

Gut microbiota-generated metabolites in animal health and disease

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Gut microbiota is found in virtually any metazoan, from invertebrates to vertebrates. It has long been believed that gut microbiota, more specifically, the activity of the microbiome and its metabolic products, directly influence a variety of aspects in metazoan physiology. However, the exact molecular relationship among microbe-derived gut metabolites, host signaling pathways, and host physiology remains to be elucidated. Here we review recent discoveries regarding the molecular links between gut metabolites and host physiology in different invertebrate and vertebrate animal models. We describe the different roles of gut microbiome activity and their metabolites in regulating distinct host physiology and the molecular mechanisms by which gut metabolites cause physiological homeostasis via regulating specific host signaling pathways. Future studies in this direction using different animal models will provide the key concepts to understanding the evolutionarily conserved chemical dialogues between gut microbiota and metazoan cells and also human diseases associated with gut microbiota and metabolites.

Gut microbiota and its metabolites have a pivotal role in the maintenance of homeostasis of metazoan physiology. Mucosal surfaces covered by a layer of gut epithelia represent the most critical interface between the organism and its environment. It is now commonly known that gut cell homeostasis influences diverse elements of host physiology, including development, metabolism and immunity. The relationship between gut and host health and disease is well documented, although detailed molecular mechanisms remain obscure. Over a century ago, the Russian scientist Elie Metchnikoff (1845–1916) conceptualized the role of the gut on host physiology and pathology¹. He suggested that gut homeostasis is key to many components of host physiology, including longevity. He also proposed that gut bacteria are essential modulators influencing homeostasis and that deregulation of gut homeostasis by certain bacteria leads to a diseased state owing to poisoning of the body from bacterial byproducts. Unfortunately, Metchnikoff's hypothesis of relationship between gut metabolites and host health remained dormant for nearly a century¹; his hypothesis has only recently reemerged as one of the most active areas of investigation in modern biology and medicine. Gnotobiotic animal models with genetically tractable 'microbe-host' are now enabling us to see a clearer picture of host genome interactions with the microbiome or metabolites. In this review, we will discuss the most up-to-date discoveries on the evolutionary conserved chemical dialogs between gut microbiomes and metabolites and different metazoans and also on human diseases associated with gut microbiota and metabolites.

Gut metabolites and their roles in invertebrates

Classical invertebrate animal models for developmental biology, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, are now successfully established in the field of gut-microbe interactions^{2–6}. The simplicity of the gut microbiota or microbiome, genetic tractability of host animals and easy generation of gnotobiotic animals (by simply bleaching eggs) has enabled the quick and decisive advancement of our understanding of gut-microbiota interactions compared to vertebrate models. For example, *C. elegans* and *Drosophila* harbor extremely simple gut microbiotas under laboratory conditions (for example, 1 bacterial species in *C. elegans* and 5–20 species

in *Drosophila*), and all of these bacteria can be cultured *in vitro*, enabling the genetic manipulation of microbiomes^{2,3,5,7}. Given that these animals have short life spans and hundreds of individuals can be easily used for different experiments under rigorously controlled diets and microbial conditions, *C. elegans* and *Drosophila* became the models of choice for the genetic analysis of complex phenotypes, such as animal aging and development⁸. Not surprisingly, these animal models have been used to show the biological significance of interkingdom interactions by showing the unexpected roles of the microbiome in metazoan longevity, dysbiosis and development.

Gut metabolites and host longevity. It has long been observed that metazoans live in close relationship with microorganisms. The soil-dwelling worm *C. elegans* eats microorganisms as a part of its diet. Although they consume *E. coli* OP50 strain under laboratory-rearing conditions, it was shown that they prefer to consume soil bacteria, such as *Bacillus mycoides* and *Bacillus soli*⁹. As such nutrition involves metabolically active microorganisms, it can generate different metabolites. Previously, it has been shown that the metabolically active state of microorganisms is important in conferring benefits to host growth and longevity¹⁰. Furthermore, altered *E. coli* metabolism, such as reduction of the bacterial respiration rate, can extend worm longevity¹¹. Interestingly, feeding worms with soil bacteria, as opposed to *E. coli* OP50, was found to extend worm life span⁹. All of these observations indicate that some metabolites generated by specific microbiome activity directly affect host growth and longevity. Recently, it has been shown that bacterial nitric oxide (NO) produced by bacterial NO synthase (NOS) activity enhances host longevity and stress resistance in *C. elegans*² (Fig. 1). *C. elegans*, unlike many other metazoans, is unable to produce NO in a NOS-dependent manner; thus, it relies solely on bacterial NO supply. Further genetic studies elegantly demonstrated that bacterial NO modulates DAF-16 (a homolog of FOXO) and HSF-1 (a master transcription factor controlling the expression of heat shock proteins) that in turn induce aging-related genes capable of enhancing host longevity and the anti-stress response² (Fig. 1). Many intriguing questions remain to be answered. Especially, as many bacteria including human gut microbiota are capable of producing NO, it

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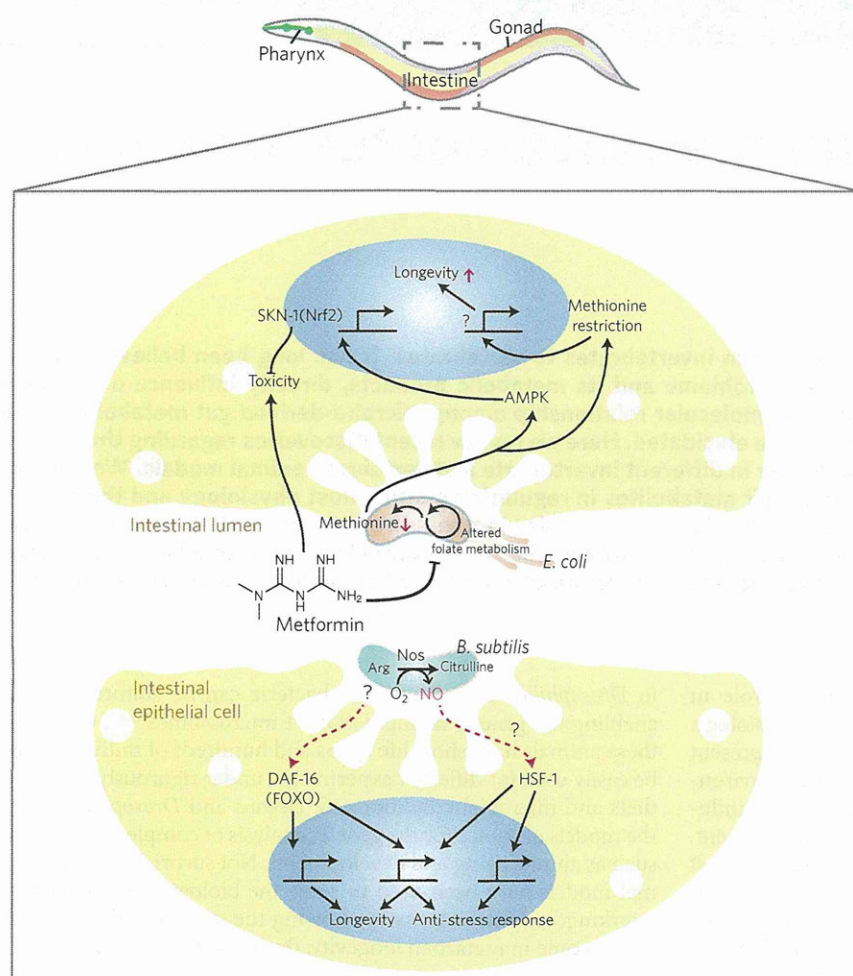


Figure 1 | Gut metabolites and metabolism and host longevity in *C. elegans*. Bacterially derived NO modulates the expression of genes involved in longevity and anti-stress responses via DAF-16 and HSF-1 activity. Metformin administration induces reduction of bacterial methionine production by disrupting the folate cycle. Reduced levels of methionine in the diet in turn induce the dietary restriction effect to promote expression of genes involved in longevity as well as genes involved in drug detoxification in an AMPK- and SKN1-dependent manner.

would be interesting to examine any health-promoting effects of bacterial NO in the human gut.

Given that bacterial metabolic activity can influence the host signaling pathway, researchers are now giving special attention to investigate whether environmental factors, such as xenobiotics or nutrition (that are capable of positively or negatively influencing host fitness), exert their effects by directly or indirectly modulating gut bacterial metabolism^{12–16}. In particular, the role of bidirectional interactions between drugs and gut microbiomes on host physiology has been extensively discussed^{15,16}. One remarkable observation has been made on the effect of metformin on host longevity. Metformin is a dimethyl-biguanide, a biguanide derivative, that is a most widely used anti-diabetic drug¹⁷. In the case of mammals, metformin administration has recently been found to alter the gut commensal community^{18,19}. It has also been shown to enhance growth of the mucin-degrading bacterium, *Akkermansia* spp., which is accompanied by increased levels of mucin-producing goblet cells in the gut and Foxp3⁺ regulatory T (T_{reg}) cells in adipose tissues¹⁹. Oral administration of *Akkermansia muciniphila* without metformin to diet-induced obese mice is sufficient to improve glucose tolerance along with reduced adipose tissue inflammation¹⁹. This observation indicates that metformin-induced dominance of a specific gut bacterium in mice mediates, at least in part, the anti-diabetic effect of

metformin. In addition to its anti-diabetic effect, metformin is also known to induce life span in rodents and *C. elegans*^{20,21}. Recently, it has been shown that metformin alters bacterial folate and methionine metabolism, resulting in methionine restriction in *C. elegans*³ (Fig. 1). The metformin-induced methionine restriction subsequently results in diet restriction, leading to a life span extension in *C. elegans* (Fig. 1). Methionine restriction in the diet is known to positively affect the longevity of other metazoans, from *Drosophila* to rodents^{22,23}, although the exact mechanism remains to be elucidated. However, addition of metformin to the diet of *Drosophila* did not enhance their life span²⁴. Considering that metformin enhances host longevity by altering metabolism of gut microbiota, one possible explanation could be that metformin may be incapable of altering bacterial folate and methionine metabolism in the case of the *Drosophila* gut bacteria. As *Acetobacter* spp. and *Lactobacillus* spp. are two major gut commensal bacteria in *Drosophila* gut²⁵, it would be interesting to examine whether folate and methionine metabolism of these bacteria is influenced by metformin treatment. All of these findings illustrate unexpected roles of the gut microbiota and microbiome on animal longevity, providing a new concept of microbiome- and metabolite-based longevity medicine.

Gut metabolites and host dysbiosis.

Gut epithelia are delicate barriers that are exposed to hostile bacterial environments. Maintaining a health-promoting bacterial community structure is of paramount importance for host fitness (the phenomenon is called gut-microbe symbiosis)²⁶. Deregulation of bacterial community structure (for example, altered bacterial

community members or overgrowth of certain bacteria) may lead to dysbiosis²⁷, resulting in disease phenotypes in the host. The sequential molecular events leading to dysbiosis are presently unclear. It was thought that symbiotic resident bacteria contribute in part to the maintenance of symbiosis by eliminating environmentally derived nonresident bacteria²⁸. For example, gut bacteria prevent colonization of potentially harmful bacteria by competing for available nutrition or by releasing antimicrobial agents²⁸. In addition to the effort made by symbiotic bacteria, host gut immunity is also known to participate to maintain gut-microbe symbiosis while suppressing dysbiosis.

In *Drosophila*, two major gut immune effectors operate to control gut bacteria; dual oxidase (DUOX)-dependent (DUOX is a member of the NADPH oxidases) microbicidal reactive oxygen species (ROS) and Relish, a homolog of p105-like NF- κ B transcription factor-mediated antimicrobial peptide (AMP)^{29,30}. It is known that the bacterially derived molecules, uracil and peptidoglycan, act as agonists for PLC β -dependent DUOX activation and immune deficiency (IMD)-dependent Relish activation (where IMD is a homolog of the mammalian NF- κ B pathway), respectively^{29,30}. It should be noted that uracil is released from pathobionts (normally benign gut bacteria that are conditionally pathogenic when the commensal community is deregulated) or environmentally driven

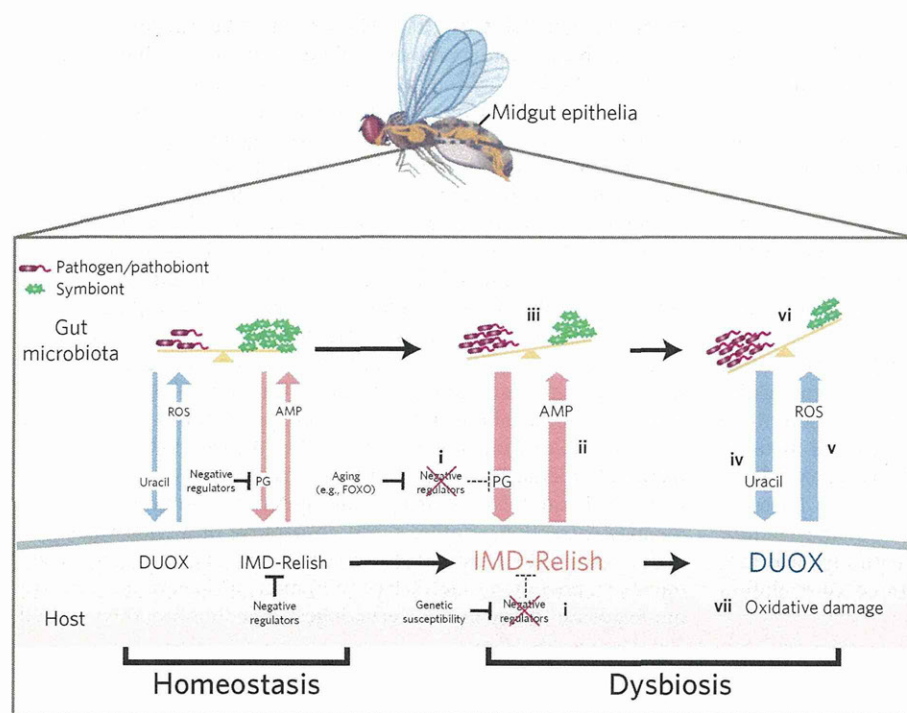


Figure 2 | A model for gut metabolites and dysbiosis in *Drosophila*. Two bacterially derived metabolites, peptidoglycan (PG) and uracil, induce adequate intensity of IMD-Relish and DUOX activation, respectively. IMD-Relish and DUOX activation induce production of two different microbicidal effectors, AMP and ROS, respectively, to control the gut bacteria, which is essential for gut-microbe homeostasis. The sequential events leading to dysbiosis are speculated on, on the basis of previous publications^{6,25,31–34}: (i) the loss of negative regulations (either by genetic susceptibility to the IMD pathway due to the mutation of the negative regulators or by repression of negative regulators, such as PG-degrading enzyme through aging-dependent FOXO activation), (ii) AMP overproduction due to constitutive IMD pathway activation, (iii) modification of the commensal community, such as overgrowth of uracil-producing pathobionts due to the activity of AMPs, (iv) DUOX overactivation due to the high level of bacterially derived uracil, (v) excess production of DUOX-dependent ROS, (vi) further modification of commensal community due to the high ROS level, (vii) oxidative damages to host leading to host death.

loads increase in the intestine, which negatively influences animal life span^{6,36}. Recently, Jasper and collaborators⁶ showed that aging is indeed a dysbiosis-inducing factor in the *Drosophila* intestine, providing an important link between aging and dysbiosis. They found that aged *Drosophila* showed enhanced FOXO activation (i.e., nuclear translocation) in the intestine, which in turn repressed a member of the peptidoglycan-degrading enzymes. This situation causes chronic IMD- and Relish-dependent AMP production, modification of the commensal community, stem cell hyperproliferation and epithelial dysplasia, resulting in host mortality⁶.

But how does the constitutive IMD pathway activation lead to host mortality? Previously, chronic IMD pathway activation (or AMP overexpression) was sufficient to promote overproliferation of a pathobiont²⁵. Recently, bacterially derived uracil molecules released from a pathobiont have been shown to act as agonists to induce chronic DUOX activation and excess ROS production, which is responsible for early host death³¹. Given that uracil feeding alone can induce DUOX activation accompanied by stem cell deregulation as well as severe oxidative stress to the host animal³¹, one could speculate that the loss of the negative regulation of the IMD pathway observed in aged flies could induce uracil-rich gut environments by supporting the expansion of uracil-producing pathobionts and/or by initiating a uracil excretion mechanism of a pathobionts (Fig. 2). Future investigations into the identification of factors directly involved in the host pathology and their modes of action will be of central importance in the improved understanding

of the relationship between bacterial metabolism and metabolites and intestinal dysbiosis.

pathogens but not from symbionts³¹. In contrast to this, peptidoglycan is released from most gut bacteria regardless of whether they are symbionts, pathogens or pathobionts³¹. Normal conventional gut environments (i.e., where symbionts are dominant) create a peptidoglycan-rich condition that could potentially induce constitutive IMD- and Relish-dependent AMP production. However, it has been found that different negative regulators suppress IMD- and Relish-dependent AMP production^{25,32–34}. These negative regulations include peptidoglycan degradation by peptidoglycan-degrading enzymes, inhibition of the IMD signal pathway and suppression of AMP gene expression by transcriptional repression^{25,32–34}. Mutant animals with a defect in one of these negative regulators showed classical features of intestinal dysbiosis^{25,32–34}, such as constitutive IMD pathway activation (high AMP production), modification of the gut commensal community, gut cell apoptosis and early host death. As the germ-free state of these mutant animals abolishes all of the above disease phenotypes, the gut commensal community is a causal element of intestinal dysbiosis. These observations also indicate that homeostasis of the IMD pathway is required for maintenance of a healthy gut commensal community and host fitness. Similarly, NF- κ B-controlled AMPs are also known to regulate the composition of the colonizing microbiota in hydra³⁵.

It has long been observed that intestinal dysbiosis is associated with aging. It is known that, when worms and flies age, bacterial

Gut metabolites and host development. It has long been observed that germ-free animals exhibit abnormal organ development and small body size, suggesting a role of gut microbiome and metabolites on animal growth and organ homeostasis. These phenomena have been observed in different invertebrates, including *C. elegans*¹⁰, *Drosophila*⁷, hydra³⁷ and squid³⁸. In addition to their roles in immune activation, bacterially derived immune agonists are known to be involved in organ development including hydra and squid³⁹. In the case of *Drosophila*, two major commensal bacteria, *Acetobacter* and *Lactobacillus*, are known to have an essential role in the animal development, especially under poor nutritional conditions^{7,40}. In the case of *Lactobacillus*, bacteria modulate the host TOR-dependent nutrition-sensing mechanism and ecdysone and insulin hormone signaling to promote animal growth⁴⁰. As this bacterium is also a commensal bacterium in many other metazoans, including humans, the genetic analyses of *Lactobacillus* and *Drosophila* may be an ideal model system to provide new insight into evolutionarily conserved probiotic effects. It has been shown that *Acetobacter* showed multiple effects on hosts, such as a larval growth-promoting effect and effects on adult cell size and number, organ size, sugar and lipid levels, intestinal stem cell regulation and animal size⁷. Genetic analyses of *Acetobacter*

genome further showed that the bacterial pyrroloquinoline quinone (PQQ)-dependent oxidative respiratory chain in the periplasmic membrane is required for all of the aforementioned effects as it induces host insulin signaling activation⁷. It is presently unclear how PQQ-dependent metabolism influences insulin signaling, thereby regulating host physiology. As absence of the growth-promoting effect in *Acetobacter* lacking PQQ-dependent metabolism (*Acetobacter-ΔPQQ*) can be ameliorated by the addition of acetic acid (an intermediate metabolite of PQQ-dependent metabolism), acetic acid production is required to achieve a host growth-promoting effect⁷. However, acetic acid alone (without the presence of *Acetobacter-ΔPQQ*) is not sufficient to promote growth, suggesting that both PQQ-dependent acetic acid and PQQ-independent metabolisms and metabolites are most likely required for insulin signaling activation and full growth-promoting effects. All of these observations in *Drosophila* suggest that specific bacterial metabolism can directly or indirectly influence a host's master metabolic signaling, such as insulin signaling. Identification of bacterial metabolites and the molecular mechanism by which they induce host development and the metabolic signaling pathways will give essential clues for unraveling the mechanisms of co-evolution between prokaryotes and eukaryotic hosts.

Physiological roles of gut metabolites in vertebrates

Gut metabolites and host metabolism. Human microbiome analyses have revealed that the intestinal microbiota is a complex community consisting of more than 500 species, with *Firmicutes* and *Bacteroidetes* as dominant phyla. The total genes of gut microbiota outnumber our genes by more than 100-fold⁴¹. Many of these microbial genes are involved in main metabolic pathways such as carbon metabolism and amino acid synthesis. Bacterial composition in human stool is diverse even among healthy subjects. However, the genes related to the metabolic pathway are stable among individuals, regardless of variation in bacterial composition⁴², suggesting that maintenance of the core microbiomes involved in carbon and amino acid metabolisms is one of the key aspects of gut commensal community. As a consequence, intestinal microbiota intimately influence the metabolism of their mammalian hosts and critically contribute to mammalian physiology (Fig. 3). The gut microbiota in mammals, including humans and mice, is known to be commensal, which in Latin means 'sharing a dining table'. Commensal bacteria mediate extraction, synthesis and absorption of a wide variety of metabolites⁴³. These metabolites have been associated with the host's metabolic phenotype. Germ-free (GF) mice, which are reared without exposure to any live bacteria, ingest 30% more food compared

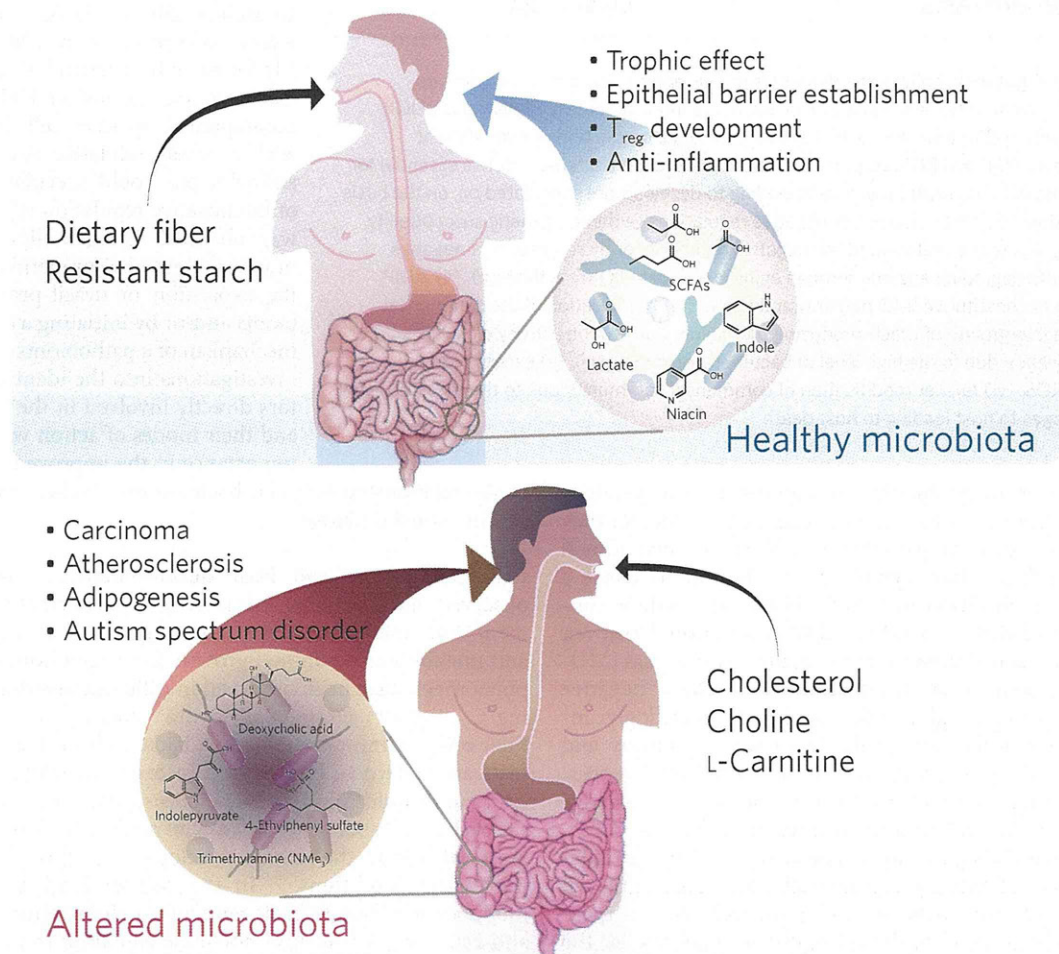


Figure 3 | Physiological and pathological roles of gut microbial metabolites. Gut microbiota are considered important environmental factors that affect the immune system, obesity, cardiovascular diseases and brain activity. Microbial metabolites are indispensable for the majority of the biological effects of gut microbiota. Under physiological conditions, soluble dietary fibers and resistant starch can be actively fermented by commensal microbiota in the large intestine. The fermentation products such as SCFAs have been appreciated for their beneficial effects on intestinal epithelium and the gut immune system. In contrast, HFD and inflammation disturb the microbial community, leading to dysbiosis (for example, the increased ratio of *Firmicutes* to *Bacteroidetes* at the phylum level and an increase in cluster XI of the genus *Clostridium*) and an altered metabolic profile in the intestinal lumen. Such dysbiosis-associated metabolites (or enhanced translocation of normal metabolites into the body) are implicated in systemic as well as gastrointestinal disorders.

with conventionally (CV) housed mice⁴⁴. Nevertheless, GF mice are leaner, with approximately 40% less total body fat compared with CV mice. Conventionalization of GF mice by inoculation of microbiota from CV mice normalizes body fat mass within 2 weeks.

Soluble dietary fibers (for example, fructans, pectin, inulin and xylans) and resistant starch can be actively fermented by commensal microbiota in the cecum (rodents) and colon (humans), producing biologically active metabolites represented by short chain fatty acids (SCFAs), namely acetate, propionate and butyrate⁴⁵ (Fig. 3). Under physiological conditions, luminal concentrations of total SCFAs reach up to 80–130 mM in the human colon⁴⁶. SCFAs serve as a major energy source for intestinal epithelial cells, in which up to 70% of energy intake is provided by gut microbiota. Colonic epithelial cells of GF mice under energy-deprived status are characterized by decreases in NADH/NAD⁺ balance and ATP levels⁴⁷. This eventually leads to phosphorylation of AMPK, a sensor of energy deprivation, and subsequent Cdkn1b/p27^{kip1} activation to not only prevent apoptosis but also induce autophagy. The induction of autophagy in GF mice reflects nutrient starvation and may be important in maintaining cellular energy levels and in facilitating amino acid recycling for new protein synthesis through degrading unnecessary proteins and intracellular organelles⁴⁸. Colonization of GF mice with the butyrate-producing bacteria *Butyrivibrio fibrisolvens*⁴⁹ rescued colonic epithelia from the energy starvation status and autophagy observed in GF colonic epithelium⁴⁷.

Production of SCFAs by commensal microbiota is implicated in adipogenesis in young mice treated with subtherapeutic doses of antibiotics⁵⁰. This treatment increases adiposity without affecting body weight later in life. This finding is analogous with the practical experience that oral administration of low-dose antimicrobial agents is effective in promoting the growth of young farm animals. In antibiotic-treated mice, the ratio of *Firmicutes* to *Bacteroides* is augmented; the change is reminiscent of genetically obese *ob/ob* mice and obese people^{51,52}. Exposure to antibiotics early in life results in enhanced production of SCFAs, increased secretion of incretin hormone GIP from K cells and upregulation of a group of hepatic genes related to lipogenesis and triglyceride synthesis in mice. These observations imply higher energy extraction from indigestible carbohydrates and enhanced adipogenesis in the liver. Thus, subtherapeutic antibiotic treatment in early life may perturb the gut microbial community, leading to long-term alterations of microbial composition. An imbalanced microbial community may affect gut microbial fermentation and host metabolism. In agreement with this view, an increase in energy harvest has been considered a predisposing factor for obesity⁵³. Therefore, manipulation of gut microbiota may be a useful approach for regulating host metabolic phenotypes. Further studies are required to identify the specific microbial species that regulate energy harvest and to understand the molecular mechanism by which these bacteria affect host metabolism.

SCFAs serve as specific activators of Gpr41, Gpr43 and Gpr109a, which are encoded by *Ffar3*, *Ffar2* and *Niacr1*, respectively. Although Gpr41 and Gpr43 are activated by all three SCFAs, Gpr109a is only activated by butyrate⁵⁴. Among these receptors, Gpr43 and Gpr109a are predominantly expressed in intestinal epithelial cells, adipocytes and myeloid cells (i.e., dendritic cells and granulocytes)^{54–57}. In contrast, lymphocytes are devoid of both receptors. Gpr41 expression is more widely observed in immunological organs with little, if any, expression in peripheral organs⁵⁵. Recently, several papers have linked Gpr43 with host-microbe metabolic interaction, although this remains controversial. It was initially reported that acetate and propionate promoted adipogenesis via Gpr43 in 3T3-L1 cells⁵⁸. Furthermore, Gpr43-deficient mice fed a high-fat diet (HFD) displayed improved glucose tolerance and decreased body fat mass⁵⁹. In contrast, other groups have reported that HFD-fed Gpr43-deficient mice exhibit obesity phenotypes characterized by an increase in white adipose in conjunction with progressive insulin tolerance

and adipose tissue inflammation^{60,61} and vice versa, i.e., the adipose tissue-specific transgene of *Gpr43* leads to a lean phenotype. Therefore, the expression of Gpr43 in adipose tissue is most likely responsible for lipid metabolism. The distinct phenotypes of Gpr43-deficient mice among different animal facilities may result from the different genetic backgrounds of the mice. An alternative interpretation may be that variation in intestinal microbiota communities, which is dependent on the environmental factors at each facility, may determine the metabolic phenotypes of host animals. It is noteworthy that Gpr43 deficiency leads to expansion of *Firmicutes* in gut microbiota and consequently raises fecal SCFA and plasma acetate levels, probably because of impaired gut immune homeostasis, for example, reduction of T_{reg} cells and uncontrolled neutrophil activation^{62,63}. Because of the diverse biological functions of Gpr43, the metabolic characteristics of Gpr43 deficiency might be determined as the net results of altered lipid metabolism, microbial imbalance, the overproduction of SCFAs and impaired immune homeostasis. Future studies using adipose tissue or immune cell type-specific Gpr43 conditional knockout mice will provide a clue to the biological significance of Gpr43 in lipid metabolism.

It should be noted that gut microbiota also has an important role in absorption and catabolism of dietary phytochemicals, such as polyphenols. For examples, gut microbiota hydrolyzes glycosylated and/or polymeric polyphenols to yield aglycons that are further subjected to ring fission, leading to the production of phenolic acids and hydroxycinnamates⁶⁴. The microbial transformations of the polyphenols lead to yield metabolites with altered bioavailabilities and bioactivities⁶⁵. Similarly, the gut microbiota has a great impact on drug metabolism and drug-induced toxicity. There are many excellent reviews on the xenobiotic metabolism^{15,16}.

Gut metabolites shape the intestinal and systemic immune system. Compelling evidence has demonstrated that colonization of commensal bacteria critically contributes to the development of the mammalian immune system⁶⁶. GF mice are defective in epithelial barrier functions⁶⁷, development of gut-associated lymphoid tissues (for example, isolated lymphoid follicles)⁶⁸, production of IgA⁶⁹ and generation of T_{reg} cells in the colon^{70,71}. Remarkably, the effect of commensal microbiota is not only confined to the gut immune system but also extends to the systemic immune response⁷². Under GF conditions, the T_H1-T_H2 balance is skewed toward the T_H2 response and, correspondingly, serum IgE is elevated. Such an immunological phenotype of GF mice is normalized by exposure to a diverse microbiota during early life; however, exposure to a limited species of microbiota in early life or exposure to a diverse microbiota in adulthood (>12 weeks old) fails to reduce serum IgE levels⁷³. Thus, exposure to diverse microbiota in early life is most likely required to shape IgE induction pathways, providing experimental evidence for the hygiene hypothesis⁷⁴.

Although the complete mechanisms by which gut microbiota regulates host immunity have yet to be elucidated, microbiota-derived products at least partly mediate the immunomodulatory effect. Such bioactive materials are produced by microbiota in both diet-dependent and independent manners⁶⁶. The diet-independent products are mainly bacterial cell wall components, such as lipopolysaccharides and peptide glycans, both of which elicit innate immune response through the activation of Toll-like receptor 4 (TLR4)-dependent and nucleotide-binding oligomerization domain (NOD) receptor-dependent signaling pathways, respectively²⁸. *Bacteroides fragilis* polysaccharide A (PSA) is another important diet-independent immunomodulatory molecule. Administration of PSA to GF mice induces T-cell expansion and corrects T_H1-T_H2 imbalance in the spleen⁷². In the gut immune system, PSA increases IL-10 production by CD4⁺ T cells and reciprocally suppresses the IL-17A response through binding to TLR2 and eventually protects against experimental colitis induced by colonization with *Helicobacter*

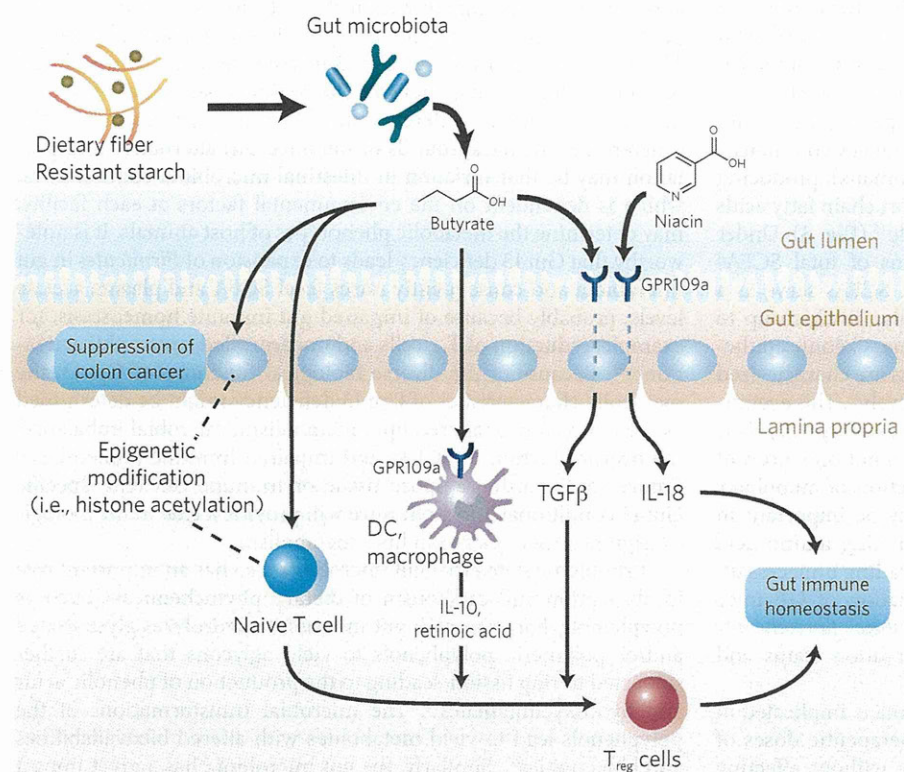


Figure 4 | Host-microbe interactions mediated by butyrate and niacin. Butyrate is mainly produced by clusters IV and XIVa of *Clostridia*. Although butyrate acts as an energy source for normal colonic epithelial cells (trophic effect), it also has the capability to suppress proliferation of cancerous epithelial cells that usually undergo the Warburg effect⁹⁹. Butyrate upregulates histone H3 acetylation at regulatory regions of the *Foxp3* gene and facilitates differentiation of naive CD4⁺ T cells into T_{reg} cells. In contrast, butyrate together with other SCFAs induce TGF- β secretion by epithelial cells through an unknown mechanism⁷¹. Gpr109a was originally described as the receptor for niacin. Butyrate and niacin bind Gpr109a on epithelial cells to trigger production of a cytoprotective cytokine IL-18. These microbial metabolites also stimulate dendritic cells and macrophages to produce IL-10 and retinoic acids, both of which are important for the development of IL-10-producing T_{reg}s in the colon. Therefore, butyrate and niacin contribute to the maintenance of intestinal homeostasis through multiple mechanisms.

hepaticus^{75,76}. Furthermore, sphingolipids of *B. fragilis* reduce cell numbers of invariant NKT (iNKT) cells in the colon. Reduction of iNKT cells in response to colonization with *B. fragilis* results in alleviation of oxazolan-induced experimental colitis^{77,78} because iNKT cells are responsible for the colitis development by producing large amounts of cytokines upon stimulation with glycosphingolipids presented by CD1d⁷⁹. All of these observations indicate that different diet-dependent metabolites have distinct roles in the maintenance of gut immune homeostasis.

So far, a variety of diet-dependent metabolites have been linked to the epithelial barrier, immune regulation and inflammation. These products include bile acids, short and long chain fatty acids and vitamins^{66,80}. Certain *Bifidobacterium* spp. actively produce folate (vitamin B₉)⁸¹, and its derivative, 6-formyl pterin (6-FP), is one of ligands of the MHC class I-like molecule MR1, which presents an antigen to mucosal-associated invariant T (MAIT) cells⁸². Notably, several riboflavin (vitamin B₂)-based metabolites, whose structures are closely related to 6-FP with an extra ribityl moiety, activate Jurkat T-cell lines (expressing invariant MAIT TCR α and β chains) to produce TNF and IFN- γ in an MR1-restricted manner. Given that B-group vitamins including riboflavin are known to be synthesized *de novo* by certain commensal bacteria, it is possible that MAIT cells may sense the growth of riboflavin-producing bacteria on the mucosa.

Indole is a quorum-sensing molecule produced from tryptophan by the tryptophanase of a variety of Gram-positive and Gram-negative intestinal bacteria. Indole enhances epithelial barrier functions *in vitro* and *in vivo* through upregulation of components of tight junction complexes^{83,84}. Acetate is another metabolite that enhances gut epithelial barrier functions (Fig. 3). GF mice succumb to *Escherichia coli* O157:H7 lethal infection; however, inoculation of mice with certain *Bifidobacteria* strains prevents *E. coli* O157-induced death⁸⁵. *Bifidobacteria*-derived acetate inhibits translocation of luminal Shiga toxin from the gut lumen to the blood by improving epithelial defense functions and suppressing colonic inflammation. These findings provide insight into the mode of action through which probiotic bacteria exert a protective effect against pathological infections and open up a new question on how bacterial metabolites regulate epithelial barrier functions.

The anti-inflammatory effect of SCFAs, including acetate, has been well characterized on both epithelial and immune cell levels^{62,86} (Fig. 4). Oral administration of acetate in drinking water suppresses not only DSS-induced experimental colitis but also inflammatory arthritis and ovalbumin-induced allergic airway inflammation. The therapeutic effect of acetate is canceled in Gpr43-deficient mice, implying a major role for the acetate-Gpr43 axis in containment of inflammation. Acetate-dependent Gpr43 activation on neutrophils results in upregulation of apoptosis-related gene clusters and thus provokes apoptosis.

SCFAs also have an impact on the terminal differentiation of CD4⁺ helper T cells⁶². Administration of an individual or a cocktail of SCFAs to GF or SPF mice

increased the frequency of colonic Foxp3⁺ regulatory T_{reg} cells^{87,88}, which have a central role in the suppression of the inflammatory and allergic immune responses⁸⁹. After oral administration, butyrate *per se* is less effective on the accumulation of colonic T_{reg}s as compared with that of the other two SCFAs⁸⁷. However, this observation may reflect enhanced migration from lymphoid tissues into the colon rather than an increase in T_{reg} development in the colon, given that a large part of the orally administered SCFAs are absorbed into the portal vein and/or used by epithelial cells (butyrate is a particularly good energy source) in the upper part of the intestine before reaching the colon. The *in vitro* T_{reg} induction assay indicated that, among SCFAs, butyrate most effectively induces the differentiation of naive T cells into T_{reg} cells^{88,90}. Propionate also shows the T_{reg}-inducing effect to a lesser extent, whereas acetate does not have any impact on T_{reg} induction. Consistent with *in vitro* observations, colonic T_{reg} cells are significantly augmented in SPF mice with a diet containing butyrylate-resistant starch and slightly augmented with propionylated resistant starch but are hardly induced with acetylated resistant starch⁹⁰. These observations show that locally produced butyrate is responsible for T_{reg} differentiation and that orally administered acetate and propionate may be important for the migration of T_{reg}s into the colon. Indeed, oral administration of propionate upregulates Gpr15 (ref. 91), a T_{reg}-specific gut-homing molecule, in a Gpr43-dependent

manner⁸⁷. Butyrate is well known to regulate gene expression epigenetically by inhibiting histone deacetylases (HDACs). Chromatin immunoprecipitation sequencing (ChIP-seq) analysis demonstrated that butyrate upregulates histone H3 acetylation at regulatory regions of the *Foxp3* gene locus and therefore facilitates *Foxp3* expression (Fig. 4). In addition to such a direct effect on CD4⁺ T cells, butyrate indirectly induces IL-10-producing T_{reg} cells by imparting anti-inflammatory properties on dendritic cells (DCs) and macrophages in the colonic lamina propria in a Gpr109a-dependent manner⁵⁶ (Fig. 4). Niacin, a pharmacological agonist for Gpr109a, also exhibits a similar anti-inflammatory activity (Fig. 4). Collectively, these findings demonstrate that microbe-derived SCFAs and niacin contribute to the maintenance of gut immune homeostasis by promoting T_{reg} accumulation in the colon through multiple mechanisms. In support of this idea, butyrate-producing bacteria decreased in the microbial community of IBD patients compared with that of healthy subjects⁹². It has also been reported that gut microbiota-derived SCFAs, particularly propionate, alleviate an allergic airway response induced by house dust mite extract in mice⁹³. The feeding of a diet rich in fermentable carbohydrates promotes the outgrowth of bacteria that belong to the *Bacteroidetes* phylum, leading to increased serum levels of acetate and propionate, which in turn increase the hematopoiesis of DC precursors⁹³. These DCs exhibit an impaired capacity to elicit allergy-prone T_H2 responses in the lung. This process requires the receptor Gpr41 but not Gpr43. This study provides key evidence that bacteria-derived metabolites may affect not only the local but the systemic immune system. Collectively, the recent data support the idea that diet-dependent metabolites shape host immunity by activating different mechanisms, including Gpr-dependent intracellular signaling activation and epigenetic modifications. Although further investigations will be required to clarify the precise molecular mechanism, the interactions between gut metabolites and host immunocompetent cells are essential for the successful establishment and maintenance of the host immune system.

Gut metabolites and disease development. Disturbance of the microbiota has been implicated in the pathogenesis of several human disorders, including IBD, obesity and cardiovascular disease⁹⁴. For example, gut microbial metabolism of dietary food-derived phosphatidylcholine and L-carnitine generates trimethylamine (Fig. 3), which is further metabolized to trimethylamine-N-oxide (TMAO), a proatherogenic agent, in mice and humans^{12,13}. A HFD alters gut microbial composition in mice, leading to expansion of deoxycholic acid-producing bacteria, namely cluster XI of the genus *Clostridium*⁹⁴. This secondary bile acid induces a phenotypic change in hepatic stellate cells to secrete proinflammatory cytokines and eventually facilitates hepatocellular carcinoma (Fig. 3).

Accumulating reports have linked gut microbiota to emotional behavior. Alteration of the gut microbial community owing to TLR5 deficiency increases appetite (about 10% increase in food intake) and induces metabolic syndrome (for example, hyperlipidemia, insulin resistance, obesity and hepatic steatosis) in mice without affecting the efficiency of dietary energy harvest⁹⁵. In addition, chronic ingestion of fermented milk with probiotics can modulate the responsiveness of an extensive brain network in healthy subjects⁹⁶. Although the exact mechanism remains to be elucidated, these observations suggest that metabolites produced by microbial fermentation have a key role in brain activity.

A current study revealed that a mouse model of autism spectrum disorder (ASD) can be triggered by the gut microbial metabolites 4-ethylphenylsulfate (4EPS) and indolepyruvate⁹⁷ (Fig. 3). Maternal immune activation induced by the administration of an immunostimulant polyinosinic-polycytidylic acid (poly I:C) during pregnancy causes ASD-like behavior in offspring, which is associated with gut barrier dysfunction and systemic leakage of luminal

metabolites. In line with these experiential observations, several metabolites (for example, *p*-cresol and indolyl-3-acryloylglycine), which are structurally similar to 4EPS and indole pyruvate, are detected as human autism biomarkers in urine⁹⁸. On the basis of these observations, the brain-microbiota-metabolite axis can be considered a potential therapeutic target for ASD and other neurodevelopmental illnesses⁹⁷. Future studies will provide mechanistic insights into how these gut microbiota-derived metabolites contribute to the pathophysiology of neurodevelopmental disorders.

Conclusions and perspectives

It is now becoming clear that the gut microbiota and its metabolites have an important role in host physiology. Of note, recent investigations indicate that different environmental factors, such as nutrition and drugs, can profoundly affect the gut bacterial community, thereby changing the gut microbiome activity, which may result in generations of bioactive metabolites (for example, health-promoting or disease-causing metabolites). These observations will have ramifications on what we eat and as such would influence food, pharmaceutical and medical sciences by providing new concepts. For example, the use of prebiotics and probiotics or folk medicines often rely on traditional beliefs rather than solid scientific evidence and still suffer because of the lack of more precise knowledge concerning their modes of action. Further investigations of the molecular mechanisms by which prebiotics and probiotics exert their effects on animal physiology will provide new opportunities for improving human health. Furthermore, many important immune and metabolic disorders, including diabetes, obesity, behavioral disorders and chronic inflammation, are now known to be in part due to the imbalance of interactions between the host and microbiota or metabolites. Accordingly, we expect that further investigation on the molecular relationship between microbiota and metabolites and host physiology will provide new concepts and strategies for the development of 'gut metabolite concept'-driven therapeutic agents. Considering the very high diversity of gut bacterial species in different metazoans, it is likely that yet-unexpected microbiota-metabolite-metazoa relationships and the molecular dynamics of these relationships remain to be explored. The use of powerful invertebrate and vertebrate genetic animal model system together with multi-omics approaches would allow us to discover and decipher the secrets of evolutionarily conserved molecular dialogs among gut microbiota, metabolites and metazoans, which will certainly open new avenues to prevent and/or treat a number of disorders associated with gut microbiota and metabolites.

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Commensal microbiota regulates T cell fate decision in the gut

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Abstract Commensal microbiota shapes the intestinal immune system by regulating T helper (T_H) cell lineage differentiation. For example, *Bacteroides fragilis* colonization not only optimizes the systemic T_H1/T_H2 balance, but also can induce regulatory T (Treg) cell differentiation in the gut. In addition, segmented filamentous bacteria (SFB) facilitate the development of T_H17 cells in the small intestine. The 17 strains within clusters IV, XIVa, and XVIII of *Clostridiales* found in human feces can also induce the differentiation and expansion of Treg cells in the colon. Thus, the regulation of T_H cell differentiation by commensal bacteria is evident; however, the molecular mechanisms underlying these processes remain uncertain. Recent studies have demonstrated that bacterial components, as well as their metabolites, play a central role in regulating T_H cell development. Furthermore, these metabolites can elicit changes in histone posttranslational modification to modify the expression of critical regulators of T cell fate. In this review, we discuss the mechanisms and biological significance of microbiota-dependent T_H differentiation.

Keywords Microbiota · T_H17 cells · Regulatory T cells · Histone modifications · DNA methylation

Introduction

Human beings harbor over 100 trillion bacterial species, comprising more than 1000 strains, in the distal intestine. Commensal bacteria facilitate the breakdown of food necessary for energy metabolism in their hosts [1, 2]. Compelling evidence suggests that colonization by commensal bacteria critically contributes to the development of the mammalian immune system. Given that patients with inflammatory bowel disease (IBD) often present with dysbiosis [3], or an imbalance of commensal bacteria, the interplay between commensals and host cells may play a critical role in the maintenance of gut immune homeostasis. This concept has been partly verified by recent studies using gnotobiotic mice, in which germ-free (GF) mice are colonized by specific strains of commensal bacteria. So far, a select number of these strains have been documented to induce the development of particular immune mediators, such as type 17 helper T cells (T_H17) and regulatory T (Treg) cells [4–6]. Although the mechanisms by which gut microbiota regulates host immunity have yet to be elucidated, their derived metabolites can partially mediate this immunomodulatory effect. Such bioactive materials are produced by microbiota through both diet-dependent and diet-independent means [7]. Here, we discuss the molecular interactions between commensal microbiota and T_H cell development.

Development of effector T_H cells by commensal bacteria

Systemic immune responses are biased to T_H2 under neonatal and GF conditions [4, 8, 9]. Microbial exposure early in life is

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