HHS Public Access

Author manuscript

Trends Pharmacol Sci. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

Trends Pharmacol Sci. 2019 June ; 40(6): 430–445. doi:10.1016/j.tips.2019.04.006.

Deciphering the Chemical Lexicon of Host-Gut Microbiota Interactions

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Abstract

The human intestine harbors an immense, diverse, and critical population of bacteria that has effects on numerous aspects of host physiology, immunity, and disease. Emerging evidence suggests that many of the interactions between the host and the gut microbiota are mediated via the microbial metabolome, or the collection of small-molecule metabolites produced by intestinal bacteria. This review summarizes findings from recent work by focusing on different classes of metabolites produced by the gut microbiota and their effects in modulating host health and disease. These metabolites ultimately serve as a form of communication between the gut microbiome and the host, and a better understanding of this chemical language could potentially lead to novel strategies for treating a wide variety of human disorders.

Gut Microbial Metabolites Mediate Host-Microbiota Interactions

Next-generation sequencing (see Glossary) technologies have catalyzed an expansion of **gut microbiome**-related research over the past decade. As a consequence, these studies have provided great insight into the composition of our microbiome and how it correlates with different health outcomes for the host. These observations, coupled with large phenotypic differences between **germ-free** and **conventionally raised mice** in models of various diseases, including metabolic syndrome (MBS), cancer, and inflammatory bowel disease (IBD), suggest that the microbiota participate in regulating many different aspects of host physiology [1,2]. There is also substantial evidence that suggests that the gut microbiome is critical to the proper development and regulation of our intestinal immune system [3]. Microbial metabolites serve as signals from the gut microbiome that can activate or inhibit endogenous signaling pathways or act as nutrient sources for host cells [1]. These chemical messengers ultimately modulate the intestinal microenvironment to be tolerant or intolerant to specific **commensal microbes**. Together, these findings highlight the importance of understanding how the gut microbiota exert these effects on the host. This review outlines

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the recent work carried out in the past several years that contribute to our understanding of how the gut microbiota communicates with us through their production of small-molecule metabolites and how these host-microbe interactions affect a wide range of diseases (Figure 1, Key Figure).

Short-Chain Fatty Acids (SCFAs) Have Pleiotropic Effects on Host Health and Disease

SCFAs, including acetate, propionate, butyrate, and pentanoate, result from bacterial fermentation of dietary fiber and are among the most abundant microbial metabolites present in the intestinal lumen. SCFAs facilitate host-microbiota communication through several mechanisms:(i) they are readily used as carbon sources for the generation of host endogenous metabolites;(ii) they serve as signaling molecules that activate host G-protein-coupled receptors (GPCRs); and (iii) they affect the expression of host genes through inhibition of histone deacetylases (HDACs) [1–3]. The following section synthesizes recent studies describing the roles of SCFAs in the host-gut microbiota axis.

SCFAs Exhibit Cell Type-Specific Effects on the Host Immune System

Tolerance of commensal microorganisms relies on a delicate balance of pro- and anti-inflammatory signals, which are regulated by various immune cell types (for a brief orientation on immune cell types referred to in this review, see Table 1). $Foxp3^+CD4^+$ regulatory T cells (T_{regs}) are critical in the downregulation of inflammatory responses in the gut and their differentiation is increased by bacterial SCFAs (Figure 2A), which are mainly produced by *Clostridia* [4–9]. Atarashi *et al*. found that a consortium of 17 different *Clostridial* strains was capable of inducing $Foxp3^+CD4^+$ T_{reg} differentiation [9]. Propionate and butyrate produced from *Bacteroides thetaiotaomicron* were found to increase the differentiation of peripheral, but not thymic, $Foxp3^+CD4^+$ T_{regs} via HDAC inhibition by causing increased acetylation at the conserved noncoding sequence 1 enhancer in the *Foxp3* promoter [5,6]. Acetate and propionate cause the proliferation of colonic $Foxp3^+CD4^+$ T_{regs} through GPR43 signaling, which is much more highly expressed in colonic T cells [4]. SCFA-mediated increases in colonic $Foxp3^+CD4^+$ T_{regs} are observed in both the steady state and during infection; however, there is also evidence of acetate increasing $T_{effector}$ cell populations (T helper cells that mediate adaptive immune responses) during *Citrobacter rodentium* infections [7]. Butyrate promotes $Foxp3^+CD4^+$ T_{reg} differentiation by activation of GPR109A on intestinal macrophages and dendritic cells (DCs), which express interleukin (IL)-10 [8]. Butyrate is also capable of downregulating proinflammatory mediators through the inhibition of HDACs in intestinal macrophages and DCs, which support its role in maintaining a commensal-tolerant environment (Figure 2A) [5,10].

SCFAs, including pentanoate, also contribute to microbial tolerance by elevating glucose oxidation, which increases regulatory B cells [11]. Pentanoate also suppresses Th17 cell responses by inhibiting HDACs [11]. Alternatively, SCFAs were found to mediate proinflammatory effects by up-regulating B cell metabolism, which increases the systemic production of IgG and IgA to regulate both homeostatic and pathogen-specific immune responses [12]. Collectively, these results suggest that SCFAs can contribute to an anti-

inflammatory environment in the gut while simultaneously bolstering host defense against **pathobionts** and pathogens.

SCFAs Ameliorate Autoimmune Diseases and Allergy

The ability of SCFAs to promote anti-inflammatory responses by increasing $Foxp3^+CD4^+$ T_{reg} differentiation ameliorates autoimmune diseases, such as type 1 diabetes (T1D), in which immune homeostasis is disrupted (Figure 1 and Table 2). Nonobese diabetic (NOD) mice, which model T1D, fed acylated starch that is fermented to acetate and butyrate showed expanded *Bacteroides* in the intestine and decreased disease severity via GPR43 activation by acetate and $Foxp3^+CD4^+$ T_{reg} induction by butyrate [13]. Miani *et al.* also demonstrated that butyrate ameliorates T1D in NOD mice via induction of IL-22 in pancreatic innate lymphoid cells (ILCs), which causes increased expression of β-defensin 14, an antimicrobial peptide that induces pancreatic regulatory macrophages and $Foxp3^+CD4^+$ T_{regs} via stimulation of Toll-like receptor 2 (TLR-2) on IL-4-secreting B cells [14].

Regulation of the $Foxp3^+CD4^+$ $T_{reg}/T_{effector}$, cell axis by SCFAs also affects experimental autoimmune encephalomyelitis (EAE) and allergic airway disease (AAD) in mice (Figure 1 and Table 2). SCFAs mitigate EAE by promoting the differentiation of $Foxp3^+CD4^+$ T_{regs} over Th1/Th17 cells via suppression of the c-Jun N-terminal kinase, JNK1, and p38 signaling pathways (Figure 2A) [15]. These metabolites also alleviate AAD through several mechanisms. Propionate treatment causes a GPR41-dependent expansion of macrophage and DC precursors in the lung that have reduced ability to induce Th2 cells [16]. The GPR41 - dependent expansion of these cells and an increase in lung $Foxp3^+CD4^+$ T_{regs} were also observed with increased levels of SCFAs resulting from intestinal helminth infection [17]. Acetate also decreased asthma severity by upregulating $Foxp3^+CD4^+$ T_{reg} populations via HDAC9 inhibition, which increases expression of Foxp3 [18]. Butyrate and acetate also suppressed immune responses to oral antigens to protect against food allergy via activation of GPR43 and GPR109a in intestinal epithelial cells (IECs) and $CD103^+$ DCs, respectively [19] (Table 2).

SCFAs Regulate Intestinal Barrier Function and Protect against IBD

Maintenance of the gut epithelial barrier is critical to immune homeostasis because exposure of the intestinal immune system to colonic contents leads to inflammation. SCFAs increase host defense by enhancing the barrier integrity of the gut epithelium via HDAC inhibition and GPCR activation [20,21]. *Clostridia*-derived butyrate increases IEC proliferation and apical junctional protein expression, which maintain the epithelial barrier, via HDAC inhibition, and as a consequence mitigates graft-versus-host disease (GVHD) [20]. SCFAs also ameliorate colitis in mouse models by enhancing gut barrier function via GPR43/109A-mediated NOD-like receptor protein 3 (NLRP3) activation (Figure 3), while the NLRP1 **inflammasome** serves as a negative regulator for SCFA-producing commensals [21,22]. It is important to note here that acetate, propionate, and butyrate activate GPR41/43, while only butyrate is known to activate GPR109A [23,24]. Butyrate also increases gut barrier integrity by stabilizing hypoxia inducible factor (HIF), an important transcription factor that maintains tissue barrier function [25]. Interestingly, butyrate inhibits the proliferation of

intestinal epithelial stem cells, which seems counter to maintaining gut barrier integrity, but the colonic crypt prevents these cells from accessing luminal butyrate [26].

SCFAs Can Exacerbate or Alleviate Symptoms of MBS

The incidence of MBS, which is characterized by obesity, reduced high-density lipoprotein (HDL) cholesterol, increased blood pressure and triglycerides, heart disease, and type 2 diabetes (T2D), is a growing crisis in many industrialized nations [1]. HDAC inhibition and GPR41/43 activation in adipose and pancreatic tissues provide a mechanism by which SCFAs attenuate pathology in this disease (Figure 1) [27–34]. SCFAs target GPR43 in white adipose tissue to decrease insulin sensitivity and fat accumulation in high-fat diet (HFD)-fed mice [27]. Adipose peroxisome proliferator-activated receptor gamma (PPAR-γ) downregulation by SCFAs attenuates HFD induced obesity and insulin resistance, while hepatic PPAR-γ downregulation limits dysregulated fat accumulation in the liver [28]. These studies describe the targeting of differentially expressed GPR43 and PPAR-γ in the liver and adipose tissue to decrease insulin resistance and fat accumulation in MBS [27,28]. Interestingly, GPR43 promotes inflammatory signals in adipose tissue by stimulating tumor necrosis factor alpha (TNF-α) expression in anti-inflammatory M2 macrophages, but this effect may be due to TNF-α suppressing fat accumulation by regulating insulin signaling in adipose tissues [27,34]. Increased acetate and butyrate levels also result in increased anorectic hormones, such as glucagon-like peptide-1 (GLP-1) and fasting peptide YY (PYY), a peptide hormone that reduces appetite, and alleviates T2D in humans [29]. Elevated SCFA levels from fiber-rich diets cause up-regulation of **intestinal gluconeogenesis (IGN)**, which decreases the glucose and energy dysmetabolism observed in MBS [30]. Butyrate serves as an energy substrate for enterocytes to increase ATP levels and, in turn, cAMP to directly upregulate IGN gene expression, while propionate activates GPR41 in the portal vein to stimulate IGN as a result of a gut-brain signaling [30]. Colonization by *Roseburia intestinalis* in the intestine increases butyrate levels to attenuate atherosclerosis by stimulating fatty acid oxidation in enterocytes and reducing **endotoxemia** [31].

In addition to regulating how the body stores and metabolizes fat, SCFAs modify behavior by regulating appetite. Acute acetate administration to HFD-fed mice was shown to decrease food in-take and induce an anorectic phenotype in the hypothalamus, potentially through the downregulation of 5′-AMP-activated kinase AMPK [32]. Alternatively, increased acetate was shown to drive MBS through upregulation of glucose-stimulated insulin secretion, hunger, insulin resistance, and hypertriglyceridemia through the parasympathetic nervous system [33].

SCFAs Regulate the Gut-Brain Axis

In addition to modulating feeding behavior, SCFAs facilitate additional crosstalk between the brain and the intestine. SCFAs are sufficient to increase α-synuclein-related inflammation in Parkinson’s disease models, and gut microbiota transfer from Parkinsonian human subjects to healthy mice replicates motor impairments observed in this neurological disease [35]. Systemic SCFAs are capable of decreasing the blood-brain barrier’s permeability through upregulation of the tight junction protein occludin, demonstrating that

the barrier integrity-promoting effects of SCFAs are not limited to the intestinal epithelial barrier [36]. Treating **gnotobiotic mice** with SCFAs also reverses global microglial maturation defects observed in germ-free mice, indicating that SCFAs regulate immune cells in the central nervous system (CNS) under homeostatic conditions [37].

Butyrate Regulates Tumorigenesis and Cancer Progression

Chronic inflammation in the gut has been associated with increased intestinal tumorigenesis [2]. As summarized above, SCFAs have anti-inflammatory effects in the gut, thereby decreasing the potential for tumor formation. The majority of the studies investigating the roles of SCFAs in intestinal cancer have been largely focused on butyrate. A consensus on butyrate's role in intestinal carcinogenesis has yet to be reached due to different models demonstrating that butyrate is capable of either suppressing or potentiating colorectal cancer [8,38,39]. Butyrate activates GPR109A in both enterocytes and immune cells, which protects against inflammation and carcinogenesis by upregulating the differentiation of IL-10-producing $Foxp3^{+}CD4^{+}$ T_{regs} and stimulating IL-18 production in $APC^{Min/+}$ mice, which lack the tumor suppressor APC and spontaneously develop intestinal adenomas [8]. In addition, depletion of the gut microbiome with antibiotics followed by treatment with GPR109A agonists exhibited reduced polyp formation in $APC^{Min/+}$ mice but not in $GPR109a^{-/-}$ $APC^{Min/+}$ [8]. By contrast, butyrate was shown to drive polyp formation and the transformation of enterocytes in an $APC^{Min/+}$ $MSH2^{-/-}$ model, which lacks the DNA mismatch repair protein MSH2 and is prone to tumor formation, at lower concentrations but had no effect at higher concentrations associated with HDAC inhibition, demonstrating that lower concentrations of butyrate can drive the proliferation of $APC^{Min/+}$ $MSH2^{-/-}$ enterocytes [38]. Butyrate was also shown to attenuate tumor progression in HFD-induced intestinal carcinogenesis in dysbiotic $K\text{-}ras^{G12Dint}$ mice by increasing DC recruitment and reversing the downregulation of barrier-promoting Muc2 and genes involved in antigen recognition [39]. In the liver, the incorporation of soluble fiber into the diets of TLR-5-deficient mice led to hepatocellular carcinoma (HCC) [40]. In this study, butyrate supplementation alone increased HCC markers but was insufficient for tumorigenesis, suggesting the need for additional tumor-promoting factors [40].

SCFAs Modulate Colonization Resistance against Intestinal Pathogens

Colonization resistance via microbiota-related factors helps to prevent harmful enteric pathogens from colonizing and expanding in the gut. Gut microbiota metabolites can mediate colonization resistance (Figure 1) by directly inhibiting gut pathogens or by indirectly modulating the host environment to make the intestinal landscape less susceptible to infection [3]. Multiple studies have demonstrated that depletion of SCFA-producing commensals drives expansion of *Salmonella typhimurium*, an enteric pathogen [41,42]. Baumler and coworkers demonstrated that butyrate prevents aerobic expansion of this pathogen by maintaining hypoxia in the gut epithelium that limits *S. typhimurium*'s access to oxygen [41]. They also demonstrated that oxygen limitation at the epithelium was mediated by butyrate stimulation of PPAR-γ in IECs [42]. Propionate has also been shown to protect against *Salmonella* infection by the disruption of intracellular pH buffering and destabilization of the *S. typhimurium* invasion virulence factor HilD, providing an example of the direct effects that SCFAs have on this pathogen [43,44].

In addition to *S. typhimurium*, butyrate was found to potentiate colonization resistance against *C. rodentium* and *Staphylococcus aureus* by promoting antimicrobial activity in intestinal macrophages through the inhibition of HDAC3 [45]. Modulation of the gut barrier also extends to the mucosal layer because dietary fiber depletion promotes the expansion of mucus-degrading bacteria, resulting in enhanced virulence of pathogens such as *C. rodentium* [46].

In summary, SCFAs are capable of enhancing host health by decreasing inflammation, ameliorating autoimmune diseases and allergy, maintaining the gut barrier, and mediating colonization resistance to enteric pathogens. SCFAs modulate these diseases and physiological processes in different tissues because of their ability to disseminate into the bloodstream, where they can access GPCRs in many tissues or inhibit HDAC activity in various cell types. The ability of SCFAs to modulate MBS and tumorigenesis appear to be tissue and context dependent.

Gut Microbial Tryptophan (TRP) Metabolism Regulates Host Physiology and Immunity

Gut microbial catabolism of TRP produces indole-containing metabolites that regulate the host immune system by activating the aryl hydrocarbon receptor (AHR), a ligand-gated transcription factor that regulates immunity. Stimulation of AHR by TRP metabolites largely upregulates anti-inflammatory responses and helps to maintain host-gut microbiota homeostasis.

Tryptophan Metabolites Mitigate Inflammation and Autoimmune Disease

Through AHR agonist activity, indole-3-lactic acid produced by *Lactobacillus reuteri* was shown to differentiate $CD4^+$ intraepithelial lymphocytes (IELs) into CD4/CD8 double-positive IELs, which are regulatory cells known to prevent intestinal inflammation (Figure 2B) [47]. Indole-3-acetic acid (IAA) and tryptamine (TRA) were found to reduce macrophage production of proinflammatory mediators, such as TNF-α, IL-1 β, and monocyte chemoattractant protein 1 (MCP-1), and indole-3-aldehyde (I3A) produced by *L. reuteri* increases the expression of IL-22 by ILCs (Figure 2B) [48,49]. Increased expression of IL-22 by ILCs due to I3A leads to the expansion of pancreatic regulatory macrophages and T cells that provides protection against T1D, similar to SCFAs [14]. Gut microbiota-derived I3A, indole-3-propionic acid (IPA), and indoxyl-3-sulfate (I3S) also regulate T cells and DCs in EAE mouse models via AHR (Table 3) and have been shown to suppress inflammation in the CNS mediated by inhibition of NF-κB in astrocytes (Figure 1) [50].

Microbially Produced TRP Metabolites Enhance Gut Barrier Function

TRP metabolites also increase intestinal barrier function, as indicated by studies describing the effects of these metabolites in ameliorating mouse colitis models. *Card9* is a risk allele of IBD and mice deficient in this gene exhibit increased susceptibility to dextran sodium sulfate (DSS) colitis and gut microbiota with impaired TRP metabolism [51]. This phenotype can be transferred to germ-free mice by transplantation of $Card9^{-/-}$ microbiota and subsequently rescued via colonization with indole metabolite-producing lactobacilli or treatment with

AHR agonists [51]. AHR expression in TRP diet-fed mice strongly correlates with the expression of IL-22 and Foxp3 as well, highlighting how multiple classes of microbial metabolites promote commensal tolerance through parallel mechanisms [13,14].

Independent of AHR, IPA was shown to decrease intestinal permeability and inflammation through a pregnane X receptor (PXR)- and TLR-4-mediated pathway [52] (Figure 3). Its levels can be increased through colonization by gut microbiota, including *Clostridium sporogenes*, which is known to produce this metabolite [53]. Indole is capable of increasing intestinal barrier function by increasing the expression of apical junction proteins (Figure 3) that mediate gut epithelial permeability, which improves pathology associated with DSS colitis [54].

Gut Microbial TRP Metabolites Protect against MBS

Gut microbial TRP metabolites largely show beneficial effects in MBS due to the involvement of AHR in the regulation of anorectic hormone secretion and glucose and insulin-regulated metabolism [55, 56]. Indole has been shown to regulate the release of the anorectic hormone GLP-1, a potential target for treating MBS (Table 3) [55]. Brief exposure to physiological levels of indole in colonic enteroendocrine L cells causes increased release of GLP-1, whereas prolonged exposure suppresses its secretion [55]. AHR activation was shown to decrease fasting glucose levels, improve glucose and insulin dysmetabolism, and increase GLP-1 secretion [56]. Similar to SCFAs, TRP metabolites such as IAA cause a reduction in cytokine-induced lipogenesis in hepatocytes, highlighting how gut microbial metabolites target lipid metabolism to attenuate MBS [48].

TRP Metabolites from the Gut Microbiota Modify Host Neurotransmitter Pools

TRP is actively metabolized by the gut microbiota into neurotransmitters that affect host serotonergic activity [57,58]. Serotonin is also a product of TRP metabolism by the gut microbiota, which has been demonstrated to significantly effect host biosynthesis and levels of this important neurotransmitter (Table 3) [57]. In addition, the neurotransmitter TRA is produced by *Clostridial* species in the gut via TRP catabolism, and it is hypothesized that sequestration of TRP reduces its bioavailability to the host, thus altering behavior by reducing serotonin biosynthesis [58]. TRA has also been demonstrated to activate the GPCR serotonin receptor-4, which in turn drives fluid secretion in the intestines to accelerate intestinal transit [59].

To summarize, microbial catabolism of TRP produces many metabolites that can serve as ligands for AHR. These metabolites have generally been shown to promote anti-inflammatory signals, maintain the gut barrier, and ameliorate MBS. Some of these metabolites also serve as neurotransmitters, such as serotonin and TRA, which regulate the gut-brain axis.

Regulation of Host Responses by Secondary Bile Acids

Primary bile acids are produced by the host in the liver to solubilize dietary lipids and fat-soluble vitamins in the small intestine. The primary bile acid pool is largely recycled back to the liver, but a small proportion of these bile acids escapes to the large intestine where they

are readily deconjugated and further metabolized by the microbiota into secondary bile acids, which have numerous effects on the host. Below we review these effects briefly.

Gut Barrier Integrity

The gut microbiota influences barrier integrity in the intestine by activation of inflammasomes to increase IL-18 levels [60]. Deconjugation of taurine-conjugated primary bile acids by the gut microbiota increases the host taurine pool, which in turn causes activation of the NLRP6 inflammasome, increasing IL-18 secretion and inflammatory responses (Figure 3) [60]. In addition, deoxycholic acid (DCA), a secondary bile acid, was found to positively regulate intestinal crypt regeneration and repair through farnesoid X receptor-mediated downregulation of elevated wound prostaglandin E2 levels, which is critical to maintaining barrier function [61].

Cancer and Colonization Resistance

HFD-induced obesity was found to drive HCC through increased levels of DCA produced by *Clostridia* and antibiotic depletion of the microbiota helped to prevent tumor formation [62]. Secondary bile acids produced by *Clostridium scindens* also promote tumorigenesis in the liver through negative regulation of CXCL16, causing a decrease in tumor-suppressing $CXCR6^+$ natural killer T cells (NKTs) [63]. Altered secondary bile acid profiles in dysbiotic microbiomes may also drive liver cancer through cooperative effects with SCFAs [40].

Secondary bile acids known for their carcinogenic effects exhibit beneficial roles in colonization resistance because DCA and lithocholic acid (LCA) inhibit the growth of *Clostridium difficile* [64] (Figure 1 and Table 3). Reconstitution of antibiotic-treated mice with *C. scindens* or a consortium of bacterial strains capable of producing secondary bile acids, including DCA and LCA, was shown to restore colonization resistance against this pathogen [64]. Furthermore, DCA and LCA inhibit the growth of *C. difficile* by enhancing the activity of TRP-derived antibiotics produced by the known DCA and LCA producers *C. scindens* and *Clostridium sordellii* [65].

Secondary bile acids are known to drive liver cancer yet also maintain the gut barrier and prevent the colonization of enteric pathogens. The beneficial and deleterious effects of secondary bile acids on the host underscore the need to understand how bacterial metabolites regulate infection and inflammatory diseases on a holistic level. Thus, further studies with various models and the effects of these metabolites with additional metabolites are needed.

Additional Microbial Metabolites That Modulate Host Health and Immune Responses

Membrane Polysaccharide A and Sphingolipids Regulate T Cells

Capsular and membrane components of gut commensals also play a role in maintaining intestinal immune homeostasis. Polysaccharide A (PSA) is a zwitterionic polysaccharide present in the capsule of the gut commensal *Bacteroides fragilis* and has been known for its immunomodulatory role in inducing IL-10 production in $Foxp3^+CD4^+$ T_{reg} cells (Figure 2C). Robust IL-10 production in T cells requires both plasmacytoid DC (PDC) presentation

of PSA fragments via MHC-II and PSA activation of TLR-2 on PDCs [66]. Recognition of the PSA-MHC-II complex by T cells was found to induce clonal expansion of $CD4^{+}CD45RB^{low}$ effector/memory cells, causing an anti-inflammatory response due to a decrease in interferon gamma (IFNγ) and an increase in IL-10 production [67]. In addition, membrane glycosphingolipids from *B. fragilis* negatively regulate in-variant NKTs (iNKTs) by inhibiting developmental iNKT proliferation and activation to attenuate proinflammatory responses and ameliorate colitis (Figure 2C) [68].

Commensal ATP Levels Mediate Anti-inflammatory Responses

Extracellular ATP in the intestinal lumen is a result of dying host cells, host export, and microbially produced ATP. In the steady state, host ectonucleoside triphosphate diphosphohydrolases (ENTPDases) hydrolyze ATP to control luminal levels, and ENTPDase-knockout mice exhibit increased differentiation of Th17 cells (Figure 2D) in the intestine and more severe inflammatory disease [69]. Commensal-derived ATP also reduced the activity of T follicular helper cells, resulting in diminished microbe-specific IgA secretion by B cells and an increase in commensal outgrowth (Figure 2D) [70].

Gut Microbial Choline Metabolism Negatively Affects Cardiovascular Health

Gut microbial metabolism of choline, phosphatidylcholine, and L-carnitine produces trimethylamine, which is oxidized by the host liver into trimethylamine *N*-oxide (TMAO). Gut microbe-dependent TMAO levels have been correlated with increased risk of cardiovascular disease (Figure 1 and Table 3) and depend on both L-carnitine and choline metabolism [71]. TMAO was also observed to increase platelet aggregation and adhesion to collagen *in vitro* and to potentiate thrombosis *in vivo* [72]. These effects could be replicated by increasing dietary choline and were significantly influenced by gut microbiota composition [72]. Gut microbial L-carnitine metabolism is not limited to cardiovascular health and also correlates with insulin resistance [73]. Effects of bacterial choline catabolism have also been shown to result in the production of TMAO and induction of a choline-deficient state, which in turn aggravates metabolic disorders through regulating DNA methylation [31].

Other Metabolites That Regulate Colonization Resistance in the Intestine

Pyruvate and Lactate—Pyruvate is generated from bacterial fermentation of dietary fiber and further reduced to produce lactate. Bacterial pyruvate and lactate induce small-intestinal CX3CR1 + mononuclear cells to extend dendrites into the intestinal lumen to capture luminal antigens and promote antigen-specific immune responses, which provides resistance to *Salmonella* infection [74].

Succinate—While some metabolites, such as secondary bile acids, may impede *C. difficile* infection, others can promote the expansion of this pathogen. One example of such a metabolite is succinate. Antibiotic treatment to cause **dysbiosis** increases local intestinal succinate levels that *C. difficile* utilizes as a metabolic source and converts into butyrate [75], causing growth of the bacterium. Increased succinate levels caused by *B. thetaiotaomicron* also promote enterohemorrhagic *Escherichia coli* strain O157:H7

pathogenesis through upregulation of the transcription factor Cra, which positively regulates virulence-associated genes [76].

Other Metabolites That Help in Maintaining Gut Barrier Function

Lactate—Microbially produced lactate is also beneficial to the maintenance of the gut barrier because it promotes the differentiation of intestinal stem cells through a GPR81-dependent mechanism [77].

Spermine and Histamine—These metabolites are produced by both the host and the gut microbiota and reduce gut epithelial barrier integrity through inhibition of the NLRP6 inflammasome, which decreases IL-18 levels [60] (Figure 3).

Concluding Remarks and Future Perspectives

Identifying the molecular mechanisms of how the gut microbiota regulates host physiology is critical to understanding the roles of these microbes in our bodies. Microbially derived metabolites produced by the gut microbiota serve as chemical messengers that mediate crosstalk between the microbes and host and can play both beneficial and deleterious roles in human health. The effects of these metabolites have been shown to influence the outcomes of many disorders, including MBS, IBD, cancer, autoimmune diseases, allergy, and neurodegenerative diseases. The gut **microbial metabolome** has also been shown to modulate colonization resistance to enteric pathogens.

A comprehensive understanding of all gut microbially produced small-molecule metabolites, their molecular targets, and their biological significance remains an important objective in the field (see Outstanding Questions). As a result, the development of new approaches for deciphering this host-gut microbiota crosstalk at the systems level are at the forefront of the field. Combining metagenomic analysis and serum metabolomic profiling has allowed bacteria capable of reductive metabolism of aromatic amino acids such as histidine, valine, leucine, and isoleucine to be correlated with improvements in insulin sensitivity and obesity [73,78–80]. Similarly, comparisons of gnotobiotic mice colonized with commensals either proficient or deficient in reductive aromatic amino acid metabolism translate to significant changes in circulating IPA levels that improve gut barrier function [53]. The effect of circulating metabolites on the host also extends to the gut-brain axis, as 4-ethylphenyl sulfate can induce autism spectrum disorder behaviors [81]. Further, use of experimental models comparing healthy and diseased fecal metabolomes have demonstrated which metabolite classes are important for IBD prevention and how their production may be impaired in individuals suffering from this disease [82]. For example, ascorbate was recently identified as a bioactive microbial metabolite associated with Crohn’s disease and was validated to exhibit suppressive effects on activated effector $CD4^+$ T cells by targeting T cell metabolism [83]. Alternatively, extrathymic $Foxp3^+CD4^+$ T_{reg} deficiency leads to increased type 2 responses against commensals and disruption of niche establishment for border-dwelling bacteria during colonization that correlated with systemic changes in lipid and amino acid metabolism in the fecal and serum metabolomes [84].

Recently, Macpherson and coworkers used isotopic labeling of metabolic precursors to label bacterially produced metabolites in the gut [85,86]. These approaches use stable isotope tracing of nonreplicating ^{13}C-labeled *E. coli* HA107 in gnotobiotic mice with high-resolution mass spectrometry, which can differentiate the ^{12}C host metabolome to identify the distribution of microbially produced metabolites in different host tissues [86]. A similar approach using ^{13}C-labeled glucose determined that maternal antibodies facilitate the transfer of certain gut microbiota-derived metabolites to the offspring [85].

Alternatively, new approaches have been pioneered to understand the effects of microbial metabolites on the host. Instead of traditional metabolomic approaches, metagenomic data can be bioinformatically mined for operons capable of biosynthesizing certain metabolites [87]. In addition, novel computational methods to analyze existing metagenomic data have enabled the identification of associations between metabolite levels and disease outcomes [88]. Ultimately, these systems-level approaches and novel methods will provide a deeper understanding of host-gut microbiota crosstalk that is mediated by microbial metabolites. Future studies will include the discovery of new microbial metabolites, the identification of bacterial species responsible for metabolite production, and understanding of the individual and systemic effects of these metabolites on host health and disease.

Acknowledgments

We sincerely apologize to those authors whose research we could not include due to space limitations. G.R.N. was supported by a National Institutes of Health Chemistry Biology Interface Training Program (T32GM008500). This work was supported by an Arnold and Mabel Beckman Young Investigator Award and the President's Council of Cornell Women Affinito-Stewart Grant (both to P.V.C.).

Glossary

Commensal microbes
microorganisms that cohabitate with the host within tissues and are generally thought to not cause harm to the host.

Conventionally raised mice
mice that harbor a diverse and largely undefined microbiome.

Dysbiosis
microbial imbalance or perturbation that is thought to cause host maladaptation.

Endotoxemia
presence of endotoxin in the bloodstream, which may case hemorrhages, kidney necrosis, or toxic shock.

Germ-free mice
mice that are raised in a sterile environment and are devoid of any microorganisms.

Gnotobiotic mice
germ-free mice that have been colonized with a defined microbiota.

Gut microbiome

trillions of microorganisms that reside in the intestinal lumen, including bacteria, viruses, fungi, parasites, and archaea.

Hepatic steatosis
accumulation of fat in the liver.

Inflammasomes
multiprotein oligomers that are responsible for the activation of inflammatory responses.

Intestinal gluconeogenesis (IGN)
metabolic pathway in the gut that results in glucose production from noncarbohydrate carbon sources.

Microbial metabolome
collection of small-molecule metabolites that are produced or modified by the gut microbiota.

Next-generation sequencing
high-throughput DNA sequencing that is processed massively in parallel.

Pathobiont
commensal microbes that have the potential to lead to disease under certain host physiological states.

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Highlights

Microbially produced metabolites serve as chemical signals between the gut microbiota and the host and regulate many tissues throughout the body, thereby influencing host physiology.

Gut microbiota metabolites modulate host immune responses and inflammation, thereby influencing host health and disease.

Disorders affected by gut microbial metabolites include metabolic syndrome, inflammatory bowel diseases, cancer, allergy, autoimmune diseases, and neurodegenerative diseases.

The gut microbial metabolome can modulate colonization resistance against intestinal infections due to direct inhibition of enteric pathogens or by improving host defense mechanisms.

Identifying the molecular mechanisms that influence these outcomes is critical to understanding the impact of the gut microbiome and their metabolites on the host.

Understanding the individual and systemic effects of these metabolites is important for deciphering the chemical lexicon of the gut microbiota.

Outstanding Questions

Which microorganisms in the gut microbiome produce the metabolites that have effects on the host?

How do host factors such as diet, age, gender, environment, and mental health affect gut microbiome composition and, in turn, the production of microbially derived metabolites?

Of the estimated thousands of unknown microbial metabolites in the gut, which of these metabolites are biologically active, what are their chemical structures, and what effects do they have on host pathways?

How are the biologically active microbial metabolites biosynthesized by the gut microbiota?

Which enzymes are involved in and which metabolites are produced by the sequential action of biosynthetic pathways in different microbes?

What is the interplay between the multiple metabolites produced by different microbes and can we establish a better understanding of the overall effect of these metabolites on host health?

What strategies can be developed to modify the metabolome or manipulate target pathways to treat inflammatory diseases?

Key Figure

Gut Microbial Metabolites Influence Many Aspects of Host Health and Disease

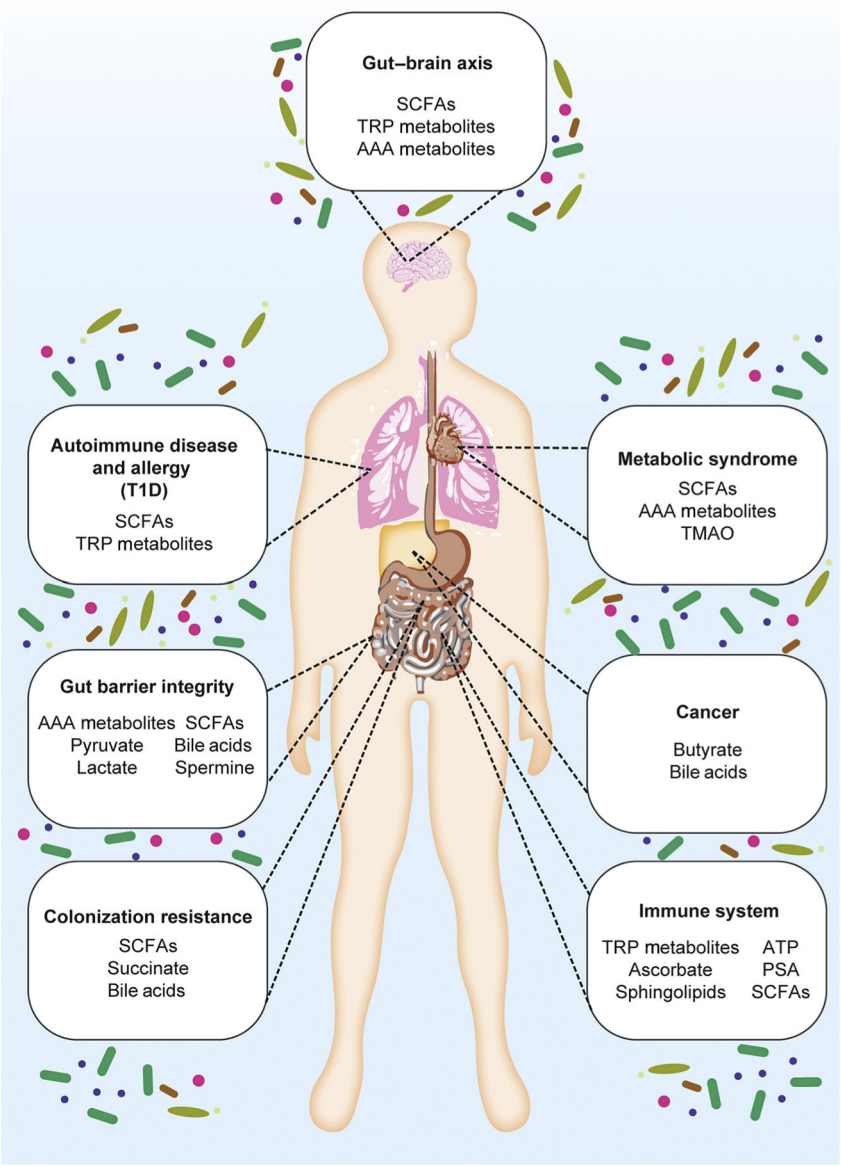

Figure 1.

Small-molecule metabolites that are produced by the gut microbiota can modulate myriad physiological processes in the host, thereby impacting disease outcomes in many inflammatory disorders, including metabolic syndrome, cancer, autoimmune diseases including type 1 diabetes (T1D), allergy, and inflammatory bowel diseases. Furthermore, these metabolites can also influence the gut-brain axis and regulate the immune system and host susceptibility to gastrointestinal infection via colonization resistance. Abbreviations: AAA, aromatic amino acid; PSA, polysaccharide A; SCFAs, short-chain fatty acids; TMAO, trimethylamine N-oxide; TRP, tryptophan.

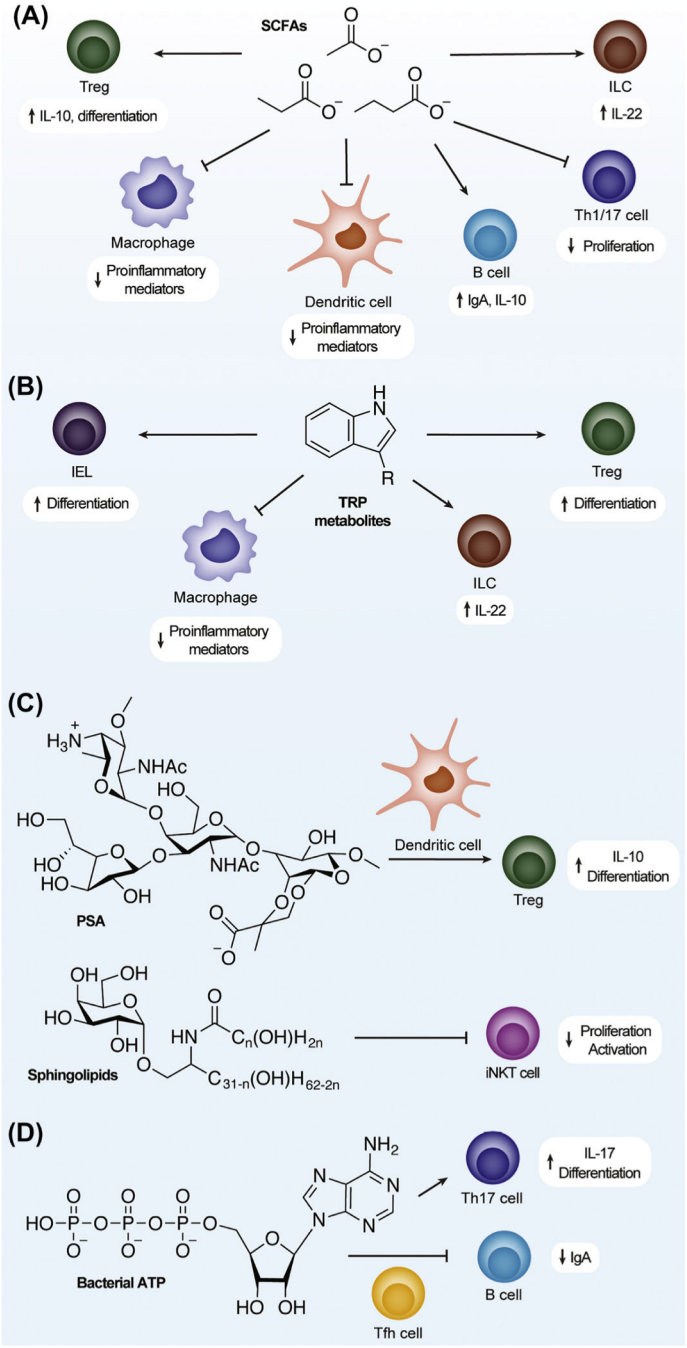

Figure 2. Gut Microbial Metabolites Regulate Specific Immune Cell Types.

Microbial metabolites regulate immune responses in the gut by modulating the activities of different immune cell types as indicated. The schematic shows immune cell types that are affected by metabolites: (A) short-chain fatty acids (SCFAs); (B) tryptophan (TRP); (C) polysaccharide A (PSA) and sphingolipids; and (D) bacterial ATP. Abbreviations: IEL, intraepithelial lymphocyte; IL, interleukin; ILC, innate lymphoid cell; iNKT, invariant natural killer T; Tfh, T follicular helper; Th1/17, T helper 1/17; Treg, T regulatory cell.

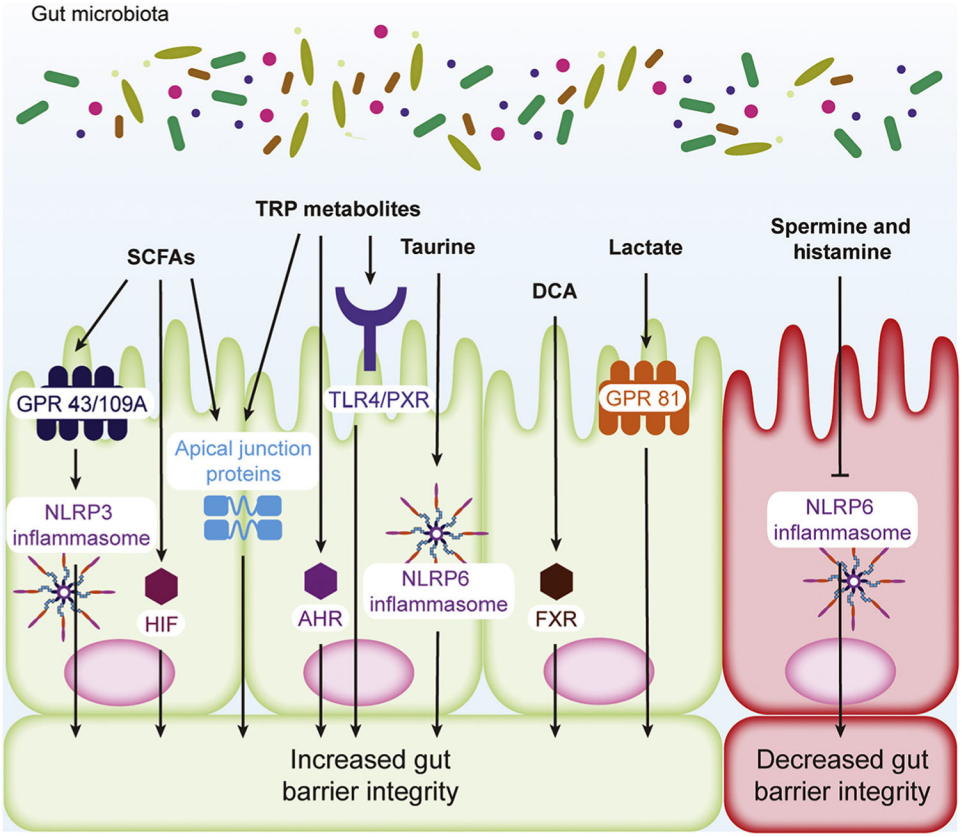

Figure 3. Gut Microbial Metabolites Regulate Intestinal Epithelial Barrier Integrity.

Metabolites produced by the gut microbiota, such as short-chain fatty acids (SCFAs), tryptophan (TRP) metabolites, taurine, deoxycholic acid (DCA), lactate, spermine, and histamine, can modulate the barrier function of the intestinal epithelium by regulating receptor expression and/or activation, transcription factor activation, increasing the expression of cytokines that confer barrier protection, and modulating apical junction proteins, which directly regulate epithelial permeability. Abbreviations: AHR, aryl hydrocarbon receptor; FXR, farnesoid X receptor; GPR, G protein receptor; HIF, hypoxia inducible factor; NLRP, NOD-like receptor protein; PXR, pregnane X receptor; TLR4, Toll-like receptor 4.

Table 1.

A List of the Immune Cell Types, Including Markers and Functions, Mentioned in This Review

Cell type	Subset	Function	Marker
T cell	T_{reg}	Suppressor T cells that downregulate inflammatory responses	Foxp3$^+$, IL-10$^+$, CD4$^+$
	Th17	$T_{effeotor}$ cells that that protect against bacteria and fungi	ROR-γt^+, IL-17$^+$, CD4$^+$
	Th1	$T_{effector}$ cells that protect against intracellular pathogens	T-bet$^+$, IFN-γ^+, CD4$^+$
	Th2	$T_{effector}$ cells that protect against extracellular pathogens	GATA3$^+$, IL-4$^+$, IL-13$^+$, IFN-γ^+, CD4$^+$
	T follicular helper	$T_{effector}$ cells critical to the regulation of germinal centers	Bcl6$^+$, PD1$^+$, ICOS$^+$, CXCR5$^+$, CD4$^+$
Macrophage	Colonic	Tissue-resident innate immune cells that phagocytose and secrete proinflammatory cytokines	CD11b$^+$, CD64$^+$, F4/80$^+$, MHCII$^+$, CXCR1$^+$, CD103$^-$
	Pancreatic, M1	Pancreatic innate immune macrophages with a proinflammatory phenotype	F4/80$^+$, CD11b$^+$, CD11c$^+$, CD206$^-$
	Pancreatic, M2	Pancreatic innate immune macrophages with an anti-inflammatory and regulatory phenotype	F4/80$^+$, CD11b$^+$, CD11c$^-$, CD206$^+$
	Adipose, M1	Adipose innate immune macrophages with a proinflammatory phenotype	CD45$^+$, CD11b$^+$, F4/80$^+$, iNOS$^+$, CD206$^-$
	Adipose, M2	Adipose innate immune macrophages with an anti-inflammatory phenotype	CD45$^+$, CD11b$^+$, F4/80$^+$, iNOS$^-$, CD206$^+$
	Microglia	Tissue-resident macrophages of the central nervous system	CD11b$^+$, CD45^{lo}, CSF1R$^+$(CD115), F4/80$^+$, CD31$^+$
DC	Splenic	Immune cells responsible for the presentation of antigen to T cells	CD11c$^+$
	Colonic, CD103$^+$	Immune cells responsible for the presentation of antigen to T cells that reside in the colon and upregulate T_{reg} differentiation	CD45$^+$, I-Ab$^+$, CD11c$^+$, CD103$^+$
	Mesenteric lymph node (MLN), CD103$^+$	DCs that migrate from the intestines to the MLN to upregulate T_{reg} differentiation and promote oral tolerance to food antigens	MHCII$^+$, CD11c^{hi}, CD103$^+$
B cell	Germinal center	Adaptive immune cells that recognize specific antigens to produce antibodies	CD19$^+$
	Pancreatic, regulatory	Adaptive immune cells that secrete IL-4 to induce regulatory macrophages, which upregulate T_{reg} induction	CD19$^+$, CD11b$^-$, CD5$^+$, CD1d$^+$, B220$^+$, CD21$^+$, CD24$^+$
ILC	Group 3	Regulatory innate immune cells that are critical to intestinal homeostasis via IL-22 secretion	CD45$^+$, CD127$^+$, CD90$^+$, Lin$^-$, IL22$^+$, ROR-γt^+
IEL	CD4$^+$	Intestinal epithelial effector T cells that recognize both autoreactive and foreign antigens	CD45$^+$, CD3$^+$, TCR$\gamma\delta^-$, CD8β^-, CD4$^+$
	CD8$^+$		CD45$^+$, CD3$^+$, TCR$\gamma\delta^-$, CD8β^-, CD8α^+
	CD4$^+$ CD8$\alpha\alpha^+$	Intestinal epithelial tolerogenic effector T cells	CD45$^+$, CD3$^+$, TCR$\gamma\delta^-$, CD8β^-, CD4$^+$, CD8α^+
NKT	CXCR6$^+$ hepatic NKT	Liver effector T cells with tumor suppressing function	TCRβ^+, CD1d$^-$, CXCR6$^+$
	Colonic invariant NKT	Effector T cells with both innate and adaptive functions	CD3+, CD1d+

Table 2.

Effects of SCFAs in the Host

Disease	Metabolite	Effect	Target	Refs
T1D	Acetate	Suppression of autoreactive T cells, increased IL-22	Decreased expression of MHC-I molecules in B cells, GPR43	[13]
	Butyrate	FOXP3$^+$/IL-10$^+$ T_{reg} expansion, increased IL-22		[13]
	Butyrate	Pancreatic regulatory macrophage and T cell expansion, IL-22 induction in pancreatic ILCs		[14]
EAE	Butyrate and propionate	Differentiation to Tregs over Th1/17 cells		[15]
AAD	Propionate	Expansion of DC precursors with an impaired ability to induce Th2 effector cells	GPR41	[16]
	Butyrate, propionate, and acetate	IL-10 and TGF-β^a-mediated T_{reg} suppressor activity	GPR41	[17]
	Acetate	Increases FOXP3$^+$ T_{reg}	Inhibition of HDAC9	[18]
Food allergy	Butyrate and acetate	Upregulate CD103$^+$ tolerogenic DCs, which induce T_{reg} differentiation	GPR109A and GPR43	[19]
GVHD	Butyrate	Protection against reactive T cell damage		[20]
MBS	Acetate	Suppression of insulin-mediated fat accumulation in adipocytes	GPR43	[27]
	Butyrate, propionate, and acetate	Prevent HFD-induced obesity, stimulate insulin sensitivity, and abrogate **hepatic steatosis**	Downregulation of PPAR-γ	[28]
	Acetate and butyrate	Improved glucose metabolism through increased post-prandial insulin, GLP-1, and fasting PYY		[29]
	Butyrate	Stimulation of IGN	cAMP-dependent IGN gene activation	[30]
	Propionate	Stimulation of IGN	GPR41	[30]
	Butyrate	Atherosclerosis prevention and stimulation of fatty acid oxidation		[31]
	Acetate	Appetite suppression	Hypothalamic AMPK inactivation	[32]
		Activation of the parasympathetic nervous system to increase glucose-stimulated insulin secretion, hunger, insulin resistance, and hypertriglyceridemia		[33]
Gut/brain	Butyrate, propionate, and acetate	Increase alpha-synuclein-related inflammation		[35]
	Butyrate	Decrease blood-brain barrier permeability	Occludin upregulation	[36]
	Butyrate, propionate, and acetate	Microglial maturation and IGN stimulation	GPR41/43	[37]
Cancer	Butyrate	Suppression of colonic tumorigenesis	HDAC inhibition and GPR109A	[8]
		Proliferation and transformation of intestinal epithelial stem cells in MSH2$^{-/-}$ mice		[38]
		Attenuation of tumor progression via increased DC recruitment and upregulation of Muc2 and antigen recognition genes		[39]
		Caused hyperbilirubinemia, hepatic inflammation, and upregulation of liver fibrosis and HCC markers in TLR-5 KO mice		[40]

Disease	Metabolite	Effect	Target	Refs
Colonization resistance	Butyrate	Maintains a hypoxic environment in the gut to prevent *Salmonella typhimurium* from accessing oxygen	PPAR-γ	[41,42]
	Propionate	Disrupts pH buffering and destabilizes virulence factors in *S. typhimurium*	HilD	[43,44]
	Butyrate	Promotion of antimicrobial activity against *Citrobacter rodentium* in intestinal macrophages	HDAC3 inhibition	[45]

[a] TGF-β, transforming growth factor beta.

Table 3.

Effects of Additional Microbial Metabolites on the Host

Disease	Metabolite	Effect	Target	Refs
TRP metabolites				
EAE	I3A, IPA, and I3S	Suppress inflammation through regulation of type I IFN signaling in astrocytes	AHR	[50]
MBS	Indole	Regulation of GLP-1 secretion in colonic L cells; acute exposure leads to GLP-1 release while longer exposures inhibit secretion	Short exposure: K^+ channel inhibition Long exposure: NADH dehydrogenase inhibition	[55]
	lAA and TRA	Prevent macrophage proinflammatory cytokine production and migration to MCP-1, abrogation of TNF-α and fatty acid-mediated effects in hepatocytes	AHR	[48]
	Indole, IAA, and TRA	Improve glucose/insulin dysmetabolism, increase GLP-1 secretion, decrease fasting glucose levels, and decrease hepatic steatosis	AHR	[56]
Gut-brain axis	Serotonin	Serotonin level regulation in colonic epithelial cells		[57]
	TRA	Enhancement of serotonergic activity		[58]
		Increased fluid secretion to accelerate intestinal transit	$5\text{-HT}_4\text{R}$[a]	[59]
Bile acids				
Cancer	DCA	Drives HCC, rescued by antibiotic depletion of HFD microbiome		[62]
	LCA	Decrease in tumor suppressing $CXCR6^+$ NKTs via downregulation of CXCL16		[63]
Colonization resistance	DCA and LCA	Reconstitution of antibiotic-treated mice with known DCA and LCA producers is sufficient to provide resistance against *Clostridium difficile*		[64]
		Enhance the activity of TRP-derived antibiotics		[65]
Additional				
MBS	TMAO	Increases cardiovascular risk and platelet aggregation and adhesion to collagen and inhibits reverse cholesterol transport		[71,72]
		Insulin resistance		[73]
		Microbial choline depletion and TMAO accumulation exacerbates metabolic disease through inguinal fat accumulation, increased levels of circulating leptin, triglycerides, and free fatty acids	DNA methylation	[31]
Colonization resistance	Pyruvate and lactate	Induce intestinal $CX3CR1^+$ mononuclear cells to promote antigen-specific immune responses and provide resistance to *Salmonella typhimuhum*		[74]
	Succinate	Nutrient source for the expansion of *C. difficile*		[75]
		Upregulation of virulence-associated genes in *Escherichia coli* O157:H7	Cra	[76]
Gut-brain axis	4EPS[b]	Induces anxious behavior in maternal immune activation mice		[81]

[a] $5\text{-HT}_4\text{R}$, serotonin receptor-4.

[b] 4EPS, 4-ethylphenyl sulfate.