

Microbiome-Modulated Metabolites at the Interface of Host Immunity

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The mammalian gastrointestinal tract and associated mucosal immune system harbor a large repertoire of metabolites of prokaryotic and eukaryotic origin that play important roles in eukaryotic development and physiology. These often bioactive small molecules originate from nutrition- and environmental-related sources, or are endogenously produced and modulated by the host and its microbiota. A complex network of interactions exists between the intestinal mucosal immune system and the microbiota. This intimate cross-talk may be driven by metabolite secretion and signaling, and features profound influences on host immunity and physiology, including the endocrine, metabolic, and nervous system function in health and disease. Alterations in microbiome-associated metabolite levels and activity are implicated in the pathogenesis of a growing number of illnesses. In this review we discuss the origin and influence of microbiome-modulated metabolites, with an emphasis on immune cell development and function. We further highlight the emerging data potentially implicating metabolite misbalance with host-microbiome-associated disease. *The Journal of Immunology*, 2017, 198: 572–580.

The gut microbiome is a microbial ecosystem that has diverse effects on physiological host functions, particularly immune development and activity. The molecular basis of host-microbiome interactions is only just beginning to be unraveled, and is mediated by both cell to cell interactions and the production, modification, and sensing of a large variety of bioactive small molecules, termed metabolites. Many gastrointestinal metabolites originate from dietary or environmental sources. They encounter the gut microbiome as part of the digestion and intestinal transit process, and link host nutrition with physiology, including immune

development and function. Conversely, nutritional composition tremendously impacts the gut microbial composition and function (1). Other metabolites are endogenously produced or modified through diverse metabolic processes, by the host or its microbiota (2). Collectively, it is estimated that more than 50% of fecal and urinary metabolites are derived from or modified by the gut microbiome (3).

Some microbiome-associated metabolites are bioactive and affect the host cellular processes including differentiation, migration, proliferation, and apoptosis, thereby featuring pleiotropic physiological or pathophysiological effects on the eukaryotic host. A number of metabolites impact mucosal and systemic immune maturation and function, in steady state and during disease (4–8). The host has evolved multiple metabolite sensing platforms, and downstream immune signaling pathways that confer reactivity to microbiome-modulated metabolites (9, 10). These sensing platforms are expressed in different combinations in mucosal cellular subsets, such as intestinal epithelial cells, macrophages, dendritic cells (DCs), T cells, and innate lymphoid cells (ILCs), where they play critical roles in host-microbiome mutualistic cross-talk. In addition to local metabolite effects on gastrointestinal mucosal immune function, many systemically absorbed metabolites may reach remote organs and modulate the immune responses in sterile host regions such as the CNS (11, 12). As such, metabolites may provide a missing link between the gut microbiome compositional and functional configuration to its remote effects on host physiology and disease risk in seemingly unrelated sterile organs. The type, composition, and concentration of metabolites coupled with the host sensor molecules repertoire orchestrate the net physiological response at a given physiological context.

In this review we provide an overview of gut microbiome-modulated metabolites, their physiological effects on major immune functions, and recent observations possibly linking metabolite misbalances with risk of immune-mediated and immune-associated disease.

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Abbreviations used in this article: AHR, aryl hydrocarbon receptor; ASD, autistic spectrum disorder; BBB, blood–brain barrier; DC, dendritic cell; FXR, farnesoid X receptor; HDAC, histone deacetylase; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; ILC, innate lymphoid cell; NA, nicotinic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SCFA, short chain fatty acid; SFB, segmented filamentous bacteria; TMAO, trimethylamine-N-oxide; Treg, regulatory T cell; Trp, tryptophan; WT, wild type.

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Microbiome modulation of the gastrointestinal metabolite configuration

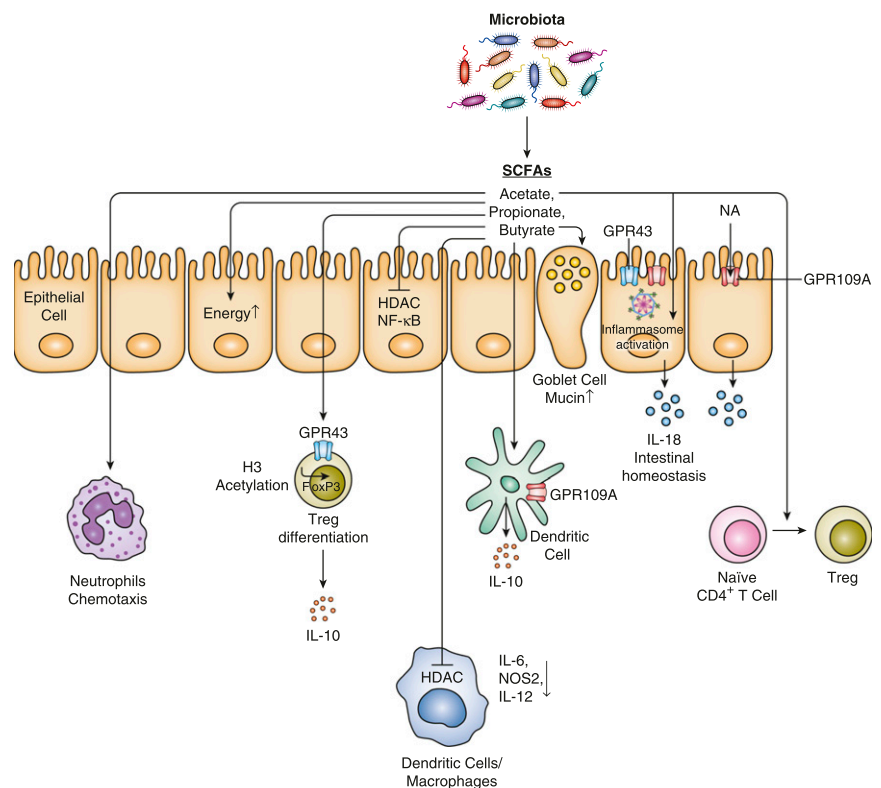
One of the essential functions provided by the gut microbiota includes the modulation of gastrointestinal metabolites, including their synthesis, digestion, fermentation, and secondary metabolism. Numerous studies have investigated the forces that drive the development of the adult microbial population in the intestine from birth (13, 14), yet the dynamics of the microbiome impact on the host metabolome remain largely unknown. The centrality of microbial activity in shaping the gut metabolome is demonstrated by profound metabolite alterations found in germ-free as compared with gnotobiotic and colonized animals, including differences in diverse metabolite biochemical groups noted in feces, urine, and the systemic circulation (15–18). Multiple commensal microbial taxonomies, including *Enterobacteriaceae*, *Enterococcus* spp., and *Lactobacillus* spp., have been shown to influence gastrointestinal metabolite concentrations (17), by processes spanning from utilization of amino acids as a nitrogen source, to byproduct generation of secondary metabolites such as short chain fatty acids (SCFAs). In the following section we will highlight key examples of these microbiome effects on dietary and endogenously generated gut metabolites, and how these microbiome effects contribute to the downstream effector functions of these metabolites on host immunity (Figs. 1, 2).

De novo microbiome production of metabolites. The gut microbiota produces a number of metabolites, some of which feature distinct bioactive functions on the eukaryotic host. A prime example of such de-novo synthesized metabolites include SCFAs, which are the products of microbial fermentation of nondigestible nutritional fibers, and include acetate (C2), propionate (C3), and butyrate (C4). Gut commensal bacteria, such as those belonging to the genera *Butyrivibrio*, *Clostridium*, and *Eubacterium*, can

produce SCFAs that locally reach a millimolar concentration (19). Additional fermentation byproducts like succinate and lactate are also generated during SCFAs production, and are used by the microbiota for maintenance and survival (20). SCFAs, in turn, mediate a number of important functions for the eukaryotic host, including usage as an energy source by intestinal epithelial cells, and a variety of anti-inflammatory properties in T cells, regulatory T cells (Tregs), neutrophils, and macrophages, where they affect migration, cytolytic activity, cytokine production, and epigenetic regulation of gene expression (discussed below).

Another example of bioactive de novo microbial-synthesized metabolites are vitamins, small bioactive nutrients that are extracted from the diet and may be modulated by the microbiome or de novo produced by intestinal commensals. One such important example involves vitamin K, a central cofactor regulating the mammalian coagulation cascade (21) and immunity (22). The vitamin K group consists of vitamin K1 (phylloquinone), derived from food, and vitamin K2 (menaquinone), which can be also produced from vitamin K1 by most of the gut microbiota species, including *Enterobacter* sp., *Eubacterium lentum*, *Veillonella* sp. and *Bacteroides* sp. (23). Members of the vitamin K group are absorbed in the small intestine in a process requiring bile salts (24). The importance of microbial production of vitamin K to its overall intestinal pool is supported by the fact that broad-spectrum antibiotic treatment induces significantly reduced vitamin K levels (25). Furthermore, a primary vitamin K deficiency animal model was not successfully generated using a vitamin K-deficient diet alone, unless germ-free mice were employed in the process, further supporting the important role of the gut microbiome in its processing (26). However, the transformation of vitamin K1 to vitamin K2 may not be solely dependent on the microbiota, as it occurs in gnotobiotic rats (27).

FIGURE 1. Microbiota-associated metabolites shape mucosal immunity. Metabolites participate in a complex host-microbiome network of communications orchestrating immune responses. The most well-studied metabolites SCFA and NA are suggested to affect multiple facets of the immune response, such as macrophage and DC function and cytokine secretion, Treg differentiation, intestinal goblet cell mucin secretion, inflammasome-mediated IL-18 activation, and neutrophil chemotaxis via the NF- κ B pathway.



Other vitamins, such as vitamin B1 (thiamine) (28) and folic acid (vitamin B9) (29), may also be synthesized by some members of the microbiome, thereby contributing to the overall vitamin pull. Vitamin B12 can be produced by bacteria, e.g., *Propionibacterium freudenreichii*, *Salmonella enterica*, *Listeria innocua*, and *Lactobacillus reuteri*, and may also be degraded by multiple members of the microbiome (30). Its binding to the eukaryotic intrinsic factor as part of the digestion process is believed to protect it from microbial degradation in the distal small intestine (30).

Microbiome modulation of nutritionally derived metabolites. Multiple bioactive gastrointestinal small molecules originate from dietary sources, undergo microbial modifications and have recently been shown to feature important immune functions. One example is the immunomodulatory amino acid tryptophan (Trp), abundantly found in foods such as milk, eggs, red meat, and vegetables (e.g., broccoli). Trp is known to undergo metabolism by *Lactobacilli*, giving rise to indole-3-aldehyde, which can bind to the aryl hydrocarbon receptor (AHR), followed by its transport through the epithelial cell layer by a transporter containing angiotensin I converting enzyme 2 (9, 31). Deficiency in murine angiotensin I converting enzyme 2, which controls the levels of neutral amino acids in the intestine, including Trp, results in higher susceptibility for intestinal inflammation and an altered gut microbial composition (32). Inflammation in this context is transmissible through transfer of the microbiota to germ-free mice, whereas dietary Trp replenishment rescues microbial dysbiosis (32).

Microbiome modulation of host-derived metabolites. The microbiome may also modulate metabolites that are produced by the eukaryotic host. An important example of such endogenously generated gastrointestinal metabolites are bile acids, which are produced from cholesterol by the liver, further conjugated to glycine (in humans) or taurine (in mice) and transported to the gallbladder, common bile duct, and the proximal small intestine (33, 34). They are considered essential for solubilizing dietary fat and cholesterol, thereby accelerating digestion and absorption.

Secondary microbial-mediated metabolism of primary to secondary bile acids increases their diversity (35), as exemplified by germ-free mice having lower levels and reduced diversity of secondary bile acids as compared with colonized mice (36). In the intestinal epithelium, many different bile acids, including microbiome-modulated deoxycholic acid and lithocholic acid (37), can bind the G-protein coupled receptor TGR5 as well as the nuclear receptor farnesoid X receptor (FXR). FXR was suggested to regulate epithelial cell integrity and bacterial composition because in the absence of FXR, mice present a disrupted epithelial barrier function and dysbiosis (38). In addition, secondary bile acid signaling may impact the host immune response by modulating proinflammatory genes through NF- κ B signaling (39, 40). As such, the addition of an FXR ligand to LPS-treated macrophages downregulated the NF- κ B-controlled genes IL-1 β , TNF- α , COX-1, COX-2, and iNOS. Moreover, *Fxr*^{-/-} mice presented a significant exacerbation of colitis symptoms, which could be mitigated by treating wild-type (WT) mice with an FXR analog (40). Furthermore, bile acid signaling was recently suggested to contribute to the effects of the microbiota on the metabolic syndrome, which may partially stem from its immune modulatory effects (41, 42).

One recently discovered role of microbiome-modulated metabolites on the intestinal immune response involves signaling through the *Nlrp6* inflammasome. The bile acid related metabolite taurine was suggested to shape the host-microbiota interface through activation of *Nlrp6* inflammasome signaling, resulting in intestinal epithelial cell IL-18 secretion and downstream modulation of anti-microbial peptide transcription (43), thereby impacting the microbiome composition and risk of auto-inflammation. The microbiome-modulated metabolites histamine and spermine, in turn, inhibit the NLRP6 inflammasome signaling, suggesting a mechanism by which the combination of bioactive metabolites at given contexts drive the host-microbiome interface through regulation of immune sensing and downstream immune modulation. Collectively, postbiotic metabolite intervention may target a variety of host-related pathways, including those exemplified above, representing a promising potential new therapeutic modality of microbiome-related metabolic and inflammatory disorders.

Mechanisms of microbiota-modulated metabolite immune regulation

Microbiome-modulated metabolites may impact the host immune response through several mechanisms that are only just beginning to be unraveled. In addition to metabolite-induced signaling in immune cell subsets that may trigger a cascade of inflammatory changes, recent studies have identified direct metabolite-mediated effects on immune cell metabolism, often resulting in substantial, previously unappreciated, functional outcomes.

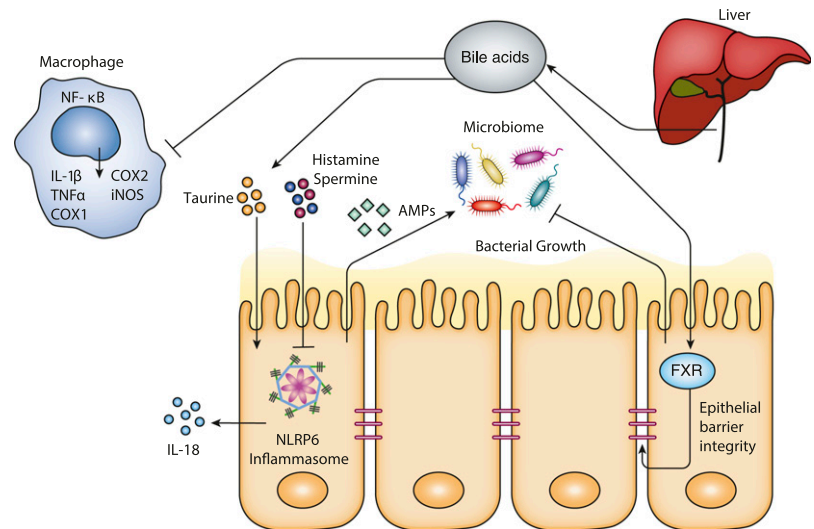
Immune cell metabolic reprogramming. One of the recently characterized roles of commensal microbiota is to provide energy to intestinal epithelial cells (IECs), through fermentation of dietary fibers by degradation of undigested complex carbohydrates into SCFAs. In the absence of the microbiota and associated SCFAs, germ-free mice feature an altered energy metabolism characterized by preferential fermentation of glucose into lactate (44), thereby leading to enhanced IEC autophagy as a consequence of nutrient starvation (45, 46). Monoinoculation of germ-free mice with the butyrate-producing *Butyrivibrio fibrisolvens* rescued IECs from autophagy and mitochondrial respiration insufficiency (45). Similar to epithelial cells, energy metabolism in leukocytes is affected by microbiota-regulated metabolites. T cell responses are dependent on nutrient catabolism, whereas intestinal T cells in germ-free mice present an immature phenotype characterized by impaired cytolytic activity (47, 48).

The molecular mechanisms by which SCFAs, such as butyrate, serve as energy sources and consequent functional modulators of intestinal epithelial cells and immune cells are beginning to be unraveled (45). Butyrate acts as a local substrate for the production of energy through the tricarboxylic acid cycle, ATP generation, and β -oxidation as well as through the suppression of autophagy in intestinal epithelial cells (45).

Transcriptional and epigenetic modulation of immune-related genes

Another key mechanism by which gut microbiome-modulated metabolites influence the immune response involves regulation of immune cell transcriptional programming through impacting their epigenetic landscape. One such example relates to the transcription of mucin-related genes by butyrate, thereby contributing to goblet cell differentiation and mucus

FIGURE 2. Bile acid effects on the host-microbiota interface and immune functions. Bile acids are produced by the liver, secreted into the proximal small intestine, and modulated by the microbiome, thereby impacting multiple immune processes. Bile acids bind FXR on intestinal epithelial cells, through which they regulate barrier integrity and bacterial community structure. In macrophages, bile acid signaling downregulates NF- κ B–controlled proinflammatory responses. The bile acid metabolite taurine shapes the host-microbiota interface through activation of *Nlrp6* inflammasome signaling, resulting in intestinal epithelial cell IL-18 secretion and downstream modulation of anti-microbial peptide transcription. Histamine and spermine negatively modulate the Nlrp6 inflammasome.



production (49–51), which are essential in host innate immune defense against invading pathogens. Furthermore, histone deacetylase (HDAC) inhibitory activity of butyrate induces gene expression changes in DCs including the suppression of IL-12, IL-6, and Relb, which contributes to Treg differentiation (52). Butyrate also plays a role in modulating the immune response in macrophages, by inhibiting transcription of proinflammatory mediators in colon lamina propria macrophages such as *Il6*, *Nos2*, and *Il12* (53), a mechanism contributing to the maintenance of tolerance toward commensal bacteria.

In addition to its possible effects on cell metabolism, butyrate was shown to influence peripheral Tregs through increased histone H3 acetylation of the *Foxp3* promoter region and at the intragenic enhancer elements conserved noncoding DNA sequence 1, associated with gene expression (54, 55). As such, butyrate was demonstrated to indirectly suppress colonic inflammation through GPR109A receptor-mediated regulation of Treg differentiation (56).

The epigenetic suppressive activity of SCFAs may be vertically transferrable to offspring, as high-fiber feeding of pregnant female mice suppressed the expression of genes related to airway response in the fetal lung (57). Similarly, SCFAs were also suggested to influence leukocyte function and neutrophil chemotaxis (58), through inhibition of HDAC activity leading to NF- κ B inactivation and suppression of proinflammatory cytokines and nitric-oxide (58, 59). A similar suppression of HDACs resulting in an anti-inflammatory phenotype was observed in DCs and intestinal macrophages (53, 60, 61).

In addition to being the receptor for butyrate, GPR109A is also the receptor for microbial-derived nicotinic acid (NA, or niacin). The microbial-derived butyrate and NA engage GPR109A on epithelial cells, macrophages, and DCs and trigger the secretion of IL-18 from epithelial cells, and IL-10 from DCs and macrophages, which further leads to suppression of inflammation as IL-10 favors the development of Tregs from naive CD4⁺ cells (56). The SCFA acetate can also bind GPR43 on IECs, which leads to NLRP3 inflammasome assembly and the downstream production of IL-18 (62). As such, acetate was shown to promote epithelial cell integrity, which contributes to protection against infection (63).

Some microbiome-mediated transcriptional and epigenetic modifications of the host may involve metabolite signaling, yet such metabolites and their putative roles in these modifications have not been identified to date. A notable example of genome-wide epigenetic effects mediated by the gut microbiome and impacting the mucosal immune response involves ILCs (64). The transcriptional landscape of ILC subsets has been recently shown to involve a complex and subset-specific epigenetic modification program. Importantly, microbiota depletion by broad-spectrum antibiotic treatment or in germ-free mice results in massive restructuring of the global epigenetic landscape of small intestinal ILCs, involving several thousand histone modifications at enhancer and promoter sites. Also, the impact of the intestinal microbiota on altered gene expression in ILCs is different between distinct, newly recognized, transcriptional subsets. This suggests that within the small intestinal ILC population, different clusters of cells feature distinct microbiota responsiveness patterns, possibly through defined sets of epigenetic modifications (64). Whether some of these microbiome-mediated epigenetic alterations in ILCs are a result of metabolite functions merit future studies. Another notable transcriptional outcome of microbiome colonization that may involve a yet unidentified metabolite signaling is the microbiome-mediated modulation of the T_H17 immune response. Following colonization with segmented filamentous bacteria (SFB), which are known to adhere to intestinal mucosa in a host-species manner, T_H17 levels of germ-free mice are restored (65), leading to induction of transcription of T_H17 associated genes including *Il17A*, *Il21*, *Ccr6*, and *Nos2*, as well as serum amyloid A and anti-microbial peptides (65). SFB-induced T_H17 cells were shown to recognize SFB Ags, through yet unrecognized mechanisms, whereas DC expression of MHCII is necessary for T_H17 cell induction (66).

Metabolite immune modulation through yet unknown mechanisms. As metabolite-mediated functions on the host are only just beginning to be discovered, some effects are not yet fully defined mechanistically. For example, some mechanisms of SCFAs modulatory effects on host immune functions have been deciphered, and are described above, but others remain cryptic and can be driven by intracellular metabolic pathways, epigenetic modification, transcription regulation, or by other yet undefined mechanism(s). Different SCFAs can be differentially sensed through the receptors GPR41, GPR43, and

GPR109A (10). Butyrate is the ligand for GPR109A, expressed by IECs and T cells, where its signaling induces substantial effects on Treg abundance and activity, yet the mechanism of this effect is still not completely understood (56). Likewise, colonic macrophages and DCs from mice deficient in GPR109A are defective in their ability to induce differentiation of naive T cells into IL-10–expressing Tregs, resulting in a general reduction in colonic Tregs and increased severity of dextrane sodium sulfate–induced colitis, through mechanisms that are still elusive (56). Germ-free and colonized mice treated with SCFAs such as acetate feature reduced inflammation in a dextrane sodium sulfate–induced colitis model as well as in a T cell transfer colitis model in a GPR43-dependent manner. Mechanistically, SCFA administration increases the numbers of Treg cells in germ-free mice as well as the secretion of its key suppressive effector cytokine IL-10, possibly through effects on immune cell metabolism (54, 67). Furthermore, a lack of GPR43 expression in *Gpr43*^{-/-} mice severely alters SCFA-mediated expansion of colonic Tregs (54). Thus, SCFAs and their GPRs represent one pathway through which the commensal microbiota regulates the inflammatory response, yet the intracellular and immune-mediated mechanisms of these metabolite effects merit further studies. Similar to their local effect in the intestine, circulating SCFAs dampen the severity of allergic airway inflammation with reduced levels of IL-4, IL-5, IL-13, and IL-17a in the lung, possibly through increased bone marrow generation of macrophages and DCs; this and other potential mechanisms merit further exploration (60).

Microbiome-modulated metabolites and the risk of disease

Defined compositional and functional microbiome alterations have been associated with the pathogenesis of common multifactorial diseases. These diseases include, among others,

pathologies primarily affecting the digestive system [such as inflammatory bowel diseases (IBD) and colorectal cancer], metabolic diseases (diabetes mellitus and nonalcoholic fatty liver disease), and even cardiovascular and neurologic disorders (68–73). As such, it is reasonable to assume that at least some of the effects of the microbiome may be mediated by altered metabolite profiles associated with these microbial disease signatures. Such altered metabolite levels and combinations may affect immune function and metabolism as well as multiple other seemingly unrelated physiological functions. Although the complex network of interactions between the microbiome, its associated metabolite profile and the immune system in the context of disease is just beginning to be unraveled, several studies, exemplified below, have identified some of the pathways underlying these interactions (Fig. 3).

Inflammatory bowel disease. The incidence of IBDs, a group of auto-inflammatory diseases involving different regions of the gastrointestinal tract, has been steadily increasing globally (70). One of the characteristic associated hallmarks of IBD is intestinal dysbiosis — a deviation from the healthy composition and function of the microbiome. Dysbiosis may contribute to the impaired cross-talk between immune cells and the microbiome in IBD, and may involve aberrant signaling by different immunomodulatory metabolites. For example, in an animal model of ulcerative colitis, an interplay between the commensal microbiota and AHR-expressing group 3 ILCs is mediated by microbiota-produced metabolites (31, 74). The absence of AHR or its ligands induces changes in microbial composition, leading to exacerbated colitis development in mice (75). A subset of commensal bacteria utilizes Trp as an energy source and produces the metabolite indole-3-aldehyde, which further activates AHR in ILCs, leading to secretion of IL-22 impacting both mucosal healing and the anti-microbial

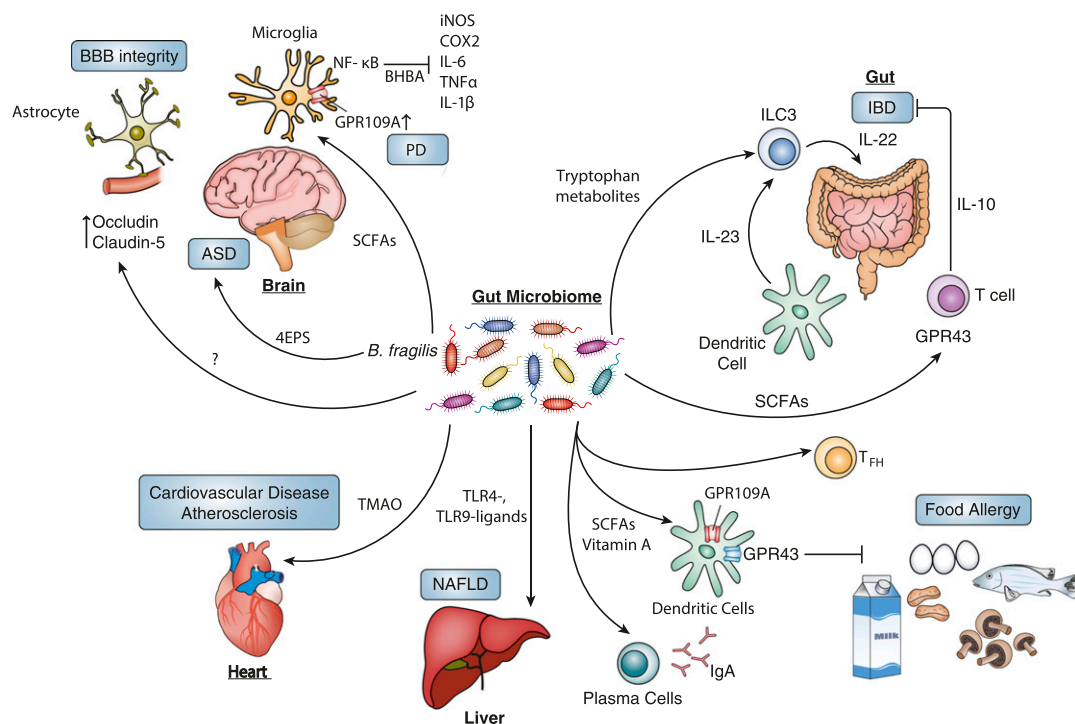


FIGURE 3. Microbiome-modulated metabolites and disease. Altered levels of microbiome-modulated metabolites have been associated with immune-mediated and immune-associated disease risk. Metabolite-mediated effects on disease processes may be localized to the gastrointestinal tract, such as in IBD or food allergy, or systemically influence remote tissues like the brain, heart and the liver.

peptides repertoire including lipocalin-2, S100A8, and S100A9 in mice (31, 74). Another possible metabolite-mediated mechanism of auto-inflammation involves SCFA-mediated inhibition of HDACs, independently of their receptors GPR41 and GPR43, leading to T cell activation of the mTOR-S6K pathway (76). Acetate supplementation in drinking water was able to mitigate anti-CD3-induced colitis severity in the ileum of WT but not of *Il10*^{-/-} mice, indicating that this protective effect requires IL-10 (76). Conversely, oral butyrate treatment enhanced IL-23 levels in DCs and exacerbated colitis symptoms in an IBD animal model (77). As the effects of SCFAs are complex and sometimes diverse, indirect, and combinatorial, future studies will have to delineate their human relevance and therapeutic potential in different combinations and clinical contexts.

Another example of the role of the microbiome-immune-metabolite axis in driving intestinal auto-inflammation is provided by the NLRP6 inflammasome model in mice. As described above, a properly functional NLRP6 inflammasome signaling, orchestrated by the microbiome-modulated metabolites taurine, histamine, and spermine (43) in mouse IEC, contributes to a healthy microbiome population. Conversely, NLRP6-deficient mice feature dysbiosis and associated intestinal auto-inflammation, which is transmittable via fecal transplantation into normal WT mice, thereby inducing exacerbated colitis symptoms, which are ameliorated by restoration of normal microbiota (43). Multiple other unknown metabolites in mice and in human IBD patients may impact immune activation and function, thereby affecting the pathogenesis of different IBD subsets, and merit further studies.

Nonalcoholic fatty liver disease. Nonalcoholic fatty liver disease (NAFLD) is the most common hepatic disease in the developed world, and is tightly associated with other features of metabolic syndrome including obesity, hyperlipidemia, and adult-onset diabetes mellitus. Whereas most affected individuals remain asymptomatic, a significant minority of NAFLD cases develop a progressive inflammatory liver disease termed nonalcoholic steatohepatitis (NASH), ultimately leading to liver dysfunction, cirrhosis, and life-risking complications (78). The microbiome was suggested in both mouse models and humans to regulate some manifestations of NAFLD and NASH via its systemic effects on the immune system (79, 80). In one such animal model, inflammasome-deficient mice feature dysbiosis mediated by an altered metabolite profile (81). When induced with NAFLD, these mice developed a context-specific form of dysbiosis characterized by a massive expansion of *Porphyromonadaceae* accompanied with enhanced TLR4 and TLR9 ligands influxing into the portal circulation and leading to massive TNF- α secretion, hepatic inflammation, and the progression to NASH (82). Interestingly, this microbiome-mediated transition into NASH was abolished by wide-spectrum antibiotic treatment and transferrable by cohabitation of inflammasome-deficient mice with WT mice. As dysbiosis in this model has been recently linked to microbiome-modulated metabolite misbalance, it hints toward the possibility of an indirect link existing between gastrointestinal metabolite alterations, dysbiosis, and influx of microbial products into the liver leading to inflammatory consequences (43). Likewise, in NAFLD human patients, dysbiosis was observed with an expansion of *Streptococcus*, *Anaerobacter*, *Lactobacillus* and *Escherichia* genera as compared with healthy subjects, accompanied by reduction of *Alistipes* and *Prevotella*.

Similar to the above animal model, NAFLD patients were found to have higher levels of TNF- α , IFN- γ , and IL-6 and disrupted microvilli morphology associated with increased gut permeability (83). These results suggest that dysbiosis may not only affect the local intestinal inflammatory response, but may also systemically influence sterile tissues through metabolites driving pathological conditions, inflammatory and metabolic alike. Other manifestations of the metabolic syndrome, such as obesity and glucose intolerance, were recently suggested to involve an altered inflammatory response in tissues such as adipose tissue, the pancreas, and the liver (84). An intriguing, yet unexplored possibility is that some of these inflammatory changes may be mediated by dysbiosis and its associated altered metabolite configuration. Exploring metabolite roles in these contexts may enable to identify new therapeutic and diagnostic modalities to these common idiopathic multifactorial disorders.

Cardiovascular disease. The catastrophic consequences of atherosclerosis, myocardial infarction, and cerebrovascular accident still account for the majority of deaths worldwide (85). Atherosclerotic plaques in arterial walls are caused by an imbalanced lipid homeostasis leading to accumulation of cholesterol-containing low-density lipoprotein particles, accompanied by chronic inflammation (86). In the past decade changes in microbiome composition and function have been associated with cardiovascular disease pathogenesis. One notable example of such an association involves trimethylamine-N-oxide (TMAO) metabolism. TMAO is a metabolite that in humans is generated by microbial metabolism of dietary phosphatidylcholine or L-carnitine, which are abundant in meat and high-fat diets. Mice fed L-carnitine featured increased levels of TMAO leading to enhanced atherosclerosis. Germ-free mice or antibiotic-treated mice were protected from the disease, thereby linking the gut microbiota, nutrition, and risk for cardiovascular disease (87). Similar findings were observed in humans, in which a time-dependent increase in plasma TMAO (and other phosphatidylcholine metabolites) was documented following ingestion of isotopic-labeled food. These increased metabolite levels were inhibited by antibiotic treatment, and reappeared after antibiotic withdrawal (88). Increased fasting TMAO plasma levels were significantly associated with major adverse cardiovascular events such as overall mortality, myocardial infarction or stroke. These results demonstrate the potential central role of this microbiome-derived metabolite in the risk factor for cardiovascular disease (88, 89).

Food allergy. Allergies to nutritional components constitute a common immune-mediated condition affecting the pediatric and adult population alike. Mucosal DCs contribute to allergy to food Ags by regulation of Treg differentiation. A high-fiber diet together with vitamin A was recently shown to drive the microbiota toward a configuration that is supportive for tolerogenic CD103⁺ DCs, which provides tolerance toward food Ags in a mouse model, thereby protecting from food allergy. This protective effect depends on epithelial GPR43 and immune GPR109A, because the protective effect was not observed in *Gpr43*^{-/-} or in *Gpr109a*^{-/-} mice. In this model, changes in the microbiota following a high-fiber diet were also associated with enhanced IgA production and T follicular helper response (90), further implicating the microbiome in this food allergy protective effect.

Neurodegenerative disease. Although neurological disorders are increasingly suggested to feature important immune components,

research on neurological immune modulation remains in its infancy. Some of the observed immunomodulatory effects on the CNS are mediated by resident immune cell subsets in the CNS, which impact disease progression, and particularly the microglia, the resident myeloid cells of the CNS. Interestingly, microglia were recently suggested to be affected by metabolites such as SCFAs in a mouse model. SCFAs secreted by the gut microbiota modulated microglial activation and maturation gene-expression profile in the mouse steady state (91). The NA receptor GPR109A was recently found to be expressed by microglia (92) and feature increased expression colocalizing with microglial markers in the substantia nigra of Parkinson's disease patients, possibly participating in disease modulation (92). Likewise, β -hydroxybutyrate treatment given to a Parkinson's disease rat model improved motor skills and protected nigro-striatal neurons by reducing neuroinflammation (92). Addition of β -hydroxybutyrate to mesencephalic neuron-glia cultures reduced the harmful effects of LPS-induced microglial activation through GPR109A inhibition of NF- κ B pathway, resulting in a reduction of proinflammatory enzymes (COX-2, iNOS) and cytokines (IL-6, TNF- α , IL-1 β) (93). Butyrate and other SCFAs influx from the gastrointestinal tract through the portal circulation or are absorbed directly into the systemic circulation through the distal colonic blood supply, and may access the CNS through the blood-brain barrier (BBB). Mice fed a fermented-fiber diet presented with a reduced endotoxin-related sickness behavior (94), and interestingly featured increased CNS IL-4 mRNA levels probably mediated by enhancement of histone acetylation increasing IL-1RA levels, which inhibits the production of IL-1 β proinflammatory cytokine (94). Furthermore, the tight-junction forming proteins occludin and claudin-5 were reduced in the brains of germ-free mice, leading to an increased permeability of their BBB (95). Treatment with butyrate-producing *Clostridium tyrobutyricum* bacteria was able to elevate occludin and claudin-5 levels in germ-free mice brains and to restore their BBB permeability to the level of specific pathogen-free mice (95).

Interestingly, other neuropsychiatric disorders such as autistic spectrum disorder (ASD) were also suggested to involve modulation by microbiota-dependent metabolites (72). ASD-prone mice feature dysbiosis, increased gut permeability, and altered serum metabolites (96). Treatment with *Bacteroides fragilis* restored gut permeability pathology and ASD neurologic symptoms, probably by regulation of 4-ethylphenylsulfate, a metabolite contributing to some ASD-like symptoms. These results suggest that the gut microbiome may influence some CNS neurological functions, including neuro-inflammation, via systemic metabolite regulation (96). Whether some of these effects may involve altered activation of the CNS immune response, and whether similar effects may be observed in humans remains elusive.

Conclusions

In this review we highlight some examples by which dietary, host derived- and microbiota-modulated metabolites may affect numerous aspects of the host immune response. In many of these observations, the mechanisms through which commensal bacteria regulate host immunity remain unclear and merit future investigation. Likewise, further investigation is required to determine the repertoire, bio-geographical distribution, and

bioactivity of metabolites in the gastrointestinal tract and how it may impact local and systemic inflammatory processes. Integration of the accumulating knowledge on microbial community structure in different disease scenarios with the corresponding changes in the metabolome and its bioactivity may enable addressing fundamental questions regarding the molecular mechanisms by which the microbiome impacts physiology, pathophysiology and even its own community function (97). Such studies will likely involve an integration of advanced next-generation sequencing related genomics, high-throughput metabolomics, and gnotobiotic experimentation that is critical in demonstrating causality and differentiating between primary driver microbial impact on disease pathogenesis, and secondary passenger microbial changes. Such integrative studies may potentially yield novel microbiota-based diagnostics and therapies of common disease. As such, colonization of germ-free animals with microbiota from humans (notwithstanding its inherent technical limitations) has recently emerged as a promising way to simplify studying these complex disorders (98). Humanized germ-free animals may allow better long-term elucidation of the roles of specific human-resident bacterial species and their associated metabolites in contributing to disease development and progression.

Furthermore, a detailed and comprehensive microbiome characterization using a combination of 16S rDNA analysis, shotgun metagenomic sequencing of the microbiome metagenome, and metatranscriptome, coupled with metabolomics and characterization of the host transcriptome, may be integrated into predictive computational modeling that may facilitate early diagnosis, patient stratification, and personalized treatment approaches (99). Last, heightened understanding of the effects of various metabolites on homeostasis and disease may pave the way for their future application as a means of postbiotic intervention that may allow the administration of metabolite combinations as a preventive or therapeutic measure in microbiome-associated disease, thereby avoiding the need for dealing with the significant variability in individual human microbiome configurations.

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References

- Veldhoen, M., and V. Brucklacher-Waldert. 2012. Dietary influences on intestinal immunity. *Nat. Rev. Immunol.* 12: 696–708.
- Lee, W.-J., and K. Hase. 2014. Gut microbiota-generated metabolites in animal health and disease. *Nat. Chem. Biol.* 10: 416–424.
- Zheng, X., G. Xie, A. Zhao, L. Zhao, C. Yao, N. H. L. Chiu, Z. Zhou, Y. Bao, W. Jia, J. K. Nicholson, and W. Jia. 2011. The footprints of gut microbial-mammalian co-metabolism. *J. Proteome Res.* 10: 5512–5522.
- Gargaro, M., M. Pirro, R. Romani, T. Zelante, and F. Fallarino. 2016. AhR-dependent pathways in immune regulation. *Am. J. Transplant.* 16: 2270–2276.
- Gaudet, R. G., A. Sintsova, C. M. Buckwalter, N. Leung, A. Cochrane, J. Li, A. D. Cox, J. Moffat, and S. D. Gray-Owen. 2015. Cytosolic detection of the bacterial metabolite HBP activates TIFA-dependent innate immunity. *Science* 348: 1251–1255.
- Hall, J. A., J. L. Cannons, J. R. Grainger, L. M. Dos Santos, T. W. Hand, S. Naik, E. A. Wohlfert, D. B. Chou, G. Oldenhove, M. Robinson, et al. 2011. Essential role for retinoic acid in the promotion of CD4(+) T cell effector responses via retinoic acid receptor alpha. *Immunity* 34: 435–447.

7. Lamas, B., M. L. Richard, V. Leducq, H.-P. Pham, M.-L. Michel, G. Da Costa, C. Bridonneau, S. Jegou, T. W. Hoffmann, J. M. Natividad, et al. 2016. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat. Med.* 22: 598–605.
8. Mader, D., M. J. Rabier, F. Boulay, and A. Peschel. 2010. Formyl peptide receptor-mediated proinflammatory consequences of peptide deformylase inhibition in *Staphylococcus aureus*. *Microbes Infect.* 12: 415–419.
9. Thorburn, A. N., L. Macia, and C. R. Mackay. 2014. Diet, metabolites, and “western-lifestyle” inflammatory diseases. *Immunity* 40: 833–842.
10. Tan, J., C. McKenzie, M. Potamitis, A. N. Thorburn, C. R. Mackay, and L. Macia. 2014. The role of short-chain fatty acids in health and disease. *Adv. Immunol.* 121: 91–119.
11. Rothhammer, V., I. D. Mascalfroni, L. Bunse, M. C. Takenaka, J. E. Kenison, L. Mayo, C.-C. Chao, B. Patel, R. Yan, M. Blain, et al. 2016. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* 22: 586–597.
12. Butovsky, O., A. E. Talpalar, K. Ben-Yaakov, and M. Schwartz. 2005. Activation of microglia by aggregated β -amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN- γ and IL-4 render them protective. *Mol. Cell. Neurosci.* 29: 381–393.
13. Koren, O., J. K. Goodrich, T. C. Cullender, A. Spor, K. Laitinen, H. K. Bäckhed, A. Gonzalez, J. J. Werner, L. T. Angenent, R. Knight, et al. 2012. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 150: 470–480.
14. Ley, R. E., D. A. Peterson, and J. I. Gordon. 2006. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124: 837–848.
15. Martin, F. P. J., N. Sprenger, I. Montoliu, S. Rezzi, S. Kochhar, and J. K. Nicholson. 2010. Dietary modulation of gut functional ecology studied by fecal metabolomics. *J. Proteome Res.* 9: 5284–5295.
16. Marcobal, A., T. Yusufaly, S. Higginbottom, M. Snyder, J. L. Sonnenburg, and G. I. Mias. 2015. Metabolome progression during early gut microbial colonization of gnotobiotic mice. *Sci. Rep.* 5: 11589.
17. Matsumoto, M., R. Kibe, T. Ooga, Y. Aiba, S. Kurihara, E. Sawaki, Y. Koga, and Y. Benno. 2012. Impact of intestinal microbiota on intestinal luminal metabolome. *Sci. Rep.* 2: 233.
18. Nicholls, A. W., R. J. Mortshire-Smith, and J. K. Nicholson. 2003. NMR spectroscopic-based metabolomic studies of urinary metabolite variation in acclimating germ-free rats. *Chem. Res. Toxicol.* 16: 1395–1404.
19. Pryde, S. E., S. H. Duncan, G. L. Hold, C. S. Stewart, and H. J. Flint. 2002. The microbiology of butyrate formation in the human colon. *FEMS Microbiol. Lett.* 217: 133–139.
20. Topping, D. L., and P. M. Clifton. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* 81: 1031–1064.
21. Card, D. J., R. Gorska, J. Cutler, and D. J. Harrington. 2014. Vitamin K metabolism: current knowledge and future research. *Mol. Nutr. Food Res.* 58: 1590–1600.
22. Xu, Z., Q. Qiu, J. Tian, J. S. Smith, G. M. Conenello, T. Morita, and A. P. Byrnes. 2013. Coagulation factor X shields adenovirus type 5 from attack by natural antibodies and complement. *Nat. Med.* 19: 452–457.
23. Biesalski, H. K. 2016. Nutrition meets the microbiome: micronutrients and the microbiota. *Ann. N. Y. Acad. Sci.* 1372: 53–64.
24. Beulens, J. W. J., S. L. Booth, E. G. H. M. van den Heuvel, E. Stoecklin, A. Baka, and C. Vermeer. 2013. The role of menaquinones (vitamin K₂) in human health. *Br. J. Nutr.* 110: 1357–1368.
25. Conly, J., and K. Stein. 1994. Reduction of vitamin K₂ concentrations in human liver associated with the use of broad spectrum antimicrobials. *Clin. Invest. Med.* 17: 531–539.
26. Komai, M., H. Shirakawa, and S. Kimura. 1988. Newly developed model for vitamin K deficiency in germfree mice. *Int. J. Vitam. Nutr. Res.* 58: 55–59.
27. Davidson, R. T., A. L. Foley, J. A. Engelke, and J. W. Suttie. 1998. Conversion of dietary phyloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. *J. Nutr.* 128: 220–223.
28. Champagne, C. P., T. A. Tompkins, N. D. Buckley, and J. M. Green-Johnson. 2010. Effect of fermentation by pure and mixed cultures of *Streptococcus thermophilus* and *Lactobacillus helveticus* on isoflavone and B-vitamin content of a fermented soy beverage. *Food Microbiol.* 27: 968–972.
29. Pompei, A., L. Cordisco, A. Amaretti, S. Zanoni, D. Matteuzzi, and M. Rossi. 2007. Folate production by bifidobacteria as a potential probiotic property. *Appl. Environ. Microbiol.* 73: 179–185.
30. Degnan, P. H., M. E. Taga, and A. L. Goodman. 2014. Vitamin B12 as a modulator of gut microbial ecology. *Cell Metab.* 20: 769–778.
31. Zelante, T., R. G. Iannitti, C. Cunha, A. De Luca, G. Giovannini, G. Pieraccini, R. Zecchi, C. D’Angelo, C. Massi-Benedetti, F. Fallarino, et al. 2013. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* 39: 372–385.
32. Hashimoto, T., T. Perlot, A. Rehman, J. Trichereau, H. Ishiguro, M. Paolino, V. Sigl, T. Hanada, R. Hanada, S. Lipinski, et al. 2012. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 487: 477–481.
33. de Aguiar Vallim, T. Q., E. J. Tarling, and P. A. Edwards. 2013. Pleiotropic roles of bile acids in metabolism. *Cell Metab.* 17: 657–669.
34. Wahlström, A., S. I. Sayin, H.-U. Marschall, and F. Bäckhed. 2016. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab.* 24: 41–50.
35. Ridlon, J. M., D.-J. Kang, and P. B. Hylemon. 2006. Bile salt biotransformations by human intestinal bacteria. *J. Lipid Res.* 47: 241–259.
36. Swann, J. R., E. J. Want, F. M. Geier, K. Spagou, I. D. Wilson, J. E. Sidaway, J. K. Nicholson, and E. Holmes. 2011. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc. Natl. Acad. Sci. USA* 108(Suppl. 1): 4523–4530.
37. Wang, H., J. Chen, K. Hollister, L. C. Sowers, and B. M. Forman. 1999. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell* 3: 543–553.
38. Inagaki, T., A. Moschetta, Y.-K. Lee, L. Peng, G. Zhao, M. Downes, R. T. Yu, J. M. Shelton, J. A. Richardson, J. J. Repa, et al. 2006. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc. Natl. Acad. Sci. USA* 103: 3920–3925.
39. Pols, T. W. H., M. Nomura, T. Harach, G. Lo Sasso, M. H. Oosterveer, C. Thomas, G. Rizzo, A. Gioiello, L. Adorini, R. Pellicciari, et al. 2011. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metab.* 14: 747–757.
40. Vavassori, P., A. Mencarelli, B. Renga, E. Distrutti, and S. Fiorucci. 2009. The bile acid receptor FXR is a modulator of intestinal innate immunity. *J. Immunol.* 183: 6251–6261.
41. Ridaura, V. K., J. J. Faith, F. E. Rey, J. Cheng, A. E. Duncan, A. L. Kau, N. W. Griffin, V. Lombard, B. Henrissat, J. R. Bain, et al. 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341: 1241214.
42. Yoshimoto, S., T. M. Loo, K. Atarashi, H. Kanda, S. Sato, S. Oyadomari, Y. Iwakura, K. Oshima, H. Morita, M. Hattori, et al. 2013. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499: 97–101.
43. Levy, M., C. A. Thaiss, D. Zeevi, L. Dohnalová, G. Zilberman-Schapiro, J. A. Mahdi, E. David, A. Savidor, T. Korem, Y. Herzog, et al. 2015. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell* 163: 1428–1443.
44. Donohoe, D. R., A. Wali, B. P. Brylawski, and S. J. Bultman. 2012. Microbial regulation of glucose metabolism and cell-cycle progression in mammalian monocytes. *PLoS One* 7: e46589.
45. Donohoe, D. R., N. Garge, X. Zhang, W. Sun, T. M. O’Connell, M. K. Bunger, and S. J. Bultman. 2011. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab.* 13: 517–526.
46. Sanderson, I. R. 2004. Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium. *J. Nutr.* 134: 2450S–2454S.
47. Buck, M. D., D. O’Sullivan, and E. L. Pearce. 2015. T cell metabolism drives immunity. *J. Exp. Med.* 212: 1345–1360.
48. Chung, H., S. J. Pamp, J. A. Hill, N. K. Surana, S. M. Edelman, E. B. Troy, N. C. Reading, E. J. Villablanca, S. Wang, J. R. Mora, et al. 2012. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* 149: 1578–1593.
49. Gaudier, E., A. Jarry, H. M. Blottière, P. de Coppet, M. P. Buisine, J. P. Aubert, C. Laboisse, C. Cherbut, and C. Hoebler. 2004. Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287: G1168–G1174.
50. Willemsen, L. E. M., M. A. Koetsier, S. J. van Deventer, and E. A. van Tol. 2003. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. *Gut* 52: 1442–1447.
51. Wrzosek, L., S. Miquel, M.-L. Noordine, S. Bouet, M. Jonquel Chevalier-Curt, V. Robert, C. Philippe, C. Bridonneau, C. Cherbuy, C. Robbe-Masselot, et al. 2013. *Bacteroides* thetaiotaomicron and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol.* 11: 61.
52. Arpaia, N., C. Campbell, X. Fan, S. Dikiy, J. van der Veeke, P. deRoos, H. Liu, J. R. Cross, K. Pfeffer, P. J. Coffey, and A. Y. Rudensky. 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504: 451–455.
53. Chang, P. V., L. Hao, S. Offermanns, and R. Medzhitov. 2014. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc. Natl. Acad. Sci. USA* 111: 2247–2252.
54. Smith, P. M., M. R. Howitt, N. Panikov, M. Michaud, C. A. Gallini, M. Bohlooly-Y, J. N. Glickman, and W. S. Garrett. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341: 569–573.
55. Furusawa, Y., Y. Obata, S. Fukuda, T. A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, et al. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504: 446–450.
56. Singh, N., A. Gurav, S. Sivaprakasam, E. Brady, R. Padia, H. Shi, M. Thangaraju, P. D. Prasad, S. Manicassamy, D. H. Munn, et al. 2014. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40: 128–139.
57. Thorburn, A. N., C. I. McKenzie, S. Shen, D. Stanley, L. Macia, L. J. Mason, L. K. Roberts, C. H. Y. Wong, R. Shim, R. Robert, et al. 2015. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* 6: 7320.
58. Vinolo, M. A. R., H. G. Rodrigues, E. Hatanaka, F. T. Sato, S. C. Sampaio, and R. Curi. 2011. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J. Nutr. Biochem.* 22: 849–855.
59. Usami, M., K. Kishimoto, A. Ohata, M. Miyoshi, M. Aoyama, Y. Fueda, and J. Kotani. 2008. Butyrate and trichostatin A attenuate nuclear factor kappaB activation and tumor necrosis factor alpha secretion and increase prostaglandin E2 secretion in human peripheral blood mononuclear cells. *Nutr. Res.* 28: 321–328.

60. Trompette, A., E. S. Gollwitzer, K. Yadava, A. K. Sichelstiel, N. Sprenger, C. Ngom-Bru, C. Blanchard, T. Jung, L. P. Nicod, N. L. Harris, and B. J. Marsland. 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20: 159–166.
61. Singh, N., M. Thangaraju, P. D. Prasad, P. M. Martín, N. A. Lambert, T. Boettger, S. Offermanns, and V. Ganapathy. 2010. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Sle5a8)-dependent inhibition of histone deacetylases. *J. Biol. Chem.* 285: 27601–27608.
62. Macia, L., J. Tan, A. T. Vieira, K. Leach, D. Stanley, S. Luong, M. Maruya, C. Ian McKenzie, A. Hijikata, C. Wong, et al. 2015. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat. Commun.* 6: 6734.
63. Fukuda, S., H. Toh, K. Hase, K. Oshima, Y. Nakanishi, K. Yoshimura, T. Tobe, J. M. Clarke, D. L. Topping, T. Suzuki, et al. 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 469: 543–547.
64. Gury-BenAri, M., C. A. Thaiss, N. Serafini, D. R. Winter, A. Giladi, D. Lara-Astiaso, M. Levy, T. M. Salame, A. Weiner, E. David, et al. 2016. The spectrum and regulatory landscape of intestinal innate lymphoid cells are shaped by the microbiome. *Cell* 166: 1231–1246.e13.
65. Ivanov, I. I., K. Atarashi, N. Manel, E. L. Brodie, T. Shima, U. Karaoz, D. Wei, K. C. Goldfarb, C. A. Santee, S. V. Lynch, et al. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139: 485–498.
66. Goto, Y., C. Panea, G. Nakato, A. Cebula, C. Lee, M. G. Diez, T. M. Laufer, L. Ignatowicz, and I. I. Ivanov. 2014. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity* 40: 594–607.
67. Maslowski, K. M., A. T. Vieira, A. Ng, J. Kranich, F. Sierro, D. Yu, H. C. Schilter, M. S. Rolph, F. Mackay, D. Artis, et al. 2009. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461: 1282–1286.
68. Giongo, A., K. A. Gano, D. B. Crabb, N. Mukherjee, L. L. Novelo, G. Casella, J. C. Drew, J. Ilonen, M. Knip, H. Hyöty, et al. 2011. Toward defining the auto-immune microbiome for type 1 diabetes. *ISME J.* 5: 82–91.
69. Heno-Mejia, J., E. Elinav, C. A. Thaiss, P. Licona-Limon, and R. A. Flavell. 2013. Role of the intestinal microbiome in liver disease. *J. Autoimmun.* 46: 66–73.
70. Kostic, A. D., R. J. Xavier, and D. Gevers. 2014. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146: 1489–1499.
71. Sears, C. L., and W. S. Garrett. 2014. Microbes, microbiota, and colon cancer. *Cell Host Microbe* 15: 317–328.
72. Parracho, H. M. R. T., M. O. Bingham, G. R. Gibson, and A. L. McCartney. 2005. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J. Med. Microbiol.* 54: 987–991.
73. Tang, W. H. W., and S. L. Hazen. 2014. The contributory role of gut microbiota in cardiovascular disease. *J. Clin. Invest.* 124: 4204–4211.
74. Qiu, J., X. Guo, Z.-M. E. Chen, L. He, G. F. Sonnenberg, D. Artis, Y.-X. Fu, and L. Zhou. 2013. Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. *Immunity* 39: 386–399.
75. Li, Y., S. Innocentin, D. R. Withers, N. A. Roberts, A. R. Gallagher, E. F. Grigorieva, C. Wilhelm, and M. Veldhoen. 2011. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell* 147: 629–640.
76. Park, J., M. Kim, S. G. Kang, A. H. Jannasch, B. Cooper, J. Patterson, and C. H. Kim. 2015. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol.* 8: 80–93.
77. Berndt, B. E., M. Zhang, S. Y. Owyang, T. S. Cole, T. W. Wang, J. Luther, N. a. Veniaminova, J. L. Merchant, C. C. Chen, G. B. Huffnagle, and J. Y. Kao. 2012. Butyrate increases IL-23 production by stimulated dendritic cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303: G1384–G1392.
78. Angulo, P. 2002. Nonalcoholic fatty liver disease. *N. Engl. J. Med.* 346: 1221–1231.
79. Miele, L., V. Valenza, G. La Torre, M. Montalto, G. Cammarota, R. Ricci, R. Mascianà, A. Forgiione, M. L. Gabrieli, G. Perotti, et al. 2009. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 49: 1877–1887.
80. Wigg, A. J., I. C. Roberts-Thomson, R. B. Dymock, P. J. McCarthy, R. H. Grose, and A. G. Cummins. 2001. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 48: 206–211.
81. Elinav, E., T. Strowig, A. L. Kau, J. Henao-Mejia, C. A. Thaiss, C. J. Booth, D. R. Peaper, J. Bertin, S. C. Eisenbarth, J. I. Gordon, and R. A. Flavell. 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145: 745–757.
82. Heno-Mejia, J., E. Elinav, C. Jin, L. Hao, W. Z. Mehal, T. Strowig, C. A. Thaiss, A. L. Kau, S. C. Eisenbarth, M. J. Jurczak, et al. 2012. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 482: 179–185.
83. Jiang, W., N. Wu, X. Wang, Y. Chi, Y. Zhang, X. Qiu, Y. Hu, J. Li, and Y. Liu. 2015. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci. Rep.* 5: 8096.
84. Thaiss, C. A., N. Zmora, M. Levy, and E. Elinav. 2016. The microbiome and innate immunity. *Nature* 535: 65–74.
85. Lusis, A. J. 2000. Atherosclerosis. *Nature* 407: 233–241.
86. Moore, K. J., F. J. Sheedy, and E. A. Fisher. 2013. Macrophages in atherosclerosis: a dynamic balance. *Nat. Rev. Immunol.* 13: 709–721.
87. Koeth, R. A., Z. Wang, B. S. Levison, J. A. Buffa, E. Org, B. T. Sheehy, E. B. Britt, X. Fu, Y. Wu, L. Li, et al. 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* 19: 576–585.
88. Tang, W. H. W., Z. Wang, B. S. Levison, R. A. Koeth, E. B. Britt, X. Fu, Y. Wu, and S. L. Hazen. 2013. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* 368: 1575–1584.
89. Wang, Z., A. B. Roberts, J. A. Buffa, B. S. Levison, W. Zhu, E. Org, X. Gu, Y. Huang, M. Zamanian-Daryoush, M. K. Culley, et al. 2015. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* 163: 1585–1595.
90. Tan, J., C. McKenzie, P. J. Vuillermin, G. Gerverse, C. G. Vinuesa, R. E. Mebius, L. Macia, and C. R. Mackay. 2016. Dietary fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. *Cell Rep.* 15: 2809–2824.
91. Erny, D., A. L. Hrabě de Angelis, D. Jaitin, P. Wieghofer, O. Staszewski, E. David, H. Keren-Shaul, T. Mahlakoiv, K. Jakobshagen, T. Buch, et al. 2015. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 18: 965–977.
92. Wakade, C., R. Chong, E. Bradley, B. Thomas, and J. Morgan. 2014. Upregulation of GPR109A in Parkinson's disease. *PLoS One* 9: e109818.
93. Fu, S.-P., J.-F. Wang, W.-J. Xue, H.-M. Liu, B. R. Liu, Y.-L. Zeng, S.-N. Li, B.-X. Huang, Q.-K. Lv, W. Wang, and J.-X. Liu. 2015. Anti-inflammatory effects of BHBA in both in vivo and in vitro Parkinson's disease models are mediated by GPR109A-dependent mechanisms. *J. Neuroinflammation* 12: 9.
94. Sherry, C. L., S. S. Kim, R. N. Dilger, L. L. Bauer, M. L. Moon, R. I. Tapping, G. C. Fahey, Jr., K. A. Tappenden, and G. G. Freund. 2010. Sickness behavior induced by endotoxin can be mitigated by the dietary soluble fiber, pectin, through up-regulation of IL-4 and Th2 polarization. *Brain Behav. Immun.* 24: 631–640.
95. Braniste, V., M. Al-Asmakh, C. Kowal, F. Anuar, A. Abbaspour, M. Toth, A. Korecka, N. Bakocevic, L. G. Ng, P. Kundu, et al. 2014. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* 6: 263ra158.
96. Hsiao, E. Y., S. W. McBride, S. Hsien, G. Sharon, E. R. Hyde, T. McCue, J. A. Codelli, J. Chow, S. E. Reisman, J. F. Petrosino, et al. 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155: 1451–1463.
97. Donaldson, G. P., S. M. Lee, and S. K. Mazmanian. 2016. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* 14: 20–32.
98. Charbonneau, M. R., D. O'Donnell, L. V. Blanton, S. M. Totten, J. C. C. Davis, M. J. Barratt, J. Cheng, J. Guruge, M. Talcott, J. R. Bain, et al. 2016. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* 164: 859–871.
99. Zeevi, D., T. Korem, N. Zmora, D. Israeli, D. Rothschild, A. Weinberger, O. Ben-Yacov, D. Lador, T. Avnit-Sagi, M. Lotan-Pompan, et al. 2015. Personalized nutrition by prediction of glycemic responses. *Cell* 163: 1079–1094.