulating factor 1) by enteric neurons, which in turn influences smooth-muscle contractions in the intestinal muscle layer[53]. The microbiome also has an influence on tissue recovery after injury. A 2015 study found that the intestinal microbiota sustains inflammation and lymphadenopathy after infection with *Yersinia pseudotuberculosis*[54], thus compromising the return to homeostatic tissue-specific immunity.

Such findings suggest that colonization by commensal microorganisms profoundly shapes the myeloid landscape of the host, both in mucosal tissues and systemically. Local concentrations of microbiota-derived metabolites, as well as systemic levels of microbial products, seem to drive myeloid-cell differentiation and function through PRR signalling. Notably, these microbiota-driven alterations in the myeloid-cell pool greatly influence the susceptibility of the host to a variety of disorders, which range from infection[55] and sepsis[44,46] to allergy, asthma[47,50] and graft-versus-host disease[56]. They also regulate the effectiveness of vaccination[57] and therapies for cancer[58].

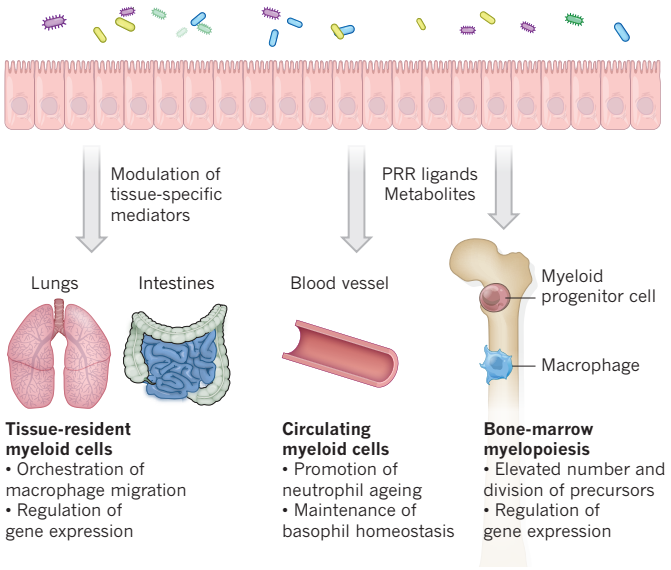

Figure 2 | The integration of microbial signals by myeloid cells. The microbiome influences the function of myeloid cells at all stages of their development. The influence of the microbiome on the migration and gene expression of tissue-resident myeloid cells is achieved mainly through the modulation of local metabolites and mediators of tissue identity. Circulating granulocytes are influenced by microbial PRR ligands. Myelopoiesis in the bone marrow is reduced in the absence of commensal bacteria and their microbial products in the blood.

Innate lymphoid cells

The influence of the microbiota is not limited to the development of the myeloid arm of the innate immune system. However, the regulation of innate lymphoid cells by the microbiota seems to follow rules and mechanisms that are different from the principles applied to myeloid-cell regulation (Fig. 3). Innate lymphoid cells (ILCs), a recently discovered lymphocyte branch of the innate immune system, develop normally in the absence of the microbiota[59], but the proper functioning of ILCs is dependent on commensal microbial colonization[60–62]. Rather than exerting their effect during lymphopoiesis, signals that stem from commensal microorganisms seem to influence the maturation and acquisition of the tissue-specific functions of ILCs.

The ILC family consists of cytotoxic cells (natural killer cells) and non-cytotoxic subsets (ILC1, ILC2 and ILC3). Most studies that examine the influence of the microbiota on ILCs have focused on ILC3. The importance of ILC3 cells in host–microbiota interactions became clear when their depletion — and the resulting abrogation of IL-22 production — was shown to produce a loss of bacterial containment in the intestine[63]. The microbiota also influences ILC3 interactions with other components of the immune system. The presentation of microbial antigens by ILC3s limits commensal-specific T-cell responses[64] to maintain tolerance to commensal bacteria[65]. Microbial sensing and the production of IL-1β by intestinal macrophages drive granulocyte–macrophage colony-stimulating factor (GM-CSF) secretion by ILC3s, which is required for macrophage function and the induction of oral tolerance[66]. Flagellin sensing by myeloid cells that carry the CD103 antigen is required for the IL-23-mediated production of IL-22 by ILCs[67]. Furthermore, the production of lymphotoxin-α (also known as tumour necrosis

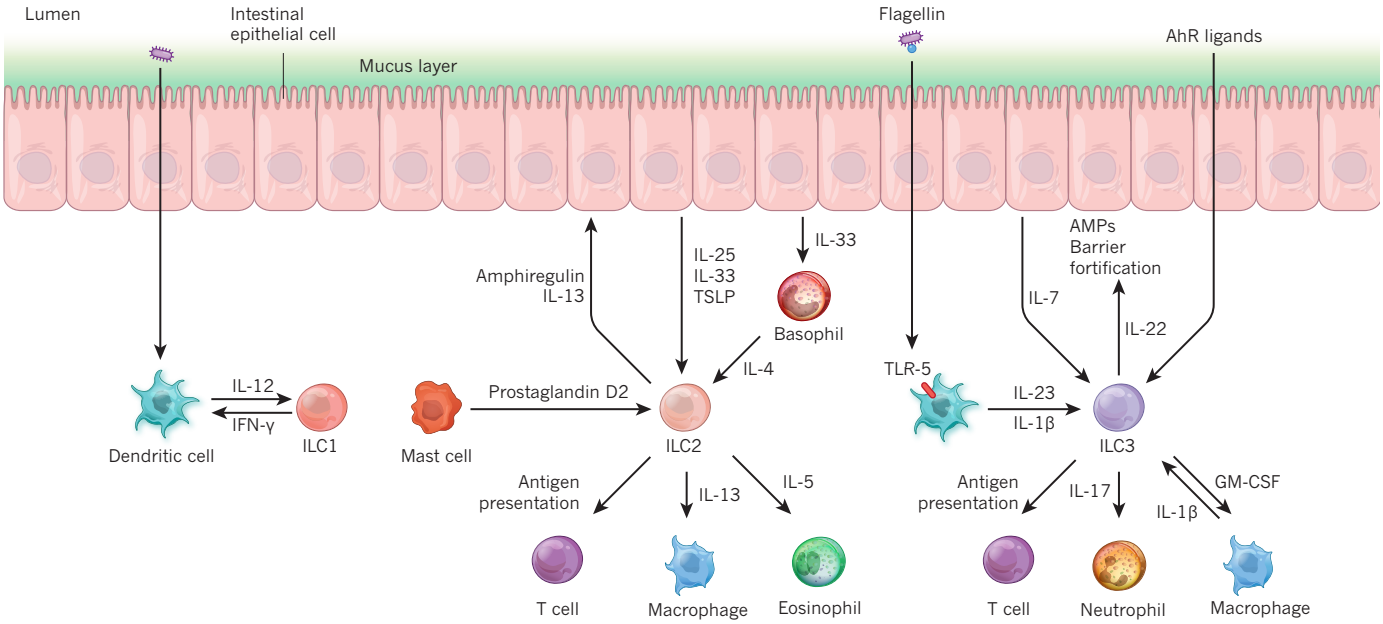

Figure 3 | The integration of microbial signals by ILCs. ILCs communicate with the local microbiota through cytokines, PRR ligands and antimicrobial peptides. In many cases, epithelial cells or myeloid cells serve as relay stations for crosstalk between ILCs and the microbiota. Group 1 ILC (ILC1) cells can be activated by myeloid-cell-derived IL-12. Group 2 ILC (ILC2) cells are activated by epithelial-derived cytokines and orchestrate type 2 immunity through their interactions with mast cells, eosinophils, basophils and macrophages. Group 3 ILC (ILC3) cells interact with cells of both the innate and adaptive immune systems. They also secrete IL-22, which initiates an antimicrobial programme as well as barrier fortification in epithelial cells. AhR, aryl hydrocarbon receptor; AMPs, antimicrobial proteins; LT, lymphotoxin; TSLP, thymic stromal lymphopoietin.

factor-β) by ILC3s is crucial for the production of IgA and for microbiota homeostasis in the intestine[68]. An equally important microbiota-instructed function of ILCs is their communication with epithelial cells. Microbiota-induced IL-22 production by ILC3s induces expression of the enzyme fucosyltransferase 2 (galactoside 2-α-L-fucosyltransferase 2) and fucosylation of surface proteins by intestinal epithelial cells, which is required for host defence against enteric pathogens[69].

Although these examples highlight the importance of microbial signals for the maturation and function of ILCs, the precise mechanisms through which they exert their influence remain unclear and are, in some cases, controversial. For instance, some studies have reported elevated levels of IL-22 by ILCs in the absence of the microbiota, whereas others have documented the abrogation of IL-22 secretion[70]. Different conclusions have also been reached in relation to whether the number of tissue-resident ILCs is altered in mice that are germ-free or have been treated with antibiotics[70]. Further studies are needed to reconcile these observations and their underlying mechanisms.

The microbiota might also influence the activity of the other ILC subsets. ILC2s are activated by epithelial tuft-cell-derived IL-25 (ref. 71), which is produced in a microbiota-dependent manner[62]. Deletion of the ILC1-lineage transcription factor T-bet (also known as T-box transcription factor TBX21) in the innate immune system results in ILC-dependent and *Helicobacter typhlonius*-driven inflammation of the intestines[72].

Collectively, the myeloid and lymphoid branches of the innate immune system are shaped by the microbiota, but the underlying mechanisms are based on distinct principles. A scenario could be envisioned in which the complexity of commensal microbial colonization is reflected in the amount of circulating PRR ligands and the concentrations of microbiota-derived metabolites in tissues, both of which tune the level of myelopoiesis, as well as the system's inflammatory capacity, over the short-term. By contrast, ILC development might be hardwired to anticipate microbial colonization. Tissue-resident ILCs would then integrate signals from the microbiota, through regulatory mechanisms that are not fully understood, to fine-tune innate and adaptive immune responses at the tissue level.

Effects of the innate immune system on the microbiome

On sensing information about the metabolic state of the microbiota, the innate immune system relays signals to the host to adapt tissue-level physiology and might also adjust the composition and function of the microbiota. Genetic evidence from humans and mice indicates that the innate immune system plays an important part in regulating variations in microbiota composition over time and between individuals[73]. Dysbiosis has been reported in several mouse models of innate immune deficiency[8], such as in mice that lack the genes *NOD2* (refs 17, 19, 74), *NLRP6* (ref. 27) or *TLR5* (ref. 75). The innate immune system might therefore function to promote the growth of beneficial members of the microbiota and to contribute to the maintenance of a stable community of microorganisms. This is best demonstrated by the induction of epithelial fucosylation by ILC3s and IL-22. During starvation that is associated with intestinal infection, the shedding of fucosylated proteins into the intestinal lumen serves as a source of energy for commensal bacteria[76]. Innate-immune-system resources therefore can be mobilized to support the microbiota during perturbations of the intestinal ecosystem. Similarly, TLR1 signalling is required to maintain the composition of the microbiota after *Yersinia enterocolitica* infection[77]. By contrast, PRRs do not seem to play a part in the development of the microbiota after treatment with antibiotics has ended[78]. However, it remains possible that activities of the microbiota that are independent of PRRs are involved in controlling the succession of microbial colonization after catastrophic events in the ecosystem.

The mechanisms through which the microbiota controls the development of the innate immune system are beginning to be understood, although the principles and purpose of innate-immune control over temporal dynamics in microbiota function remain unknown. Future mechanistic studies need to better define the characteristics of a 'healthy' microbiome that the host immune system attempts to preserve. Insights into such mechanisms came from the finding that dysbiosis in NLRP6-deficient mice was associated with similar metagenomic functions as were being studied in different animal facilities[25]. Dysbiosis developed *de novo* after the colonization of germ-free NLRP6-deficient mice, which indicates that certain PRRs might create specific

antimicrobial landscapes that are associated with the preservation of distinct functions of the microbiome.

Mechanisms of system crosstalk

A wide range of physiological contexts are influenced by communication between the microbiota and the innate immune system, and it is interesting to consider the molecular and cellular mechanisms that mediate this communication at the functional level. Commensal microbial colonization is known to influence the activity of the innate immune system according to a number of common principles.

Transcriptional reprogramming

One of the most striking observations made in germ-free mice was the reprogramming of intestinal gene expression in animals that were colonized with a single commensal bacterium[79] or a single enteric virus[80]. This includes the expression of genes that are involved in host nutrient absorption and processing, barrier functions, gut motility, intestinal immune responses, angiogenesis and the metabolism of xenobiotics. Studies of germ-free mice and of natural microbial colonization during postnatal development have substantiated such findings by showing that transcriptional reprogramming of the intestine by the microbiota spans different regions of the gastrointestinal tract and is partially dependent on microbial sensing receptors of the innate immune system[81,82]. The impact of the microbiome on transcription reaches beyond the intestine. For instance, the livers of germ-free mice show massive alterations in the expression of a range of genes with metabolic and non-metabolic functions[83].

The transcriptional responses of the host to bacterial colonization are in part evolutionarily conserved, as shown by reciprocal microbiota transplantations between mice and zebrafish[84]. Yet there is a considerable degree of species specificity in host responses to microbial colonization, especially with respect to the maturation of the immune system[85]. Although such examples underline the importance of transcriptional responses to commensal colonization for the innate immune system, several lines of evidence suggest that regulation also occurs through mechanisms other than gene expression. Constituents of the microbiota have been implied in the regulation of ubiquitin signalling[86], protein neddylation[87,88], the nuclear translocation of RelA (also known as transcription factor p65) (ref. 89) and vesicle trafficking[90], which indicates that the full regulatory reach of commensal microorganisms is yet to be defined.

Epigenetic programming

Because a large fraction of the transcriptome is shaped by the microbiome in an organ-specific manner, gene regulatory mechanisms must integrate microbial signals into the orchestration of gene expression. Although it is appreciated that bacterial pathogens can modulate host epigenomics, the epigenetic interpretation of commensal microbial colonization by the innate immune system is only starting to be investigated. On an organismal scale, mediation of the transcriptional reprogramming of gene expression in the intestine by the open chromatin landscape was ruled out because the chromatin accessibility in germ-free mice is similar to that in colonized mice[91]. Instead, microbial regulation of gene transcription in the host might be achieved by differential expression of specific transcription factors and their binding to chromatin. The exploration of this possibility on an organismal scale could reveal potential regulatory pathways through which information on the state of the microbiota is integrated into the chromatin landscape of host tissues.

Specific examples of this phenomenon exist in the context of the innate immune system. Analysis of epigenetic modifications in the intestinal epithelial cells of germ-free mice revealed a low level of methylation on the gene that encodes the lipopolysaccharide sensor TLR4, which indicates that commensal bacteria might induce tolerance through the epigenetic repression of PRRs[92]. Microbial colonization of germ-free neonatal mice was found to decrease the methylation level of the chemokine-encoding gene *Cxcl16*, which reduced its expression and diminished the recruitment of invariant natural killer T cells, ameliorating colitis and allergic asthma[93]. A comparison of mononuclear phagocytes from colonized and germ-free mice revealed that the microbiota promotes the trimethylation of histone H3 at lysine 4 at the loci of inflammatory genes, including those which encode the type I interferons[55]. The acetylation of histones is similarly involved in the crosstalk between the microbiota and the innate arm of the immune system. When histone deacetylase 3 is specifically deleted from intestinal epithelial cells, gene expression is massively altered and the integrity of the epithelial barrier is lost[94]. These aberrations are known to be microbiota-dependent because germ-free mice that lacked intestinal histone deacetylase 3 do not present the same phenotype as their colonized counterparts[94].

Although the microbial signals that are responsible for specific epigenetic alterations are mostly unknown, it seems probable that microbial metabolites, rather than just the presence or absence of microorganisms, mechanistically influence the orchestration of histone modifications. For instance, the microbiota-derived SCFA butyrate was shown to modulate the immune response of colonic macrophages through the inhibition of histone deacetylases[51], with a potential contribution to the maintenance of immunological tolerance to commensal microorganisms. Transcriptional reprogramming through epigenetic modifications is therefore a prominent mechanism by which the microbiota exerts its influence on host innate immunity. The elucidation of the precise mechanisms through which microbial molecules influence host-cell epigenomes and adjust the transcriptome to respond to the state of microbial colonization is an exciting area for future research.

Hierarchical feedback loops

The local containment and functional maintenance of a microbial ecosystem within the host is a formidable challenge for the mammalian innate immune system. Co-evolution between the microbiota and

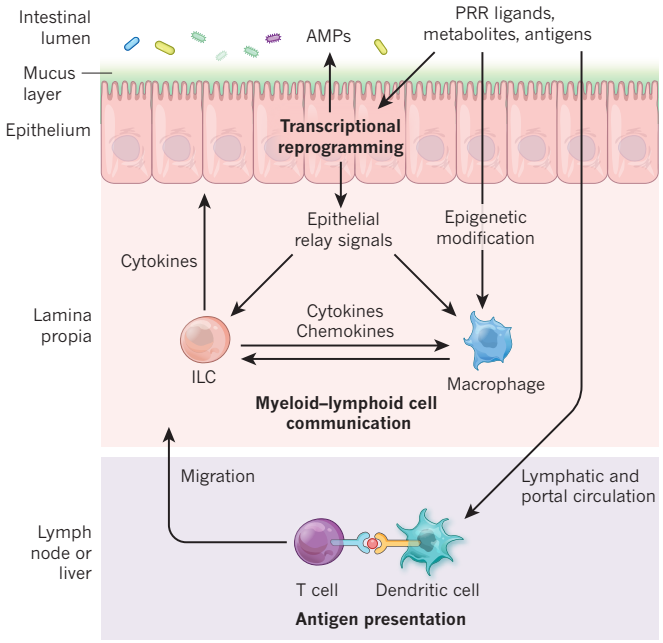

Figure 4 | The hierarchy of anatomy in microbiome–innate-immune-system interactions. Feedback loops between the host and the microbiome can be restricted to the epithelial layer of the intestinal wall, in which they consist of a brief circuit that links microbial sensing with transcriptional reprogramming and antimicrobial responses. A prototypical cytokine for such communication is the paracrine IL-18. Feedback loops that extend to the underlying lamina propria involve communication between epithelial, myeloid and lymphoid cells using cytokines and chemokines. Examples of cytokines that mediate such interactions are IL-22 and IL-23. Microbial products can also reach the draining lymph node and liver, where dendritic cells regulate anticommensal T-cell immunity to promote microbial containment. AMPs, antimicrobial peptides.

the host has led to the development of sophisticated feedback loops to accomplish this task. These loops can be regulated by various layers of cells within the intestinal wall. Although they are often restricted to the epithelium, which is directly exposed to the microbiota, they sometimes extend into the underlying mucosal lamina propria or even the lymphatic and portal circulation (Fig. 4).

In evolutionary terms, feedback loops that are restricted to the epithelium could represent the most ancient form of host–microbiota interaction. Such loops consist of only three steps: first, the recognition of microbes by PRRs; second, the transcriptional response of the host; and third, the secretion of effector molecules. The advantage of using such confined regulatory circuits is that the inflammatory response can be limited to the epithelial layer, without involving entire tissues or multiple organs. Examples include the epithelial-autonomous regulation of antimicrobial-peptide and mucus secretion by NLRP6 and NOD2, as well as the control of intestinal epithelial cell death by NLRC4, which all occur without the apparent contribution of other regulatory layers of cells[17–19,21,22,25–28,74,95–97].

The crosstalk between the innate immune system and the microbiome can also extend to the lamina propria. Microbial sensing by myeloid cells of the lamina propria provides regulatory signals that are crucial for the maintenance of commensal mutualism and the initiation of inflammatory responses in the host[67,98]. Myeloid cells modulate important pathways such as IL-22 production by ILCs, which induces the production of epithelial regenerating islet-derived protein 3 (RegIII)β and RegIIIγ, antimicrobial peptides that are important for maintaining a spatial separation between the majority of commensal bacteria and the intestinal epithelial layer, and this modulation is also pivotal for the local containment of commensals[11,63,99].

Regulatory circuits that reach the lymphatic and portal circulation represent a further level of interaction between the microbiome and the immune system. Migration to the mesenteric lymph nodes of antigen-presenting cells that carry material from commensal gut microbes is essential for the induction of commensal-specific adaptive immune responses[100–102]. Likewise, dendritic cells carry microbial antigens from colonized skin to the draining lymph nodes, where the production of cytokines determines the signature of the anticommensal immune response[103]. A similar 'firewall' might apply in the liver, which microbial products access through the portal vein[104].

Multiple levels of anatomy therefore contribute to the innate immune-mediated containment of the microbiota and to the tailoring of the immune response to the tissue-specific characteristics of host–microbiota interactions.

Impact on diseases

The interactions between the host and its microbiota are crucial for the preservation of tissue homeostasis. It is unsurprising therefore that perturbed interactions have emerged as a pivotal driver of various chronic disease states (see page 94). Three concurrent themes of interactions between the microbiome and the innate immune system are emerging as important contributors to microbiome-mediated disease phenotypes. First, microbial products might serve as perpetual stimuli of chronic immune responses, which contribute to the occurrence of non-resolving inflammation. For instance, microbial signals can sustain inflammation and tissue damage after infection-induced injury to the mucosa[54]. Second, abnormal microbial development during maturation of the innate immune system results in a failure to induce immunological tolerance, which then leads to exacerbated autoimmune and autoinflammatory disorders later in life. An example of this is the condition allergen-induced airway hyperreactivity[105]. Third, the microbiome greatly influences the factors that control tissue-specific immunity through mechanisms that can be active even at sites that are distant from the microbiome[106]. Therefore, dysbiosis can trigger pathophysiologies at remote organs and manifest as distinct symptoms in the context of 'sterile' tissues. For instance, intestinal dysbiosis drives the remodelling of the haematopoietic stem-cell niche in the bone marrow, and it also alters the differentiation of progenitor cells in the context of obesity[107].

A number of medical conditions that occur in people, or the equivalent conditions in animals, demonstrate how aberrations in the crosstalk between the innate immune system and the microbiome can contribute to pathogenesis on a molecular and cellular level (Fig. 5).

Infection

The microbiota contributes to the health of the host by colonizing the mucosal entry sites of pathogens, where it occupies biological niches and prevents invasion of the ecosystem by foreign elements — a concept known as colonization resistance (see page 85). In addition to its direct mediation of niche competition, the microbiota mediates resistance to infection indirectly by stimulating the innate immune response.

A prominent example of this is the intestinal immunity to viral infections that occurs when the host response is impaired by antibiotic-mediated depletion of commensal bacteria[108,109]. Effective antiviral innate immunity in the intestine is achieved through the induction of interferon (IFN)-λ and IL-18 or IL-22 (refs 110, 111) pathways, which then cooperate to induce the activation of signal transducer and activator of transcription 1 (STAT1) and antiviral genes[112]. Although IL-18 and IL-22 are induced by commensal bacteria, the expression of IFN-λ is suppressed by the microbiota, which enables efficient viral persistence[113]. Similarly, certain viruses can hijack interactions between bacterial molecules and the innate immune system, such as LPS–TLR4 signalling, to ensure their efficient transmission[114,115].

The microbiome and innate immune system also cooperate in the eradication of bacterial infection. Sometimes, neither innate immunity nor colonization resistance is sufficient to ensure the expulsion of pathogens. Instead, a combination of the two is required, as in the case of cooperation in the host defence against *Citrobacter rodentium*[116,117], a bacterium that can cause disease in mice. However, such combinatorial responses can be subverted by the pathogen. During infection with *Salmonella* Typhimurium, microbiota-induced IL-22 elicits a response that targets commensal bacteria and liberates a colonization niche for the pathogenic bacterium[118]. *Porphyromonas gingivalis*, an oral bacterium that is associated with periodontitis, evades the host by modulating the TLR2 pathway to support a niche for dysbiosis and subsequent inflammation[119].

Autoimmunity and autoinflammation

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders of multifactorial aetiology that affect the gastrointestinal tract and extraintestinal organs. These disorders provide models for studying perturbed crosstalk between the microbiota and the innate immune system because they integrate all aspects of mucosal immunology at the interface between microbial colonization and innate-immune-system activation. They also clearly demonstrate how limitations in our mechanistic understanding of this crosstalk hamper the development of treatments for common human disorders. Dysbiosis has a central role in the pathogenesis of IBD, and the introduction of bacteria that are associated with IBD into a murine model of colitis resulted in chronic disease[120], which suggests that immune dysfunction as an adjunct to specific microbial alteration is necessary for the development of IBD. Despite large-scale efforts, however, no particular species or group of commensal or pathogenic microorganisms has been identified as the cause of IBD in humans. Instead, multiple mechanisms at the interface between the innate immune system and the microbiome, such as microbial sensing, the release of reactive oxygen species and antigen processing, were hypothesized to contribute to the molecular pathophysiology of IBD[121]. Genome-wide association studies in humans have found allelic variance in several of the genes that regulate the innate immune system. These include: *NOD2* (refs 122, 123), which is linked to activation of the immune system by peptidoglycans; *ATG16L1* (refs 124, 125), which has a role in autophagy; and *CLEC7A*[126], which is involved in the recognition of fungi by dendritic cells.

Dysbiosis might also promote other extraintestinal inflammatory

and autoimmune disorders, although the underlying mechanism is yet to be completely unravelled. Type 1 diabetes is associated with microbiota compositions that are characterized by low diversity and the expansion of distinct groups of bacteria[127]. Non-obese diabetic mice, an animal model for type 1 diabetes, could be phenotypically rescued by the deletion of the gene *Myd88*. However, germ-free, MyD88-deficient non-obese diabetic mice do develop type 1 diabetes, which could be attenuated by faecal transplantation, demonstrating that microbiota–innate-immune-system interactions can modify the disease[128]. Rheumatoid arthritis was found to associate with an overabundance of *Prevotella copri* and a propensity to develop colitis[129]. Such examples suggest that even classic autoimmune diseases might contain an autoinflammatory component that is driven by perturbed communication between the host and the microbiota.

Interactions between the microbiota and the innate immune system also participate in pulmonary and atopic phenomena. Commensal bacteria have been shown to protect against food allergy and allergic airway inflammation; germ-free mice and mice treated with antibiotics develop exacerbated disease[47,50,130]. Mice that are deficient in TLR2 or TLR4 develop pulmonary damage on the chronic intake of a high-fat diet. This damage is abrogated in germ-free mice or mice that consume antibiotics, and it can be transmitted to wild-type mice by faecal transplantation[131]. Together, these findings reveal the trialogue that exists between the microbiota, the host and environmental factors and that contributes to common idiopathic diseases.

Metabolic syndrome

Obesity has become a global-health problem; in 2014, approximately 40% of the population worldwide was overweight and 13% was obese, according to the World Health Organization. The association of obesity with other metabolic derangements, such as type 2 diabetes, hypertension, dyslipidaemia and non-alcoholic fatty liver disease, is known as metabolic syndrome. This complex of conditions is highly associated with cardiovascular morbidity and mortality, and it has become the leading cause of death worldwide (see page 56).

Obesity and type 2 diabetes are associated with chronic low-grade inflammation and an increased expression of PRRs in adipose tissue, muscle tissue and in circulating monocytes[132]. Both conditions also trigger dysbiosis, which is consistent with the idea that diet and PRR activation shape the microbial composition of the gut[133]. In mice, certain deficiencies of innate-immune receptors induce metabolic aberrations and dysbiosis, which can be transferred to wild-type mice by faecal transplantation and abrogated by treatment with antibiotics[75,106]. The microbiota, innate immunity and metabolic syndrome are directly linked through the secretion of IL-22 by ILCs, a mechanism that was found to preserve the integrity of the intestinal mucosal barrier, thereby alleviating metabolic disorders[134].

Other constituent conditions of metabolic syndrome, such as hypertension and dyslipidaemia, have also been linked to intestinal bacteria. The bacterial composition of stool samples obtained from people with these conditions feature dysbiosis and reduced taxonomic diversity[135,136]. The pathogenesis of non-alcoholic fatty liver disease is linked to interactions between the microbiota and the innate immune system of the host. Deficiencies in inflammasome components exacerbate non-alcoholic fatty liver disease owing to the induction of colonic inflammation and a subsequent increase in the release of TLR agonists from the gut and their arrival at the liver through the portal circulation[106].

Atherosclerosis, a progressive inflammatory process that is another component disorder of metabolic syndrome, involves the accumulation of lipids and the formation of plaques around arterial walls. This pathology was linked to the intestinal microbiota as a result of several observations.

First, the administration of antibiotics was shown to confer beneficial effects on cardiovascular risk factors in a murine model of atherosclerosis[137]. Second, some of the bacterial species in atherosclerotic plaques are common to both the oral and intestinal microbiota, and the presence or absence of these groups correlate with levels of cholesterol in the blood plasma[138]. Third, metabolomic analysis revealed that trimethylamine *N*-oxide, a phospholipid that is found in red meat and is metabolized exclusively by intestinal microbiota, promotes atherosclerosis and increases the risk of cardiovascular diseases[139,140]. Intriguingly, the targeted inhibition of trimethylamine *N*-oxide attenuates features of atherosclerosis, which paves the way for a microbiota-mediated therapeutic approach to the treatment of cardiovascular diseases[141]. Atherosclerosis is also dependent on the host's innate immunity, because a deficiency in *Myd88*, specific TLRs or components of the inflammasome suppresses the condition in murine models[142].

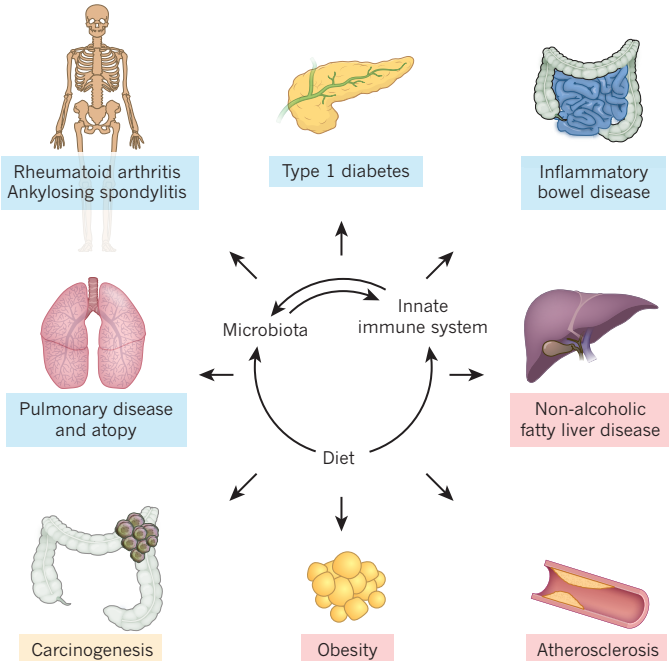

Figure 5 | Microbiome–innate-immune-system interactions are involved in multifactorial diseases. Many inflammatory disorders are influenced by alterations in the crosstalk between innate immunity and the microbiome. These include metabolic (red boxes), neoplastic (orange box) and autoimmune or autoinflammatory (blue boxes) disorders. Modulation of the severity of a disorder through dietary interventions and their influence on microbiome–immune interactions is an exciting area of research.

Cancer

The idea that chronic inflammation drives carcinogenesis has been widely established in various tissues. For example, hepatocellular carcinomas arise in people with chronic hepatitis, colorectal cancer can occur in people with longstanding untreated IBD and Marjolin's ulcers develop on chronically inflamed skin. The presence of bacteria at tumour sites was first described more than a century ago, so it is surprising that the role of the microbiota in tumourigenesis has only recently been recognized. Colorectal carcinogenesis is triggered by a combination of microbiota- and host-dependent mechanisms. Certain bacteria promote carcinogenesis directly, through the secretion of substances that elicit DNA damage[143]. Prominent examples include the excessive release of nitric oxide from immune cells that is triggered by *Helicobacter hepaticus*, the production of reactive oxygen species by *Enterococcus faecalis* and the secretion of an enterotoxin by *Bacteroides fragilis*, which activates the oncogene *c-MYC*. Other bacteria drive carcinogenesis indirectly by sustaining a proinflammatory microenvironment, such as the production by *Fusobacterium nucleatum* of the virulence factor FadA, which increases the paracellular permeability of colonic epithelial cells.

Inflammation might also promote community-level alterations in the microbiome and facilitate bacterial translocation into neoplastic tissue, which further promotes the expression of inflammatory cytokines and leads to the increased growth of tumours[144]. Dysbiosis that arises in

the absence of NLRP6 promotes the development of cancer through IL-6-induced epithelial proliferation[17].

The influence of the microbiota on innate immunity has been shown to affect the host response to cancer therapy. For example, germ-free mice and mice that are treated with antibiotics both show a diminished response to immunotherapy by CpG oligonucleotides and chemotherapy owing to the impaired function of myeloid-derived cells in the tumour microenvironment[58]. Furthermore, commensal *Bifidobacterium* enhances immunity to tumours through antibodies directed against programmed cell death 1 ligand 1 (PD-L1) through the augmentation of dendritic-cell function[145]. These studies might open up a fascinating avenue of research to prevent cancer and develop cancer therapeutics through manipulation of the microbiota.

Future directions

The importance of the innate-immune sensing of commensal microorganisms was recognized merely a decade ago. Since then, multiple levels of interaction between the microbiota and the cells of the innate immune system have been uncovered, which range from molecular events at the level of individual cells to the physiology of entire organs. The importance of the microbiome in mammalian health and disease is clearly recognized, and in many cases the innate immune system provides the causal link between disease-associated microbial alterations and the pathophysiological mechanisms of the host. Nonetheless, very few of the insights gained from the study of microbiome–innate-immune-system interactions have been used to develop clinical therapies for inflammatory diseases. In the next decade, research in the field must therefore reach a number of milestones that will help to harness our knowledge to provide clinical applications.

First, the majority of insights so far have been gained from studies of mouse models. The relevance of these principles for microbiome–innate-immune-system interactions in humans remains to be determined.

Second, knowledge of how the microbiome influences the innate immune response is based mostly on well-known examples and might not fully represent the scope of possible mechanisms. Systematic studies that screened members of the microbiome for their effects on the immune system suggest that the range of commensal bacteria that modulate the maturation of the immune system might be far larger than was previously anticipated[146]. Whether the entirety of microbiota–innate-immune-system interactions can be classified according to a limited number of paradigms — that is, whether certain groups of bacteria use common mechanisms to modulate the innate immune system — is still to be uncovered[39].

Third, in comparison to its effect on the adaptive immune system (see page 75), very little is known about the bacterial species, effector molecules and molecular mechanisms through which the microbiota exerts its immune-modulating effect on the cells of the innate arm of the immune system. Because it lacks antigen specificity, the innate immune system might act by broadly evaluating the activity of the microbiome through tissue-level microbial sensing rather than by responding to particular species of bacteria. A comprehensive characterization of the bacterial components and metabolites that are sensed by the innate immune system, through either PRRs or other sensors, as well as their effects on the transcriptional and post-transcriptional landscape of the host, will greatly facilitate our ability to understand the molecular aetiology of microbiome-driven disorders.

Fourth, our deepening knowledge about the interactions between the innate immune system and the microbiome will ultimately result in the development of therapeutic approaches that target these processes. Such interventional strategies, especially when applied to humans, should take into account the enormous variation in both microbiome configurations and innate immune responses that exists between individuals[147]. However, the fact that the microbiome is amenable to rapid change through dietary interventions could be exploited to construct tailored diets that alter microbiome function and downstream innate immune responses to influence common, multifactorial disorders. Dietary modification might alter the microbiome in a way that would enable it to be primed for subsequent immunomodulatory interventions, thereby integrating both treatment modalities (Fig. 5). Alternatively, the identification of 'postbiotic' bioactive microbiome-modulated compounds might allow common downstream pathways in the host to be targeted, thereby influencing the development and outcome of disorders. The future of immunotherapy might therefore combine direct, drug-based immune modulation with microbiome and metabolome modification to collectively target both microbial and host components of the molecular aetiology of disease. ■

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