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Regulation of inflammation by microbiota interactions with the host

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The study of the intestinal microbiota has begun to shift from cataloging individual members of the commensal community to understanding their contributions to the physiology of the host organism in health and disease. Here, we review the effects of the microbiome on innate and adaptive immunological players from epithelial cells and antigen-presenting cells to innate lymphoid cells and regulatory T cells. We discuss recent studies that have identified diverse microbiota-derived bioactive molecules and their effects on inflammation within the intestine and distally at sites as anatomically remote as the brain. Finally, we highlight new insights into how the microbiome influences the host response to infection, vaccination and cancer, as well as susceptibility to autoimmune and neurodegenerative disorders.

An astounding number and diversity of microorganisms coexist with mammalian organisms[1]. Recent years have seen an increase in understanding of the complexity and sophistication of the host–microbiota relationship and its effects on human health[2–4]. Several technological advances have bolstered the study of mammalian microbiomes. Sequencing of 16S-rRNA-encoding genes has identified the constituent bacterial species of the human intestinal microbiota as belonging predominantly to the Bacteroidetes and Firmicutes phyla. Deep sequencing of the internal-transcribed-spacer regions ITS1 and ITS2 of the fungal ribosomal DNA and improved downstream analyses[5,6] have unveiled the presence of rich fungal communities, dubbed the mycobiome, within the mammalian intestinal tract[7]. Sequencing of total DNA, the metagenome, from fecal specimens has enabled systematic studies on the virome and has yielded valuable information about the complex interaction of these commensals with their host. Large-scale endeavors have been launched to characterize the human microbiome: the US National Institutes of Health (NIH)-funded Human Microbiome Project (HMP) and the European Metagenomics of the Human Intestinal Tract (MetaHIT)[8,9]. Concurrently, gnotobiotic resources and treatment of mice with antibiotics have shown how specific compositions of the mouse or human gut microbiota contribute to disease development and have enabled mechanistic dissection of host–microbiota interactions. Targeted phenotypic culturing by subjecting fecal samples to selection for a desired phenotype and subsequent whole-genome sequencing and phylogenetic analysis has revealed that almost 75% of the intestinal microbiota is culturable[10]. Selection for sporulation has indicated that 50–60% of intestinal bacterial genera produce resilient spores adapted for survival and dispersal[10], thus potentially explaining why, in humans, the intestinal microbiota of family members with close contact have Ruminococcaceae and Lachnospiraceae spore-forming bacteria in common[11]. *Ex vivo* organ cultures of the mouse intestine have allowed for the introduction of molecules and microbes into the gut lumen in a setting that recapitulates luminal flow and features spontaneous peristaltic-like contractions and an intact tissue architecture and cellular network[12].

Microbiome-wide studies have revealed important correlations between specific microbes and a range of diseases including inflammatory bowel disease (IBD), autoimmune disease[13], cancer[14] and metabolic[4] and neurodegenerative disorders[15]. Chronic inflammation is a driver of many of these conditions. Here, we focus on the most recent insights into the molecular underpinnings of host–microbiota interactions that influence inflammation within the intestine and distal organs. We consider the properties of the microbiota that most critically affect the immune response, including its biogeography, metagenome and metabolome, and how the microbiome modulates the host response to infection, autoimmunity, neuroinflammation, vaccination and tumor immunotherapy.

Toward identification of an immune-modulatory microbiota

Physical and biochemical barriers anatomically segregate the microbiota from mammalian immune cells in the intestine[3,16]. This 'demilitarized zone' is essential to limit inappropriate immune activation[16]. On the host side of this zone lies the intestinal epithelium[17], which comprises a single layer of intestinal epithelial cells whose frequent cycles of apoptosis and renewal[18] maintain cellular fitness and orchestrate intestinal immune homeostasis[19].

The demilitarized zone is not impermeable, and certain commensals, such as segmented filamentous bacteria (SFB), *Acinetobacter* spp., *Bacteroides fragilis* and Proteobacteria, can associate with the intestinal epithelium[20]. Proximity to the epithelium evokes the

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strongest effects on the host. For example, the capsular polysaccharide A of the human commensal *B. fragilis* stimulates production of the anti-inflammatory cytokine IL-10 by Foxp3$^+$ regulatory CD4$^+$ T (T_{reg}) cells, thus facilitating colonization while promoting beneficial immunosuppression in the intestine[20]. Outer-membrane vesicles produced by *B. fragilis* activate noncanonical autophagy (involving the autophagy-related protein ATG16L1 and the receptor Nod2), thereby inducing T_{reg} cells and suppressing mucosal inflammation[21]. Intestinal SFB colonization induces a response by IL-17-producing helper T (T_H17) cells positive for the transcription factor RORγt, thus protecting mice from infection with the enteric rodent pathogen *Citrobacter rodentium*[3]. Similarly, *Clostridium* spp. and the human symbiont *Clostridium ramosum* are potent inducers of colonic T_{reg} cells[3,12]. T cell–dependent immunoglobulin A (IgA) production is activated by epithelium-associated commensal bacteria, such as *Mucispirillum* and SFB[22]. These observations highlight the importance of defining the immunologically relevant microbiome, especially because many of the mucosal responses regulated by the microbiota are critical for intestinal homeostasis and are disrupted in IBD.

The mouse circadian clock is synchronized according to diurnal oscillations in the composition and activities of the microbiota[23–25]. The numbers and species of epithelial-associated commensals in mice fluctuate almost tenfold in the dark phase compared with the light phase, and diurnal oscillations in species such as *Mucispirillum schaedleri*, *Lactobacillus reuteri* and *Bacteroides acidifaciens* are associated with the feeding cycle[24]. Bacterial adherence to the epithelium controls reprogramming of transcriptional oscillations not only in the colon but also at distant sites, such as the liver, through rhythmic chromatin remodeling and the activity of promoter and enhancer regions[24]. The diurnal detoxification of acetaminophen, regulated by circadian liver functions, is disrupted by changes in the microbiota[24].

The aforementioned immunologically relevant microbiome includes several keystone pathosymbionts identified through sorting and sequencing of IgA-coated microbiota (a technique termed IgA-seq or Bug-FACS)[22,26,27]. During the first two years of life in humans and gnotobiotic mice, age-specific bacterial taxa define distinct temporal patterns of mucosal IgA responses[28]. IgA can cross-link bacteria in the mammalian intestine, thereby inhibiting bacterial pathogenesis or the genetic spread of antimicrobial resistance[29]. Fecal IgA varies independently of bacterial phylogeny and can be perturbed during disease[30]. Enrichment of *Enterobacteriaceae* and *Lachnospiraceae* in IgA-coated and IgA-negative microbiota, respectively, in both Crohn's disease–associated spondyloarthritis[31] and malnutrition[26], suggest that a potential core IgA response may exist in various inflammatory conditions.

Keystone pathosymbionts may similarly affect mucosal T cell responses. Human-derived adherent-invasive *Escherichia coli* and *Bifidobacteria adolescentis* induce both mucosal and systemic inflammatory T_H17 cells[31,32]. Although both of these pathosymbionts recapitulate the close epithelial adherence that has been observed for SFB, *B. adolescentis* triggers an epithelial transcriptional response distinct from that of SFB, thus suggesting the potential for shared and distinct pathways in microbial induction of T_H17 cells. Whereas cluster IV, cluster XIVa and cluster XVIII *Clostridium* support T_{reg} induction[33], nearly one-quarter of the 53 species recently profiled similarly induce colonic T_{reg} cells. This potential redundancy by a diverse group of bacteria may serve to ensure consistency in mucosal homeostasis. However, the immunomodulatory properties of different bacterial species do not necessarily cluster by phylum or genus, thus highlighting the importance of considering strain-specific traits when assessing immunological phenotypes.

The subset of microbes that colonize lymphoid tissues are known as lymphoid-tissue-resident commensal (LRC) bacteria and include alpha- and betaproteobacteria, such as *Alcaligenes*, *Achromobacter*, *Bordetella* and *Ochrobactrum* species[34–37]. LRC bacteria selectively colonize the Peyer's patches, isolated lymphoid follicles and mesenteric lymph nodes in healthy humans, nonhuman primates and mice, and their entry to these tissues depends in part on M cells, IgA and the cytokine IL-22 (refs. 34,36,38). LRC bacteria colonize dendritic cells and uniquely modulate cytokines that promote responses by local T_H17 cells and group 3 innate lymphoid cells (ILC3)[34]. Innate lymphoid cells are ubiquitously distributed in humans and mice but are enriched at mucosal surfaces and rapidly respond to cytokine milieus after colonization with microbes[37]. Among subsets of innate lymphoid cells, ILC3 are most heterogeneous, uniquely express RORγt and broadly comprise two subsets on the basis of expression of the chemokine receptor CCR6 or the transcription factor T-bet. CCR6$^+$ ILC3 lymphoid-tissue-inducer-like cells persist after birth in secondary lymphoid tissues, cryptopatches and isolated lymphoid follicles. CCR6$^+$ ILC3 promote gut-associated lymphoid-tissue maturation and IgA production, and contribute to the innate host defense to enteric pathogens[37]. CCR6$^+$ ILC3 are also antigen-presenting cells that regulate homeostasis with beneficial microbes by limiting the development of microbiota-specific CD4$^+$ T cell–effector responses in the intestine[37]. In contrast, T-bet$^+$ ILC3 are localized diffusely in the intestinal lamina propria, require the aryl hydrocarbon receptor (AHR) and expand after microbiota colonization[37,39]. AHR protects mucosal sites from pathogenic infection and inflammation[40]. T-bet$^+$ ILC3 are responsive to microbial sensing by mononuclear phagocytes positive for the chemokine receptor CX3CR1, and subsequent ILC3 production of IL-22 has been linked to intestinal-tissue repair and barrier function by acting directly on intestinal epithelial stem cells[37,41]. IL-22 production by ILC3 also regulates epithelial fucosylation and supports diverse microbiota colonization[37].

LRC bacteria also induce IL-10 production by dendritic cells and provide tissue-protective functions in the context of intestinal-barrier damage[34]. ILC3 promote anatomical containment of LRC bacteria, because ILC3 depletion results in systemic bacterial dissemination and chronic immunological activation[37]. Additional research is required to define the mechanisms by which LRC bacteria colonize dendritic cells and mammalian lymphoid tissue, as well as to interrogate the functional potential and compositional changes of LRC bacteria in the context of chronic inflammatory diseases.

Interaction with symbiotic fungi, protozoa, worms and viruses

Rich and diverse fungal communities (mycobiota) colonize the mammalian barrier surfaces. Mycobiota diversity increases in the lower gastrointestinal tract, and several genera such as *Candida*, *Saccharomyces*, *Aspergillus*, *Cryptococcus*, *Malassezia*, *Cladosporium*, *Galactomyces* and *Trichosporon* have the potential to colonize the intestines[7,42–44]. Fungal-community changes with outgrowth of *Candida spp*. have been documented in people with IBD[43,45–47]. Deficiencies in the receptor Dectin-1 (also known as CLEC7A) and the downstream adaptor protein CARD9 lead to susceptibility to more severe experimental colitis as well as fungal and bacterial dysbiosis[6,7,48]. *Clec7a*$^{-/-}$ mice colonized with *Candida tropicalis* show aggravated experimental colitis, whereas the absence of *Candida* leads to less severe disease[6,49]. Fungi and bacteria share similar niches in the intestine, and these communities influence each other. Antibiotic treatment promotes gut *Candida* colonization[7,50], which can have immunological outcomes at distant sites such as the lung[7,51]. Bacteria affect fungal colonization both directly and indirectly. *Bacteroidetes thetaiotamicron*, which induces the

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production of the antimicrobial peptide CRAMP by the transcription factor HIF-1α, prevents *Candida albicans* gut colonization[52]. In addition to fungi, the common mouse protozoan *Tritrichomonas musculis* is a transmissible microorganism in mice that increases susceptibility to T cell–dependent intestinal inflammation while providing protection from intestinal infections through inflammasome activation and production of the cytokine IL-18 by intestinal epithelial cells[53,54].

The mammalian gastrointestinal tract is also colonized with eukaryotic viruses, which may substantially affect intestinal health and disease. Colonization with common murine norovirus is able to compensate for several, but not all, functional and immunological defects in germ-free or antibiotic-exposed mice[55]. In the presence of a diverse microbiota, several enteric eukaryotic viruses interact with the commensal microbiota and consequently induce immunological-evasion pathways and ensure their own replication and transmission[56,57]. Although the contributions of colonizing eukaryotic viruses and bacteriophages to human health are only beginning to be interrogated, early analyses have suggested substantial changes in these populations in the context of IBD and progressing HIV infection[58–60]. Finally, intestinal worms or helminths have long been known to influence intestinal immune responses and physiology, and may be an ancient intestinal symbiont lost in industrialized nations. In the developing world, helminths affect bacterial composition and colonization resistance[61], and independently impair host immunity to eukaryotic viruses[62,63] through induction of intestinal type 2 immune responses. These data highlight intestinal symbionts other than bacteria and the importance of considering multiple cross-kingdom interactions in future basic and translational studies of the microbiota.

Microbiota small molecules mediate interspecies interaction

The gut microbiota is influenced in part by long-term dietary habits and is responsive to daily variations in food[4], and it contributes to the metabolite profile in the plasma[64]. Bacterial metabolites exhibit rhythmicity, owing to the oscillation of several bacterial biosynthetic pathways, such as those for biotin and proline[24]. Concordantly, homeostatic circadian oscillations in the serum levels of amino acids and polyamines are sensitive to dysbiosis and dietary polyamine content[24]. Dietary-fiber deficiency promotes the proliferation of mucus-degrading bacteria, thus leading to colonic mucus erosion, association of luminal bacteria with the intestinal epithelium and increased susceptibility to *Citrobacter*[65] (**Fig. 1**). Short-chain fatty acids (SCFA) derived from the anaerobic fermentation of nondigestible polysaccharides such as dietary fiber, particularly by *Clostridia* spp., counter inflammation and maintain gut homeostasis[66] (**Fig. 1**). Among SCFAs, butyrate uniquely inhibits intestinal stem-cell and progenitor-cell proliferation during mucosal injury, and this inhibition is likely to prevent their potential transformation under genotoxic stress in response to luminal contents[67]. Colonocyte localization at the crypt mouth ensures the preferential consumption of butyrate before it reaches stem cells at the crypt base[67] (**Fig. 1**). Microbiota-derived butyrate promotes colonic oxygen consumption stabilizing the transcription factor HIF-1 and its target barrier-protective genes[68].

Gut bacteria are also an important source of potent anti-inflammatory polyamines such as putrescine and spermine. Ingestion of the probiotic *Bifidobacteria* LKM512 by elderly people increases intestinal polyamine concentrations and inhibits intestinal inflammation, particularly when it is administered with arginine[69]. Importantly, microbiota-derived histamine, putrescine and spermine suppress cleavage of the protease caspase-1 and secretion of IL-18 as well as the colonic expression of antimicrobial peptides that predispose the colon to inflammation[70] (**Fig. 2**). The suppressive activity of these polyamines is countered by the bile-acid conjugate taurine, which induces activation of the NLRP6 inflammasome and production of IL-18 after intestinal microbial colonization and promotes microbial diversity and intestinal homeostasis[70] (**Fig. 2**).

In humans, specific bacteria such as *Lactobacilli* spp. metabolize dietary tryptophan, thus generating indole ligands for AHR[40]. These ligands can also be derived from the breakdown of glucosinolate glucobrassicin from cruciferous vegetables[40]. *Lactobacillus* production of AHR ligands promotes resistance to intestinal colonization by *C. albicans*[71]. The intestinal microbiota of *Card9*$^{-/-}$ mice has altered *Lactobacilli* populations, thus leading to impaired tryptophan metabolism, defective production of AHR ligands and decreased gut expression of IL-22 and the antimicrobial proteins Reg3γ and Reg3β (ref. 48). AHR activity is controlled by the cytochrome P4501 enzymes, which terminate AHR signaling by metabolizing AHR ligands[72]. Constitutive expression of cytochrome P450 1A in mice or its specific deletion in intestinal epithelial cells deprives T_H17 and ILC3 of AHR ligands, thus leading to the loss of these cell populations from the intestine as well as to increased susceptibility to *C. rodentium*; however, these effects are reversed by dietary supplementation with AHR ligands[72].

Trimethylamine-*N*-oxide generated through the metabolism of diet-derived choline, phosphatidylcholine and carnitine, sequentially by gut microbes and the liver, increase platelet hyper-responsiveness and thrombosis risk[73] and accelerate heart and liver disease[74–76]. Despite abundant representation of the glycyl radical enzyme (GRE) superfamily that catalyze this enzymatic conversion by the human microbiota, little is known about the activity and roles of GREs in health and disease. The use of chemically guided functional profiling–coupled protein sequence-similarity networks combined with quantitative metagenomics has allowed for the discovery and functional characterization of *trans*-4-hydroxy-L-proline dehydratase[77], the most abundant GRE in the NIH HMP stool microbiota. This enzyme allows the microbiota to chemically reverse C4-hydroxylation of L-proline (the most common eukaryotic post-translational modification), thereby acquiring additional sources of carbon and nitrogen (**Fig. 2**). Chemically guided functional profiling has also led to the functional characterization of novel coenzyme B_{12}–independent propanediol dehydratase, which converts L-fucose to SCFA (**Fig. 1**). Although propanediol dehydratase might be the major contributor to propionate production at steady state, coenzyme B_{12}–dependent propanediol dehydratase is required for T_H17 induction by adherent-invasive *E. coli*[31].

A survey of biosynthetic-gene clusters from stool samples from the NIH HMP has identified thousands of biosynthetic loci with no known functions[78]. Nonribosomal peptide synthetase–encoding gene clusters have been identified as an abundant gene cluster exclusive to gut-associated bacterial species, predominantly in anaerobic *Firmicutes* from the class *Clostridia*, and several clusters in Gram-negative *Bacteroides* and *Desulfovibrio*[78]. Their absence in free-living or nonintestinal niche-colonizing microorgansims suggests adaptation to intestinal colonization[79]. Heterologous expression combined with quantitative and unbiased chemical proteomics has led to the discovery of dipeptide aldehydes[79]. The dipeptide aldehyde Phe-Phe-H is stable and acts as a cell-permeable inhibitor of cathepsins (**Fig. 2**), thus suggesting active blockade of innate and adaptive immunity by microbiota-derived dipeptide aldehydes, given that cathepsins are important for antigen processing and presentation, as well as endosomal activation of the Toll-like receptor TLR9 (ref. 80). There is great potential for the discovery of novel mechanisms of immune modulation through the functional characterization of yet-undiscovered microbiota-derived molecules.

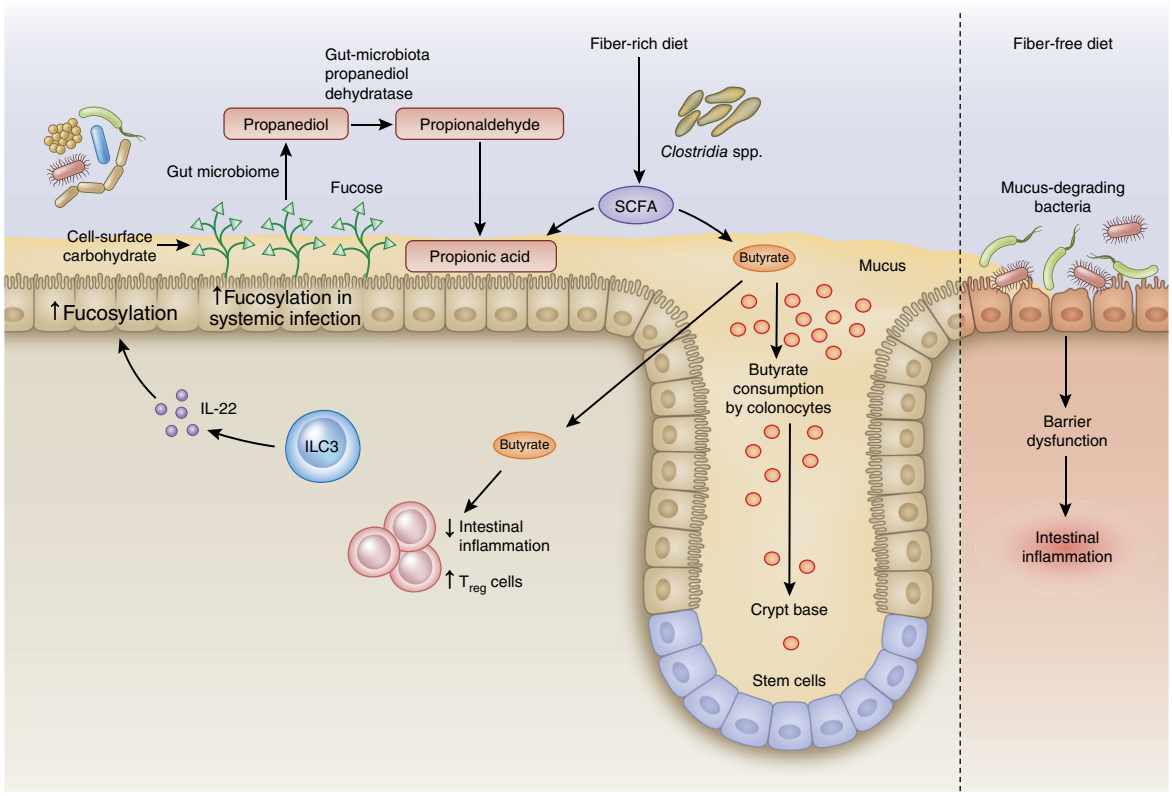

Figure 1 Dietary fiber and SCFAs in intestinal homeostasis. Anaerobic fermentation of dietary fiber by members of the commensal microbiota, particularly by *Clostridia* spp., serves as a source of SCFAs, which help to maintain T_{reg} cell expansion, immunosuppressive function and overall intestinal homeostasis. Butyrate is the preferred metabolic energy source for colonocytes but is detrimental to stem cells, inhibiting their proliferation and wound-repair functions[67]. The strategic positioning of colonocytes and stem cells within the colon mirrors the concentration gradient of butyrate: colonocytes are positioned at the location of highest concentrations near the lumen, where they consume butyrate, thus decreasing the concentration to which distally located stem cells within the colonic crypts are exposed. Propionate is the end product of fucose metabolism by the microbiota. The host increases fucosylation of epithelial-cell carbohydrates during infection, thereby protecting its gut commensals[116]. Fucose-using *B. acidifaciens* increase in abundance and elevate their metabolism of fucose, thus leading to propanediol formation. Propanediol dehydratase converts propanediol to propionaldehyde, thereby generating propionic acid[77], which in turn tempers inflammation and protects host tissues from collateral damage during the immune response to infection.

Gut microbiota modulate inflammation at distant sites

Both bacterial and fungal dysbiosis have been linked to autoimmune and immune-mediated diseases[13,51,81]. The prevalence of *Bacteroides* spp. within Finnish and Estonian infants is associated with early-onset autoimmune disease[82] (**Fig. 3**). Relative to Russia, Finland has an incidence two- to sixfold higher for allergies and five- to sixfold higher for type 1 diabetes and other autoimmune disorders. Compared with the hexa-acylated lipopolysaccharide (LPS) expressed by the more abundant *E. coli* in Russian infants, the less stimulatory tetra- and penta-acylated LPS characteristic of *Bacteroides* spp. impairs endotoxin tolerance, thereby leading to a propensity for higher immunological stimulation[82] (**Fig. 3**). These data are concordant with the hygiene hypothesis, in which early-life exposure to specific microbes and parasites confers protection against allergic and autoimmune disease[83,84], and they highlight how perinatal environmental influences on the microbiota can determine susceptibility to immune-mediated disease later in life.

The effects of commensal microbiota on mucosal and systemic immunity highlight a potential role for keystone species in autoimmunity. Antigen-specific T_H17 responses develop to the intestinal microbiota in mice[3] and in people with Crohn's disease[85] as well as to the intestinal epithelium during mouse colonic infection associated with apoptosis of intestinal epithelial cells[86]. Severe gastrointestinal infection in mice leads to loss of T cell tolerance to commensal antigens and results in long-lived inflammatory effector T cells that drive chronic intestinal and extraintestinal inflammatory pathology[87]. In mice, infection-induced apoptosis of intestinal epithelial cells triggers the loss of $CD4^+$ T cell tolerance to self-antigen derived from intestinal epithelial cells. Under these conditions, self-reactive $CD4^+$ T cells differentiate into T_H17 cells alongside pathogen-specific $CD4^+$ T cells and mediate intestinal inflammation[86,88]. Notably, the T_H17 response to SFB is not disrupted by concurrent infection with the T_H1-cell inducer *Listeria monocytogenes*[89]. SFB-induced T_H17 cells are sufficient to induce extraintestinal inflammatory disease including inflammatory joint disease[90] and experimental autoimmune encephalomyelitis[91].

A role for mucosa-associated microbiota is coming to light in systemic autoimmunity. IgA-coated mucin-degrading *Akkermansia muciniphila* are enriched in an HLA-B27-antigen transgenic rat model of inflammatory arthritis[92]. An enrichment in adherent-invasive *E. coli* in the IgA-coated microbiota has also been found in people with Crohn's disease–associated spondyloarthritis, and this observation correlates with systemic T_H17 cell activation and *E. coli* seroreactivity[31] (**Fig. 3**). Adherent and invasive bacteria are enriched in ileal biopsies from people with HLA-$B27^+$ ankylosing spondylitis[93]. Induction and egress of intestinal T follicular helper cells enable the

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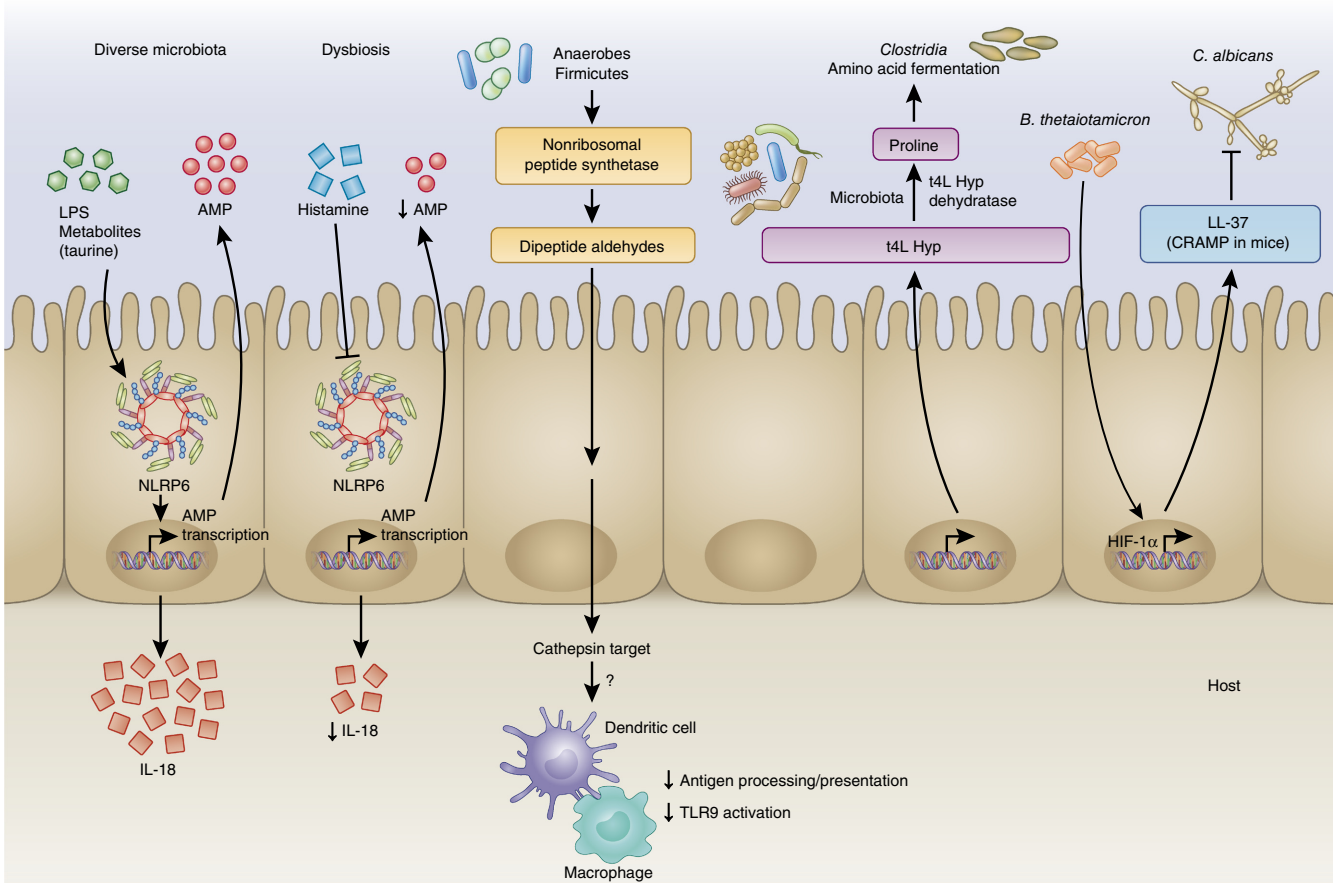

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Figure 2 Examples of mechanisms mediating host–microbiota interactions. A diverse microbiota provides two signals for NLRP6 inflammasome activation in intestinal epithelial cells: signal 1 is in the form of LPS, and signal 2 is in the form of metabolites such as the bile-acid conjugate taurine. Together, these signals activate the NLRP6 inflammasome in intestinal epithelial cells and lead to the production of epithelial IL-18 and downstream antimicrobial peptides (AMP). Under dysbiotic conditions, such as those in mice lacking the inflammasome adaptor ASC, microbiota-derived histamine, putrescine and spermine are increased, thus suppressing NLRP6 inflammasome signaling in intestinal epithelial cells, decreasing production of epithelial IL-18 and AMP in the colon and promoting intestinal inflammation[70]. *B. thetaiotamicron* induces the transcription factor HIF-1α in intestinal epithelial cells, thereby activating transcription and production of the antimicrobial peptide LL-37 (CRAMP in mice), which in turn promotes resistance to *C. albicans* colonization[52]. Gut anaerobic Firmicutes from the class Clostridia, and several clusters in Gram-negative *Bacteroides* and *Desulfovibrio*, express nonribosomal peptide synthetase–encoding gene clusters that mediate the synthesis of dipeptide aldehydes[79]. The dipeptide aldehyde Phe-Phe-H is cell permeable and has been shown to inhibit cathepsins in macrophages, an activity that might modulate antigen processing and innate immune function[79]. The generation of the nonproteinogenic amino acid *trans*-4-hydroxy-L-proline (t4L Hyp) is one of the most common post-translational modifications in eukaryotic cells but is rare in bacteria. Intestinal commensals such as Clostridiales and human pathogens such as *C. difficile* chemically reverse proline hydroxylation through the activity of the GRE *trans*-4-hydroxy-L-proline dehydratase, which generates L-proline[77]. Many Clostridiales then use L-proline as an electron acceptor in amino acid fermentation.

gut microbiota to regulate systemic autoimmunity[94], but additional models are needed to understand the contribution of microbe-specific autoimmunity to the pathophysiology of inflammatory disease.

Both the gut microbiome and the immune system are integral parts of gut–brain communication, which relies on neuroendocrine and autonomic nervous systems[95,96]. Enteric afferent neurons communicate intestinal conditions to intestinal muscularis macrophages via β2-adrenergic receptors[97] and to the brain through the vagus nerve[95,96]. Intestinal infections of mice with *C. rodentium*, *Campylobacter jejuni* or *Salmonella enterica* var. Typhimurium increase levels of the transcription factor c-Fos in visceral and vagal neurons in select brain regions, events requiring an intact vagus nerve[98]. Multiple members of the microbiota such as *Escherichia*, *Lactobaccillus*, *Bifidobacterium*, *Enterococcus* and *Truchuris* produce neurotransmitters and neuropeptides including dopamine, acetylcholine, gamma-aminobutyric acid, serotonin (5-hydroxytryptamine) and brain-derived neurotrophic factor[98] (**Fig. 4**). These metabolites induce mouse intestinal epithelial cells to release molecules that modulate signaling within the enteric nervous system. Spore-forming bacteria, primarily *Clostridium* spp., modulate the colonic luminal metabolome, including SCFAs, thereby inducing serotonin biosynthesis by enterochromaffin cells—the major producers of serotonin—and consequently affecting intestinal motility and platelet function in mice[99,100] (**Fig. 4**). Serotonin has a wide range of physiological effects including the development and function of the immune system[101], and it will be important to determine its role in intestinal inflammation and to elucidate how serotonin control by the microbiota affects function and inflammation in distal tissues including the brain. Microbiota-dependent signals also stimulate enteric-nervous-system nociceptors known to regulate inflammation[12]. Immunomodulatory colonic RORγt$^+$ T_{reg}–inducing *C. ramosum* represses neuronal-specific transcripts, particularly those encoding

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nociceptive neuropeptides, in microfluidics-supported mouse intestinal organ cultures (**Fig. 4**), thus suggesting an unappreciated inverse functional link between neuronal activation and T_{reg} cell differentiation[12].

The blood–brain barrier and brain lymphatic vasculature allow the passage of various immune cells, macromolecules and metabolites into the brain[96]. Disruption or absence of the microbiota in mice impairs the function of the blood–brain barrier (**Fig. 4**), alters cortical myelination and hippocampal neurogenesis, decreases cognitive function and memory formation, and decreases social and anxiety-like behavior[96]. Microbiota-derived SCFAs promote the differentiation and function of microglia, the resident macrophages in the brain[102,103] (**Fig. 4**), and play a significant role in accelerating the appearance of motor deficits mediated by the neuronal protein α-synuclein as well as brain pathology in a mouse model of Parkinson's disease[104]. Gut microbiota from people with Parkinson's disease induce enhanced motor dysfunction when they are transplanted into α-synuclein transgenic mice[104], thus suggesting that Parkinson's disease–associated microbes can trigger disease symptoms in this genetically susceptible mouse model. However, microbiota-dependent metabolism of tryptophan into AHR ligands targets AHR on astrocytes, which are critical in neuronal transmission and development and repair of the central nervous system, thereby limiting central-nervous-system inflammation in mice[105] (**Fig. 4**). Dietary supplementation with tryptophan ameliorates autoimmune encephalomyelitis scores, whereas treatment of mice with ampicillin worsens disease[105].

Microbiota-driven modulation of the host immune response

Significant associations between fungal- and bacterial-induced cytokine responses and specific gut bacterial species and genera have been found through the Human Functional Genomics Project[106]. For example, production of the cytokines IFN-γ and TNF by peripheral blood mononuclear cells is more strongly associated with the microbiome than are the cytokines IL-6 and T_H17-derived IL-17 and IL-22. *Staphylococcus aureus*–induced IL-17 is positively associated with five genera, including species from *Clostridium* clades IV and XIV, and is negatively associated with *Fecalibacterium*, including *Fecalibacterium prausnitzii*; however, multiple diet-sensitive bacteria, such as *Alistipes* spp., *Clostridium* spp. and *Bilophila* spp., are negatively associated with LPS-induced TNF production[106]. Although these findings have identified targetable regulators of systemic inflammation, analysis of the metabolic pathways and gene-ontology categories explaining the cytokine variation has indicated that microbiome functions have a greater effect on the cytokine response than do taxonomic classifications; for example, IFN-γ and TNF are strongly modulated by microbial palmitoleic acid metabolism and degradation of tryptophan to tryptophol[106].

Microbiota-driven variations in the inflammatory response have been predicted to regulate the host response to infection[106]. The intestinal microbiota can mediate colonization resistance against enteric pathogens. The conversion of primary to secondary bile salts in *Clostridium scindens* is associated with resistance to *Clostridium difficile* infection in mice and humans[107]. In *Caenorhabditis elegans*, the peptidoglycan hydrolase activity of the secreted antigen A from the commensal *Enterococcus faecium* protects against *Salmonella* pathogenesis[108]. In *Drosophila*, gut-microbiota-derived peptidoglycans, particularly from the commensal *Acetobacter pomorum*, prime intestinal induction of a secreted factor that is released after enteric viral infection and stimulates antiviral signaling by extracellular-signal-regulated kinases in intestinal epithelial cells[109]. Colonization resistance by the intestinal microbiota can be extended to systemic

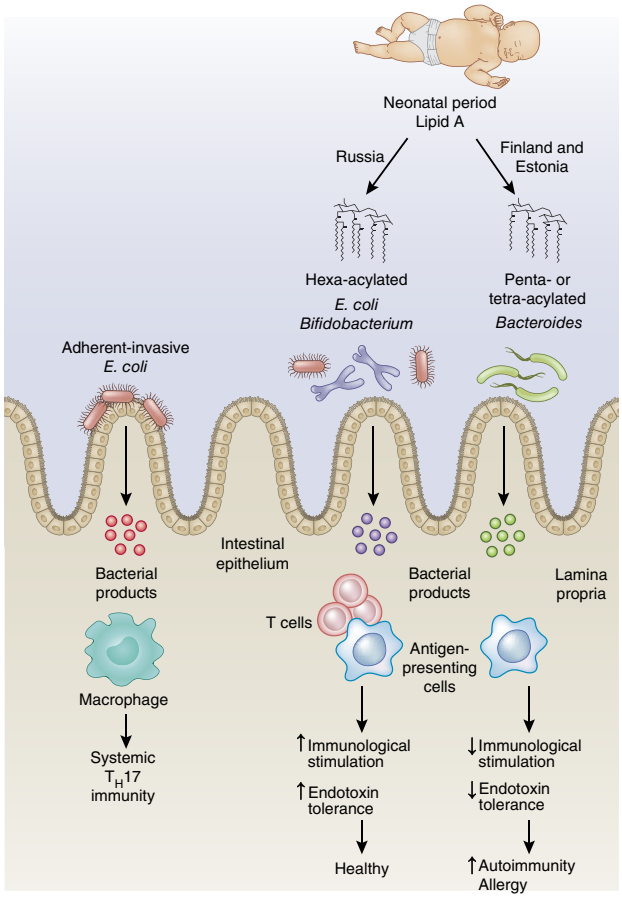

Figure 3 Associations between the intestinal microbiota and autoimmune disorders. Infants from Russia have more abundant *E. coli* species expressing stimulatory hexa-acylated LPS, whereas infants from Finland and Estonia have more abundant *Bacteroides* spp. expressing the less stimulatory tetra- and penta-acylated LPS[82]. Hexa-acetylated LPS induces greater Immunological stimulation but also endotoxin tolerance thought to dampen the capacity for immunological education in early life. However, the less stimulatory LPS from *Bacteroides* spp. impairs LPS tolerance, thus increasing susceptibility to immunological disease later in life. Enrichment of adherent-invasive *E. coli* in the IgA-coated microbiota in patients with Crohn's disease–associated spondyloarthritis correlates with *E. coli* seroreactivity and systemic T_H17 cell activation[31].

infections or pathogens infecting distant sites such as the lung. In the absence of the microbiota, hematopoietic defects in tissue-resident myeloid cells confer susceptibility to intravenous infection with *L. monocytogenes*[110]. Gut-microbiota-derived products preprime inflammasome-dependent cytokines that promote dendritic-cell migration from the lung during respiratory influenza A virus infection[111] and enhance innate immune responses of neutrophils in a manner dependent on the receptor Nod1 (ref. 112). The protection afforded by intestinal microbiota against enteric pathogens such as *C. difficile* has paved the way toward therapeutic development of probiotics that enhance host resistance against life-threatening antibiotic-resistant pathogens, such as vancomycin-resistant enterococci. These bacteria expand not because of their antibiotic resistance but because the antibiotic kills the protective commensal bacterial species that provide colonization resistance[113].

Beyond conferring resistance, endosymbionts confer disease tolerance to infection in insects and in mice[114]. Disease tolerance does not target the infecting pathogen but instead protects against

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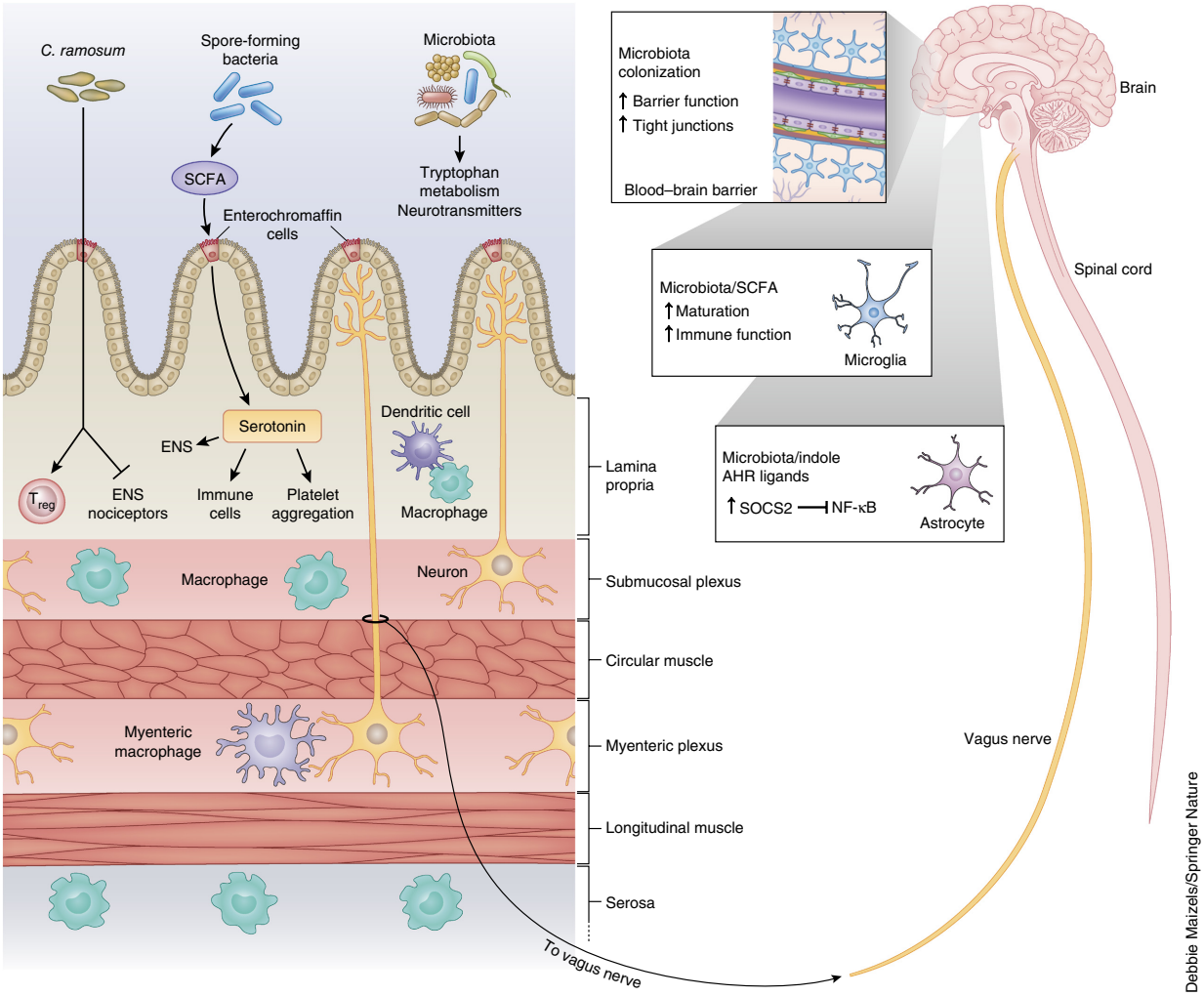

Figure 4 Links between the intestinal microbiota and neuroinflammation. Multiple members of the microbiota, such as *Escherichia*, *Lactobaccillus*, *Bifidobacterium*, *Enterococcus* and *Truchuris*, produce neurotransmitters and neuropeptides including dopamine, acetylcholine, gamma-aminobutyric acid, serotonin and brain-derived neurotrophic factor[98]. Spore-forming bacteria, primarily *Clostridium* spp., modulate the colonic luminal metabolome, including SCFAs, thus inducing serotonin biosynthesis by enterochromaffin cells—the major producers of serotonin—and thereby affect intestinal motility and platelet function in mice[99,100]. In the colon, *C. ramosum* induces RORγt$^+$ T_{reg} cells but also represses neuronal-specific transcripts, particularly those encoding nociceptive neuropeptides[12]. Afferent neurons within the enteric nervous system (ENS) can communicate intestinal conditions to intestinal muscularis macrophages via β2-adrenergic receptors[97] and also to the brain via the vagus nerve[95,96]. Intestinal colonization by the microbiota increases blood–brain tight junctions and barrier function, although microbiota-derived SCFAs can gain access to the brain and promote microglia differentiation and function[102,103]. Microbiota-dependent metabolism of tryptophan into AHR ligands engages AHR on astrocytes, thus leading to an increase in astrocyte expression of the inhibitor protein SOCS2 and consequently inhibiting activation of the transcription factor NF-κB and thereby limiting inflammation[105].

physiological damage such as cachexia, muscle wasting or endotoxic shock in response to infection[114]. The endosymbiont *E. coli* O21:H$^+$ protects mice against muscle wasting and loss of fat during enteric *S.* Typhimurium or respiratory *Burkholderia thailandensis* infections by activating the NLRC4 inflammasome[115]. Subsequent IL-18 sustains production of the growth factor IGF-1, which in turn activates signaling by the PI3K–AKT kinase pathway in skeletal muscle, thereby countering muscle wasting[115]. Increased fucosylation of the intestinal epithelium during systemic exposure to Toll-like-receptor ligands is sensed by the intestinal microbiota, thus leading to an abundance of fucose-using *B. acidifaciens*[116] (**Fig. 1**). Fucose is a substrate for microbial production of propionate[117] and may thus promote host-protective SCFA-mediated effects. This adaptation of the intestinal microbiota to conditions of host stress confers host tolerance to *C. rodentium* but notably without affecting colonic bacterial burdens[116]. The microbiota can also contribute to negative outcomes after acute infection with *Yersinia pseudotuberculosis*, in which sustained intestinal inflammation and lymphatic leakage after pathogen clearance is mediated by the microbiota[118]. Distinct readouts are necessary to identify whole-microbiome associations with interindividual variations in disease tolerance.

Evidence in mice has suggested that the microbiota can modulate vaccine responses. Differentiation of T follicular helper cells and plasma cells in response to intranasal immunization is promoted by the nasal microbiota of mice, particularly *Staphylococcus sciuri*, via signaling by Nod2 and the kinase RIPK2 in CD11c$^+$ phagocytes[119]. Toll-like-receptor stimulation by microbiota-derived signals conditions IgA class-switching in mouse-lung CD103$^+$ dendritic cells after intranasal immunization[120]. Treatment of mice with antibiotics diminishes specific antibody and CD8$^+$ T cell responses to a trivalent

inactivated influenza vaccine[111,121]. Sensing of the microbiota by the Toll-like receptor TLR5 promotes plasma-cell differentiation after parenteral administration of trivalent inactivated influenza vaccine, probably through flagellin detection[121]. In humans, early TLR5 expression directly correlates with the magnitude of the antibody response to the trivalent inactivated influenza vaccine[122]. Numerous vaccines and boosters are administered to children within the first 15 months of life, when the microbiota is highly sensitive to environmental factors such as hygiene, breast milk versus formula diet, and vaginal versus Caesarean-section delivery[123,124]. Emerging considerations in determining vaccination efficacy are the microbiota composition and diversity, as well as the therapeutic potential of the critical perinatal period to imprint protective host defenses in adult life. A concomitant assessment of the microbiome in prospective vaccination studies in babies and older humans will be necessary to establish and mechanistically understand the link between commensal microbial communities and vaccine effectiveness.

The microbiota plays a complex role in modulating both pro- and antitumor responses. Microbial translocation and chronic inflammation secondary to the loss of intestinal barrier function enhances intestinal tumor progression[125,126] and may account for an increased risk of colorectal cancer in people with IBD[127]. Inflammation also facilitates the expansion of microbes with oncogenic potential, including *Fusobacterium nucleatum*, enterotoxigenic *B. fragilis* or genotoxic *E. coli*[128–130]. The microbiota is also essential for the efficacy of antitumor immunity after chemotherapy or immunotherapy[14,131]. In mouse models, antitumor immunity induced by chemotherapy or blockade with antibodies to the checkpoint inhibitors CTLA-4 and PD-1 is abrogated after dysbiosis or in the absence of intestinal microbiota. Chemotherapy and checkpoint-inhibitor blockade may induce microbial translation or outgrowth of immunostimulatory microbiota such as *Bacteriodes* or *Bifidobacterium* species, which can enhance dendritic-cell function and tumor-specific $CD8^+$ T cell responses[132,133]. These data are provocative and suggest that in some contexts, modulating the microbiota may enhance cancer immunotherapies.

Perspectives and future directions

Host and commensal microbiota interactions follow rules of engagement different from those between host and pathogen. Future studies will undoubtedly yield exciting new insights into how the commensal microbiota modulate immune-cell function and inflammation within the intestine and at distal-tissue sites. It will be important to gain a full understanding of the composition and characteristics of the microbiome that affect vaccine efficacy as well as modulate susceptibility not only to IBD but also to neurological, metabolic and autoimmune diseases. More studies are also needed to define the microbiota constituents that promote health as well as the environmental factors early in life that favor colonization with such microbiota. Such studies should inform new approaches for manipulating the microbiome to alter disease susceptibility and improve vaccine efficacy.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Refs. 132 and 133 show that the intestinal microbiota affects the outcome of checkpoint-blockade-based cancer immunotherapy.

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