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Immune regulation by microbiome metabolites

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Summary

Commensal microbes and the host immune system have been co-evolved for mutual regulation. Microbes regulate the host immune system, in part, by producing metabolites. A mounting body of evidence indicates that diverse microbial metabolites profoundly regulate the immune system via host receptors and other target molecules. Immune cells express metabolite-specific receptors such as $P2X_7$, GPR41, GPR43, GPR109A, aryl hydrocarbon receptor precursor (AhR), pregnane X receptor (PXR), farnesoid X receptor (FXR), TGR5 and other molecular targets. Microbial metabolites and their receptors form an extensive array of signals to respond to changes in nutrition, health and immunological status. As a consequence, microbial metabolite signals contribute to nutrient harvest from diet, and regulate host metabolism and the immune system. Importantly, microbial metabolites bidirectionally function to promote both tolerance and immunity to effectively fight infection without developing inflammatory diseases. In pathogenic conditions, adverse effects of microbial metabolites have been observed as well. Key immune-regulatory functions of the metabolites, generated from carbohydrates, proteins and bile acids, are reviewed in this article.

Keywords: barrier function; bile acids; immunity; indole; inflammation; metabolites; microbiome; short-chain fatty acids.

Introduction

Commensal microbiota functionally maturate the host immune system. This effect is mediated by microbial factors that stimulate host cells. Microbial factors work through a variety of host receptors and molecular targets on or within host cells. Host receptors for microbial factors include pattern recognition receptors such as Toll-like receptors, nucleotide oligomerization receptors (NLRs), C-type lectin receptors and RIG-1-like receptors, which sense major microbial constituents such as DNA, RNA, proteins and cell wall components. However, these microbial constituents are just a fraction of what commensal microbes produce to regulate the host immune system. In the alimentary tract, host enzymes process dietary materials, such as starch, dietary fibres, proteins/peptides and lipids for nutrients harvest in the small intestine. However, some dietary materials inevitably reach the colon for microbial fermentation due to incomplete digestion and absorption. These materials feed the microbiota for production of a variety of microbial metabolites, some of which have immune regulatory roles. It is thought that there are 500–1000 microbial operating taxonomic units in the mammalian gut. While each microbial species has a finite number of genes that code for nutrient transporters and metabolizing enzymes, the combined number of these genes for the whole microbiota would be enormous.[1] Moreover, the microbiota employ a wide variety of nutrient-utilizing enzymes in numbers that far exceed that of the human enzyme repertoire. Because the products of microbial enzymes are absorbed in the colon and utilized by the host, the gut microbiota increase nutrient or energy recovery from consumed diets. Microbial metabolites have significant effects on the host metabolism.[2–4] Moreover, some of the metabolites also condition and activate the immune system to increase the immune function but decrease harmful inflammatory responses.[5–8]

The concentrations of certain microbial metabolites, best exemplified by short-chain fatty acids (SCFAs), reach very high concentrations in the colon,[9] lowering pH, fulfilling nutritional needs, regulating microbial function and composition, and conditioning the immune system. Host cells express various receptors to sense microbial

metabolites, which include purinergic receptors such as $P2X_7$ to detect microbial and host-derived nucleotides [e.g. adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD)];[10] GPR43 and GPR41 to detect SCFAs; membrane bile acid receptor (M-BAR/TGR5) and farnesoid X receptor (FXR) to detect bile acid and xenobiotic metabolites;[11,12] and aryl hydrocarbon receptor precursor (AhR) and pregnane X receptor (PXR) to detect tryptophan, indole, bile acid and toxicant metabolites.[13–15] Moreover, the roles of dietary factors that activate immune cells, including certain phytochemicals, even without microbial modifications should not be ignored. This article is to review our current understanding of the origin, host receptors and target cells of major microbial metabolites in the immune system. Detailed impacts of these metabolites on the innate and adaptive arms of the immune system are discussed.

Major groups of microbial metabolites and their receptors

Carbohydrate metabolites

Bacteria produce SCFAs as the result of carbohydrate fermentation in the colon (Fig. 1). Bacteria express carbohydrate-active enzymes, such as glycoside hydrolases and polysaccharide lyases.[16,17] These enzymes often form enzyme complexes (e.g. cellulosomes) to process long

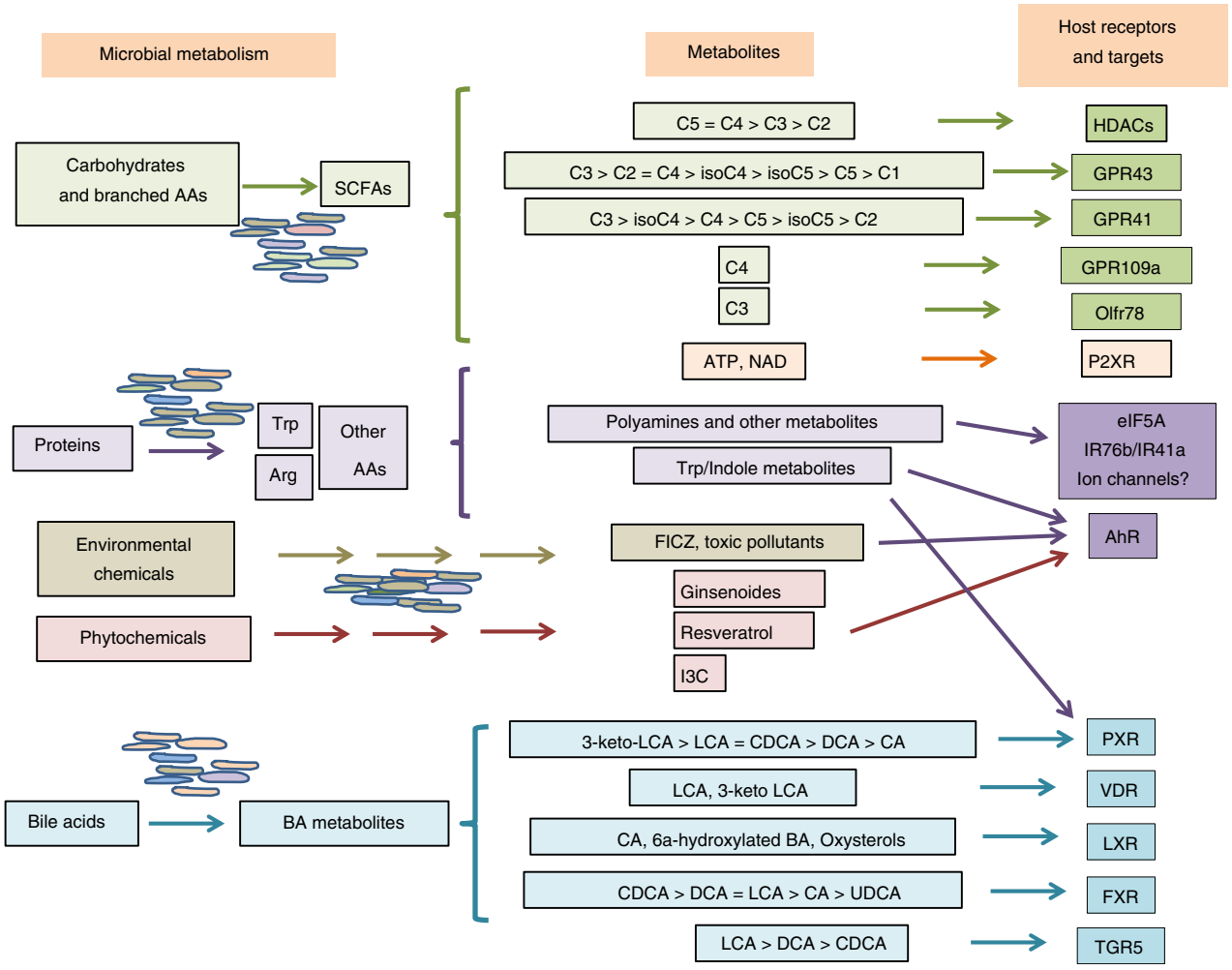

Figure 1. Production of microbial metabolites and their major receptors in the immune system. The gut microbiota can metabolize a variety of dietary materials, which include carbohydrates, proteins, lipids, plant-derived molecules, bile acids and environmental contaminants. These materials are metabolized into short-chain fatty acids (SCFAs), polyamines, ATP, indoles, phytochemical metabolites and bile acid metabolites. SCFAs function as histone deacetylase (HDAC) inhibitors to regulate gene expression and activate G-protein-coupled receptors (GPCRs) such as GPR43, GPR41, GPR109A (C4) and Olfr78 (C3). Other metabolites collectively activate nuclear receptors [aryl hydrocarbon receptor precursor (AhR), pregnane X receptor (PXR), VDR, LXR and farnesoid X receptor (FXR)], TGR5 and P2XRs. These receptors are expressed by various cells in the innate and adaptive immune systems to sense the presence of the gut microbial metabolites.

carbohydrate fibres into simple sugars.[18] Released component sugars, such as glucose and xylose, are transported into bacteria via the phosphotransferase system for uptake and phosphorylation of carbohydrates for fermentation.[19] While the human genome encodes 17 enzymes to breakdown carbohydrate nutrients, some gut bacteria such as *Bacteroides thetaiotaomicron* encode over 260 glycoside hydrolases.[20] Because there are a few hundred bacterial and yeast species in the gut, the total number of carbohydrate-active enzymes and their overall combined capacity to handle different dietary fibres are expected to be sufficient to handle most of the consumed polysaccharides. While both soluble and insoluble dietary fibres can be processed by bacteria, soluble fibres such as arabinoxylan, pectin, inulin and hemicellulose are preferentially utilized to produce SCFAs in the gut over insoluble fibres such as cellulose and chitin.[21] Also, digestion-resistant oligosaccharides, such as fructooligosaccharide and xylooligosaccharide, and resistant starches along with host glycoproteins, such as mucins, can be processed to produce SCFAs. Microbes greatly differ in their ability to ferment dietary fibres and sugars to produce different SCFAs. In general, members of the Bacteroidetes phylum are good producers of acetate (C2) and propionate (C3), whereas bacteria in the Firmicutes phylum are efficient butyrate (C4) producers.[22] More specifically, *Akkermansia municiphilla* produces C3 from mucin.[23] *Roseburia inulinivorans* and *Coprococcus catus* produce both C3 and C4.[24–26] *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Eubacterium hallii* and *Ruminococcus bromii* are good producers of C4.[27] Also, *Roseburia intestinalis*, *Eubacterium rectale* and *Clostridium symbiosum* are good C4-producers and are increased in numbers with a high-fibre diet.[28] *Ruminococcus bromii* produces C4 from resistant starch.[29] SCFAs are absorbed by colonocytes and other cells via solute transporters and simple diffusion. SLC16a1 and SLC5a8 are major transporters for SCFAs.[8] C4 is mainly used by colonocytes, whereas C2 and C3 are transported out to the portal circulation. C2 and C3 are transported to the liver, muscle, brain and other organs. C2 is converted into acetyl-CoA for lipogenesis or oxidation in peripheral muscles. Most C3 is metabolized in the liver and contributes to gluconeogenesis. SCFAs affect the metabolism of host cells, activating multiple metabolic pathways to produce energy and building blocks and regulating host metabolism.[30,31] Also produced by microbes are lactate and succinate, which can be converted to C3 by many bacterial species.[32] SCFAs activate several cell surface G-protein-coupled receptors (GPCRs), such as GPR43, GPR41, GPR109A and Olfr78.[33–36] GPR43 and GPR41 are highly expressed by intestinal epithelial cells.[37] T- and B-cells do not express these SCFA receptors, but certain myeloid cells, such as neutrophils, macrophages and dendritic cells (DCs), express GPR43 and GPR109A at variable levels to sense the concentration of SCFAs in tissue environments.[33,34,38–40]

Amino acid and related metabolites

Another abundant group of dietary materials includes proteins. Proteins are digested into oligopeptides and amino acids, which are largely absorbed in the small intestine. Some proteins, oligopeptides and amino acids, not processed or absorbed in the small intestine, reach the colon for bacterial catabolism and utilization. Many bacterial groups, including certain Clostridium, Bacillus, Lactobacillus, Streptococcus and Proteobacteria groups, are effective metabolizers of proteins, causing 'protein putrefaction'.[41] Among amino acids, glycine, lysine, arginine, leucine, isoleucine and valine are preferred amino acid substrates for gut bacteria.[42] Microbial catabolism of these amino acids generates ammonia, biogenic amines (monoamines and polyamines) and other metabolites. Polyamines are produced from ornithine, arginine, lysine and methionine.[43] Decarboxylation of amino acids generates histamine (from histidine), agmatine (from arginine) and cadaverine (from lysine).[44] For example, *Lactobacillus plantarum* decarboxylates ornithine to produce putrescine, a major polyamine.[45] Moreover, branched-chain SCFAs, such as isobutyrate, valerate and isovalerate, are produced from respective branched amino acids (i.e. leucine, valine and isoleucine).[46] Similar to C4, branched-chain SCFAs are potent histone deacetylase (HDAC) inhibitors and, therefore, their functions in regulating host cells are expected to be similar to that of C4. The luminal concentrations of branched-chain SCFAs are relatively lower than those of the major SCFAs (C2–C4).

Indole is produced from tryptophan and metabolized into kynurenine, indole-3-acetic acid and tryptamine. Bacterial trytophanase produces indole, and host-produced indoleamine-pyrrole 2,3-dioxygenase (IDO) generates kynurenine. These indole metabolites activate AhR and PXR.[15,47,48] Non-protein materials such as glucobrassicin, a compound found in cruciferous vegetables, are metabolized to indole-3-carbinole, which also activates AhR.[49] AhR acts as a transcription factor to induce expression of genes such as CYP4501A1, which detoxifies chemicals and toxins.[50]

Lipid and bile acid metabolites

Gut bacteria produce many lipid-modifying and metabolizing enzymes. For example, hydroxy fatty acids are generated from polyunsaturated fatty acids by gastrointestinal microorganisms such as *Lactobacillus plantarum*, which encodes polyunsaturated fatty acid-saturating enzymes.[51,52] Roseburia species are active in metabolizing linoleic acid (cis-9,cis-12-18:2).[53] They produce vaccenic acid, a precursor of health-promoting linoleic acids. These fatty acid-modifying functions of the microbiota can change the lipid profile in the gut lumen. Bile acids are produced from the liver and secreted into the small intestine to emulsify

triglyceride and fatty acids. Primary bile acids, such as cholic acid and chenodeoxycholic acid, are produced in the gall bladder and secreted into the duodenum as conjugated forms to glycine and taurine. Most of the secreted bile acids are reabsorbed in the terminal ileum. Gut microbiota alter bile acids through various modifications, including hydrolysis of the C24N-acyl amide bond, oxidation and epimerization of hydroxyl groups, 7α-dehydroxylation, esterification and desulphatation.[54] These processes generate more than 20 different secondary bile acids, including deoxycholic acid (DCA) and lithocholic acid (LCA). Also, phosphatidylcholine is metabolized to produce important metabolites such as trimethylamine-N-oxide (TMAO). Gut microbes convert choline to trimethylamine, which is absorbed and then converted by the flavin monooxygenase system in the liver to TMAO.[55] Other substrates of gut bacteria to produce trimethylamin include betaine (trimethylglycine) and carnitine (a lysine derivative). Bile acids activate many cell types through cell receptors, such as FXR, VDR, PXR and TGR5 (also called GP-BAR1 or M-BAR).[12,56] FXR, VDR and PXR are nuclear receptor family members, whereas TGR5 is a G-protein-coupled receptor. FXR is expressed by many cell types, including epithelial and endothelial cells in the liver, gastrointestinal tract and kidney.[57,58] PXR is expressed by cells in the liver, small intestine and colon.[14,59] TGR5 is widely expressed in the body. Its expression is particularly high in the intestines, pancreas, lungs, lymphoid tissues and brain.[60]

Altered microbial metabolites in diseases

Metabolites are produced by the microbiota and, thus, changes in the microbiota or dysbiosis can alter metabolite profiles. Particularly, metabolites are altered in many pathogenic conditions such as obesity, type I diabetes, alcoholic- and hepatitis B virus-induced liver diseases, inflammatory bowel diseases (IBD), cancer and other chronic diseases. Alteration of SCFA levels and/or microbial producers has been observed in many diseases. It has been observed that C2 and C3 are decreased in some patients with type I diabetes or colitis.[61,62] Similarly, C2 and C4 levels were decreased in patients with colitis, colon cancer or other intestinal disorders.[63,64] Also, low dietary fibre intake and decreased levels of SCFAs and some other longer-chain fatty acids have been observed in allergy patients.[65–67] Increased levels of C2 and C4 along with the expansion of Firmicutes species are associated with obesity.[68] In addition, indole derivatives are also altered in some diseases. For example, tryptophan metabolism seems to be increased in patients with IBD, and the levels of tryptophan metabolites such as kynurenine and indole-3-acetate were altered in some colitis patients.[69,70] However, more data are needed to firmly connect the changes of specific metabolites to diseases. Assuming that these metabolites regulate many cell types in most parts of the body, changes in the metabolites could have comprehensive effects, potentially altering immune and inflammatory responses, metabolism, neuronal functions, cancer development and other processes.

Immune regulatory functions of SCFAs

As the most abundant microbial metabolites among all in the colonic lumen, SCFAs play multi-faceted regulatory roles in the immune system (Fig. 2). First, SCFAs are used by colonocytes as a major energy source, and they influence gene expression necessary for epithelial barrier and defense functions. Second, SCFAs regulate innate immune cells such as macrophages, neutrophils and DCs. Third, SCFAs bidirectionally regulate antigen-specific adaptive immunity mediated by T-cells and B-cells.

Dietary fibre and SCFAs support intestinal epithelial proliferation.[71,72] SCFAs are converted to acetyl-CoA for energy production through the tricarboxylic acid cycle and lipid synthesis.[73] SCFAs also inhibit HDACs to promote gene expression in epithelial cells.[74] In the colonic lumen, SCFAs decrease intracellular pH and facilitate sodium ion absorption through multiple mechanisms, mediated in part through the Na^+-H^+ and Cl^--HCO_3^- exchange pumps.[71,75,76] SCFAs are weak acids and play important roles in lowering pH in the gut lumen. SCFAs induce cancer cell differentiation and apoptosis and, therefore, are generally perceived as tumour suppresors.[77] This function is closely associated with the HDAC inhibition and the histone hyperacetylation effects of SCFAs to regulate genes that control key cellular processes.[78] A caveat for this role is that C4 promotes hyperproliferation of mutated $(MSH2^{-/})^-$ colon epithelial cells, and thus has the potential to even promote certain types of tumours.[79] A function important for barrier immunity is the positive effects of SCFAs on epithelial cell production of certain cytokines such as IL-18 and antimicrobial peptides.[80,81] SCFAs also enhance the expression of epithelial barrier-forming molecules and mucin production, which are mediated, in part, by HDAC inhibition and AMP-activated protein kinase (AMPK) activation.[82–85]

Short-chain fatty acids regulate myeloid cells. Neutrophils are the prototype myeloid cells that express the SCFA receptor GPR43. GPR43 activation by SCFAs induces chemotaxis and functional activation of neutrophils.[32,33,37,86] SCFAs also regulate macrophages. C4 conditions intestinal macrophages through GPR109A to induce IL-10-producing T-cells.[87] While GPCR activation by SCFAs is involved in regulating macrophages, the HDAC inhibitor function of SCFAs is also important. For example, C4 downregulates lipopolysaccharide-induced production of nitric oxide and inflammatory cytokines such as IL-6 and IL-12 in a GPCR-independent manner presumably through HDAC inhibition.[88] C4 suppresses production of inflammatory cytokines, such as TNF-α,

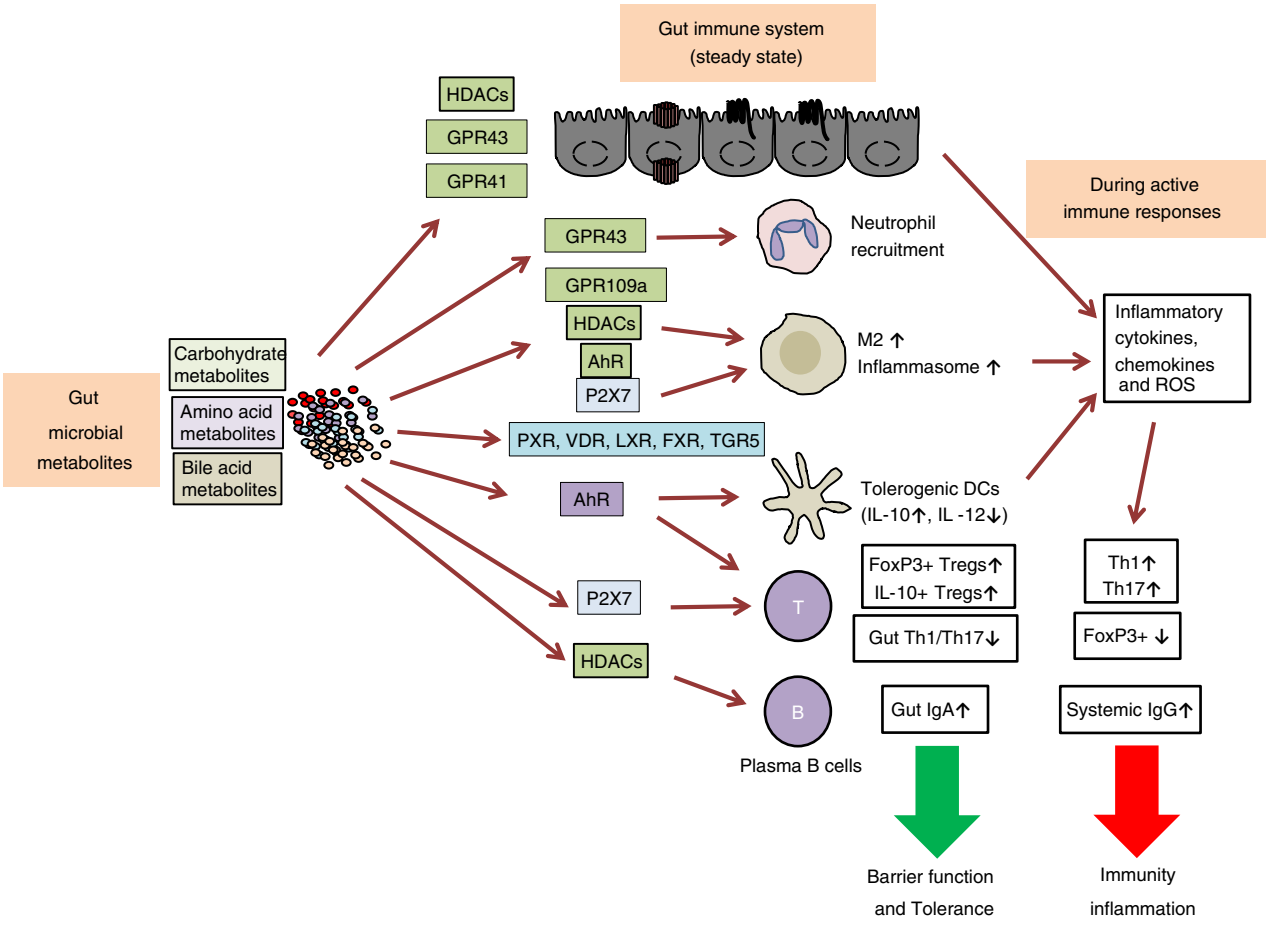

Figure 2. Impacts of microbial metabolites on the immune system. Gut microbial metabolites exert far-reaching influences on the host. They regulate the immune system through histone deacetylases (HDACs), receptors and/or metabolic integration. Short-chain fatty acids (SCFAs) fuel and fortify epithelial cells for promoting barrier functions. SCFAs also function as a neutrophil chemotaxin, and regulate macrophages and dendritic cells (DCs) through G-protein-coupled receptors (GPCRs) and HDACs. Bile acid metabolites also induce tolerogenic DCs and type 2 macrophages (M2), effects mediated by multiple receptors. Aryl hydrocarbon receptor precursor (AhR) activation by gut microbial metabolites induces regulatory T-cells that express FoxP3 and IL-10. Many gut microbial metabolites support the generation of induced Tregs for immune tolerance. SCFAs also fuel B-cells and promote their differentiation into IgA- or IgG-producing plasma B-cells, an effect mediated by HDAC inhibition and metabolic regulation by SCFAs. However, during infection, gut microbial metabolites promote the generation of effector T-cells such as Th1 and Th17 cells to fight pathogens. P2X7 activation by adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) exert both positive and negative regulatory roles in the immune system. Both tolerogenic and inflammatory functions of gut microbial metabolites have been reported, indicating that the overall functions of gut microbial metabolites are determined by the immune status of the host.

MCP-1 and IL-6 in macrophages.[89] SCFAs also regulate DCs. Human monocyte-derived DCs cultured with C3 or C4 were less inflammatory with decreased production of pro-inflammatory cytokines and chemokines.[90] It was documented that SCFAs also indirectly affect DCs by inducing retinaldehyde dehydrogenase 1 (RALDH1) expression in intestinal epithelial cells, which leads to retinoic acid (RA) production and subsequent generation of RA-regulated DCs with a tolerogenic phenotype.[91]

Short-chain fatty acids can also directly and indirectly regulate T-cell differentiation into functionally specialized cells.[8] This occurs when T-cells undergo antigen priming by antigen presenting cells in the presence of SCFAs. It was initially reported that C4 suppresses $CD4^+$ T-cell proliferation but does not induce $FoxP3^+$ T-cells.[92] However, several groups reported that C4 and C3 can increase colonic $FoxP3^+$ Tregs *in vivo* and/or *in vitro* settings, potentially through their HDAC inhibitor activity.[93–95] In our own study, all major SCFAs (i.e. C2, C3 and C4) enhanced the generation of effector T-cells such as Th17 and Th1 as well as $IL\text{-}10^+$ $CD4^+$ T-cells in both *in vitro* and *in vivo* settings.[38] However, the number of $FoxP3^+$ T-cells was not necessarily increased *in vivo* and *in vitro*, whereas the number of $IL\text{-}10^+$ T-cells was increased by SCFAs. However, the $IL\text{-}10^+$ T-cell-inducing effect of SCFAs is lost during active immune responses to *C. rodentium*.[38] During active immune responses, the numbers of Th17 cells and Th1 cells were increased by SCFAs.

Overall, SCFAs have significant impacts on regulatory T-cells and effector T-cells depending on immunological context. This is perhaps due to the booster effect of SCFAs on gene expression during lymphocyte activation. HDAC inhibition increases histone acetylation but it does not have selectivity toward Treg versus effector T-cells. Rather, SCFAs have the tendency to boost the polarization effects set by cytokine milieus present at the time of T-cell priming and differentiation. Because SCFA levels are high in the colonic tissues and gut-associated lymphoid tissues, intestinal T-cells are likely to be a major target of SCFAs.

It has long been recognized that consumption of dietary fibre increases host antibody responses in animals.[96] *In vitro*, C4 increased B-cell production of antibodies, which was associated with increased histone acetylation.[97] Our laboratory systematically studied the impact of dietary fibre and SCFAs on antibody responses to commensal bacteria and pathogens.[98] SCFAs exert strong epigenetic regulatory effects on B-cells through their HDAC inhibitory activity, and promote B-cell differentiation into plasma B-cells. SCFAs can boost the production of both IgA and IgG isotypes. They increase IgA-coated intestinal bacteria and promote IgA and IgG production in response to *C. rodentium* infection. This indicates that both mucosal and systemic antibody responses are boosted by SCFAs. SCFAs upregulate three important metabolic processes, such as glycolysis, oxidative phosphorylation and lipogenesis, in B-cells, which are necessary to produce cellular building blocks and energy to support plasma B-cell differentiation. It is believed that HDAC inhibition by SCFAs promotes histone acetylation to enhance gene expression necessary for plasma B-cell differentiation. Another potentially important regulatory mechanism of SCFAs is mediated by their impact on cellular metabolism. SCFAs increase the levels of acetyl-CoA and ATP but decrease AMP level and AMPK activity, leading to increased mTOR activity that sustains the high metabolic demand of differentiating B-cells.[98] High metabolic activity is necessary to fully support plasma B-cell differentiation.

Through the effects on many cell types, SCFAs exert comprehensive regulatory effects on inflammatory diseases. Compared with wild-type (WT) mice, GPR43$^{-/-}$ mice suffer more from dextran sulphate sodium (DSS)-induced colitis.[99] However, GPR43 signalling promotes also acute inflammatory responses required to mount normal immune responses to microbes, which is delayed in GPR43$^{-/-}$ mice.[100] C4 enema has been reported to suppress chronic colitis in mice and humans.[101,102] However, C4 was not effective in suppressing acute colitis responses such as 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced inflammation.[103] SCFAs and dietary fibre also suppress inflammatory responses associated with type I diabetes and food allergy responses in animal models.[104,105] SCFAs can also suppress acute kidney injury in an animal model.[106] However, chronic administration of SCFAs induced urethritis in mice characterized by smooth muscle cell and epithelial hyperplasia leading to hydronephrosis.[107] Therefore, SCFAs can promote also inflammatory responses depending on their concentrations, organs and host condition.

Immune regulatory functions of amino acid and indole-related metabolites

Major indole derivatives, generated either from tryptophan or plant-derived glucobrassicin, activate AhR. AhR is activated by many natural and synthetic chemicals such as indole-3-acetate, indole-3-carbinol, kynurenine, resveratrol, indirubin, flavonoids, omeprazole and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD). Therefore, AhR is important not only for dietary factor-regulated but also for environmental pollutant-modulated immune responses. Upon ligand binding, cytoplasmic AhR translocates to the nucleus and heterodimerizes with AhR nuclear translocator. Dimerized AhR binds the consensus xenobiotic responsive element (XRE, 5′-GCGTG-3′) in many genes encoding toxicant-modifying enzymes or immune-regulatory molecules. The XRE is thought to be created by insertion of transposable elements such as LINE-1 and Alu.[108] AhR regulates many genes, including the genes coding for CYP450 1A1, IDO, IL-10, Aiolos, FoxP3, IL-21 and CD39. For T-cells, AhR activation supports Th17 but suppresses Treg differentiation.[109] AhR works together with other transcription factors, such as STAT3, to turn on Aiolos or regulates STAT1.[110,111] Interestingly, AhR activation by TCDD had an opposite effect, boosting Tregs but suppressing Th17 cell differentiation. In this case, AhR works with another transcription factor c-Maf, which promotes IL-27-induced IL-10^{+} T-cells.[112] AhR activation promotes monocyte differentiation into DCs over macrophages.[113] *In vivo*, dietary indoles suppress Th17 but increase Treg responses.[114] Thus, the effects of AhR ligands on T-cell responses are complex, mediated by many factors and cell types. AhR function is not necessarily equal to that of AhR ligands in overall immune responses. This is probably because AhR functions as a transcription regulator regardless of the presence of ligands, and its function is further regulated by ligands. Moreover, different ligands and cooperating transcription factors could lead to heterogenous outcomes.

Polyamines regulate transcription, protein translation, stress protein responses and cellular metabolism. They have the potential to exert regulatory functions on immune cells. Polyamines have anti-inflammatory effects, in part, by suppressing inflammatory T-cells and the production of cytokines and nitric oxide (NO).[115] Blocking of polyamine synthesizing enzymes in host cells can break tolerogenic tumour microenvironments.[116] Spermine can regulate autophagy and apoptosis and suppress the

production of IL-12 but increase that of IL-10.[117,118] Polyamines also exert repair functions following tissue damages. However, the functions and mechanisms of actions of various polyamines in regulating the immune system remain unclear at the molecular level.[42] Particularly it remains to be determined how gut microbiota-derived polyamines are transported through the gut barrier and regulate immune cells.

Immune regulatory functions of bile acid and related cholesterol metabolites

Bile acids play important anti-inflammatory roles in cholestatic and metabolically driven inflammatory diseases. Low bile acid levels correlate with increased susceptibility to infection.[119] However, chronic exposure to high levels of bile acids can induce inflammation and cancer.[120] Secondary bile acids, such as DCA and LCA, regulate the immune system, in part, through their receptors, such as TGR5 (also called GPBAR1 or M-BAR) and two nuclear receptors, FXR and PXR. Animals deficient in TGR5 develop more severe colitis induced by T-cell-activating haptens (TNBS and oxazolone).[121] Similarly, $FXR^{-/-}$ mice were more susceptible to TNBS-induced colitis than WT mice.[122] Also, DSS-induced colitis was more severe in $PXR^{-/-}$ mice.[123] These data suggest that bile acid receptors promote immune tolerance. One caveat is that not all the phenotype of $FXR^{-/-}$ mice can be attributed to the function of bile acid metabolites, because these receptors, particularly PXR and FXR, are activated also by non-bile acid metabolite ligands. Moreover, these receptors, particularly those receptors that function as transcription factors, can have ligand-independent regulatory functions. TGR5 ligands, such as LCA and DCA, suppress TNF-α production by macrophages.[124] TGR5 activation induces cAMP production and subsequent phosphorylation of c-Fos, leading to NF-κB inhibition. In this regard, TGR5 ligands generate DCs that have decreased production of IL-12.[125] Consistently, instillation of DCA into the colon exacerbated colitis responses.[126,127] Bile acid metabolites regulate NLRP3 inflammasome activation, but their exact roles in this regard are controversial.[128,129] While the functions of bile acid metabolites appear to be complex and are probably determined by receptors, cell types, tissue sites and immunological context, these metabolites play important roles in regulating the immune system.

Concluding remarks

Gut commensal bacteria produce a myriad of microbial metabolites. These metabolites function as nutrients and/or activate host receptors, including GPR43, GPR41, GPR109A, PXR, FXR and TGR5. While not discussed in detail, P2X receptors ($P2X_{1-7}$) are expressed by many cell types, including tissue, myeloid, mast and T-cells to sense ATP,[130] which is produced by both host and microbial cells. Activation of $P2X_7$, for example, leads to Ca^{2+} influx and subsequent inflammasome activation or cell death for positive and negative regulation of the immune system.[131–133] The host receptors for microbial metabolites are expressed by diverse cell types in barrier and systemic tissues. Therefore, gut microbial metabolites and their receptors create an extensive array of signalling to sense and respond to nutritional status and host conditions reflected in microbial activity. It is apparent that gut microbial metabolites can promote both immunity and tolerance, both of which are required to maintain health by preventing chronic infection and inflammatory diseases. Microbial metabolites strengthen barrier tissues and train the immune system to prevent and prepare for possible infection by pathogens. Beyond the immune system, gut metabolites and their receptors maintain homeostasis of metabolism, which is important to maintain host health by balancing nutrients intake and utilization. Diseases and pathological conditions cause gut dysbiosis and altered production of microbial metabolites, leading to dysregulation of the immune system and metabolism. More research is required to identify the immune regulatory functions of individual metabolites in health and disease. More importantly, how these microbial metabolites in combination regulate the host immune system remains to be elucidated. Furthermore, how altered composition of microbial metabolites is associated with specific diseases should be studied in an effort to identify biomarkers for pathological conditions.

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Disclosure

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