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Regulation of gastrointestinal immunity by metabolites

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Abstract: The gastrointestinal tract contains multiple types of immune cells that maintain the balance between tolerance and activation at the first line of host defense facing non-self antigens, including dietary antigens, commensal bacteria, and sometimes unexpected pathogens. Maintaining homeostasis at the gastrointestinal tract requires stringent regulation of the immune responses against various environmental conditions. Diet can be converted into gut metabolites which have unique functional activities through host as well as microbial enzymatic activities. Accumulating evidences demonstrate that gastrointestinal metabolites have significant impacts on the regulation of intestinal immunity and further integrate immune response of distal mucosal tissue. Metabolites, especially derived from microbiota, regulate immune cell functions by various ways including recognition and activation of cell surface receptors, controlling of gene expression by epigenetic regulation and integration of cellular metabolism. These mucosal immune regulations are key to understand underlying mechanism for the development of gastrointestinal disorders.

Here, we review the recent advancement of our understanding on the role of gut metabolites in the regulation of gastrointestinal immunity with highlighting the cellular and molecular regulatory mechanisms by macronutrients-derived metabolites.

Introduction

The phrase 'You are what you eat' emphasizes the importance of diet on human health. Diet-containing various nutrients including, proteins, lipids, carbohydrates, and nucleic acids build blocks to supply energy through their metabolism. Metabolic reactions regulated by series of enzymatic reactions convert dietary nutrients into diverse metabolites and the

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most of responses are happening in the gastrointestinal tract. Secreted enzymes from host intestinal tissues digest dietary components to produce various dietary metabolites, which mostly absorbed into small intestine. In the gastrointestinal tract, unlike other tissue, a huge amount of gut microbiota colonizes and plays diverse functions including integration of gut metabolism. Undigested nutrients or gut metabolites are further processed through gut microbial fermentation, which produce new types of microbial metabolites by microbial enzyme activities. As the spectrum of gut microbial enzymes are different from those of host enzyme, one could expect more diverse gut metabolite profiles in the gastrointestinal tract. On the other hand, composition of gut microbiota is easily influenced by diet and different microbiota community produces distinct gut microbial metabolites. Thus, types of diet are considered as a key regulatory factor for gut metabolite profile through manipulation of microbiota community. Collectively, diet components convert into diverse dietary and microbial metabolites by complex enzymatic reactions and microbial fermentation, respectively.

Maintenance of immune-homeostasis in mucosal tissues is quite challenging as the mucosal barrier is vulnerable to damage by various extrinsic factors including diet, microbiota and toxins. To maintain immune-homeostasis, inflammatory responses at the steady-state are restrained through mechanisms promoting regulatory function of immune cells and fortifying barrier functions in mucosal tissues. Disruption of such regulation contributes to inflammatory and, if severe, autoimmune diseases. It has been generally accepted that the occurrence and severity of these inflammatory diseases are regulated by various extrinsic factors including diet and the microbiota. It is worthwhile to note that the most prominent way of diet and the microbiota affecting mucosal immunity is through

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production of metabolites. Undoubtedly, the intestinal immunity is the major target for the regulation of gut metabolite. Dietary and microbial metabolites directly regulate epithelial cell and intestinal immune cell function, which plays significant roles in maintenance of immune homeostasis in the gut. Accumulating reports further suggest that metabolites influence largely on the development of gut inflammatory diseases such as inflammatory bowel diseases and colorectal cancer through diverse immune-regulatory mechanism [1,2]. Integrative approach with immunology, nutrition, microbiology is needed to better understand the link between diet and immune diseases. In this article, we reviewed current progress of nutritional immunology in linked with gut metabolites for the regulation of gastrointestinal immunity.

1. Intestinal immunity

The intestine is highly complex organ, composed of the intestinal epithelium layer, microorganisms, and local immune system. The organized immune structures of gut-associated lymphoid tissue (GALT) and draining lymph nodes in the intestine act as a principal site for priming adaptive immune responses [3]. The GALT contains microfold (M) cells and PPs and comprises subepithelial lymphoid aggregates that lie in the mucosa and submucosa. M cells are specialized cell type for the uptake of relatively large size of antigens that normally unable to enter from the lumen into lamina propria, where dendritic cells (DCs) process and present antigens to T cells [4]. Thus, it provides an important route for intestinal immune cells recognizing luminal antigens. PPs lie in the small intestine that contain numerous B cell lymphoid follicle together with T cell area [5]. PPs are known as a major source for small intestinal IgA, which is one of the key components of the mucosal protective

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immunity. GALT also includes smaller lymphoid aggregates such as cryptopatches and isolated lymphoid follicles. These lymphoid structures also contain adaptive immune cells, particularly B cells. The composition of immune cells and even structural formation of these smaller lymphoid aggregates are changed by ageing together with colonization and diversity of the gut microbiota [6]. Mesenteric lymph nodes (MLNs) are the largest lymphoid tissue in the body, where constant exposure of immune-stimulatory antigens to the gastro intestinal area and interactive response between innate and adaptive immune cells are observed.It is worthwhile to note that antigens from each anatomical location throughout gastrointestinal tract drains into distinct lymph nodes [7]. This suggests that dynamic interactive responses between innate immune cells and adaptive immune cells occur differently at various lymphoid tissue sites.

The intestine contains the largest number of immune cells compared with any other tissues in the body [3]. The epithelium and lamina propria are the effector sites of the intestinal immune system containing different immune cell population. While intestinal epithelium has relatively smaller number of immune cells than those of lamina propria, they act as a central coordinator of mucosal immunity. Accumulating reports suggest that dietary and microbial metabolites control epithelial functions [8–10]. Intestinal epithelial cells (IECs) composed of several subtypes, including enterocyte, goblet cell, paneth cell, tuft cell, enteroendocrine cell, and M cell. They, differently distributed in small and large intestine, play distinct roles in intestinal biology [11]. Major immunological function of IECs is providing a barrier between inner and external (luminar) side. Intestinal epithelial layer provides physical and chemical barrier between luminal immunogenic agents and host immune system to prevent undesired activation of immune cells in the intestinal tissue.

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Therefore, epithelial barrier integrity is critical factor to maintain intestinal tissue-homeostasis that is influenced by gut microbiota and metabolites [10,12]. IECs also actively participate in orchestrating intestinal immune responses through recognition of luminal antigens and producing immune modulatory molecules. IECs produce a variety of functional molecules including cytokines, chemokines and anti-microbial peptides that act as a key regulator for intestinal immune responses. IECs express multiple types of pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), C-type lectin receptors, retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and AIM2-like receptors (ALRs) [13,14]. Like other innate immune cells, PRRs on IECs recognize pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). This epithelial recognition provides a critical initial step for crosstalk between luminal microbes and intestinal immunity. After antigen recognition, IECs produce various immune-modulatory molecules, including antimicrobial peptides (AMPs), which mediate gut barrier function. In addition, IEC-derived cytokines shape intestinal immune cell responses in lamina propria. For example, IECs produce IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) that induce the activation of type 2 innate lymphoid cell (ILC2) to secret interleukin (IL)-5 and IL-13 in the context of type 2 immunity [15–17]. IECs also receive internal signal such as cytokines that are recognized by their receptors to respond and control intestinal immune responses. In response to IL-22, paneth cells secret AMPs such as lysozyme and defensins, critical for anatomical compartmentalization and maintenance of symbiotic microbes [18]. IECs also secrete IL-18 in linked with an inflammasome activation. Inflammasome sensors detect microbial and host-derived molecules through both direct and

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indirect manner [18]. As IL-18 is critical cytokine to induce AMPs, an impaired inflammasome activation in IECs induces dysbiosis of gut microbiota resulting in inflammatory disease [19,20]. Collectively, IECs are central mediators to orchestrate intestinal immune responses in the middle of external stimuli and intestinal immune cells.

Mononuclear phagocytes, consisting macrophages and DCs, share the expression of certain markers such as CD11b, CD11c, and major histocompatibility complex (MHC)II [21]. They uptake antigens and present antigenic peptide to adaptive immune cells with pro-inflammatory cytokine production. Majority of intestinal macrophages are found in healthy intestinal lamina propria [21]. They play critical roles in controlling intestinal homeostasis through clearance of pathogens and cellular debris. Subset of macrophages called M2 (or alternative) macrophages produce regulatory molecules such as IL-10, which mediates epithelial healing process after tissue damage and lessen the inflammatory responses. Intestinal DCs in lamina propria can be subdivided by their CD11b and CD103 expression into four subsets, differently distribute throughout the intestinal tissues. For example, $CD11b^{+}CD103^{+}$ DCs are the major subset in the small intestine while $CD11b^{-}CD103^{+}$ DCs are the major DC subsets in the colon. It has been reported that $CD11b^{+}CD103^{+}$ DCs correlated with Th17 responses [21]. Although, molecular mechanism for balancing T cell homeostasis by specific type of DCs is yet to be further defined, reduced Th17-skewing cytokine IL-6 can be a reason to explain such regulation. In lamina propria, plasmacytoid DCs (pDCs) are also observed, but smaller numbers than conventional DCs. The pDCs appear to be recruited to the colonic mucosa in response to inflammation or microbial infection [22,23]. It has been reported that pDCs sensing microbiota induce naïve T cells into IL-10 producing $CD4^{+}$ T cells [24]. Intestinal DCs play a role in the regulation of intestinal immunity through manipulating

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intestinal T cell responses. It is noteworthy that regulatory functions and specific subset distributions of DCs and macrophages can be influenced by dietary and microbial metabolites.

The intestine contains both innate and adaptive lymphocytes. ILCs, most recently identified, are a type of innate lymphocytes and primarily localized in the mucosal tissues such as intestine. ILC1, ILC2, and ILC3 resemble to Th1, Th2, and Th17, respectively in terms of their cytokine expression and transcription factors involved [24]. Intestinal ILCs receive immune-stimulatory signals from microbes, metabolites or dietary antigens to produce cytokines, that significantly affect intestinal homeostasis and inflammation [24]. Occasionally, eosinophils and mast cells are increased in the intestine where they work together with ILC2 for type 2 immunity [25,26], which is involved in tissue repair process. T and B lymphocytes provide a specific immunity to luminal antigens. T cells locate in both intestinal epithelium and lamina propria. Intraepithelial lymphocytes (IELs) have regulatory and effector activities via induction of intestinal inflammation and epithelial homeostasis [27]. In lamina propria, distinct $CD4^+$ T cell subsets, Th1, Th2, Th17 and regulatory T cells (Tregs) exist depends on immune milieu in the intestinal tissue. Naïve T cells recognize antigens by antigen presenting cells (APCs) in induction site of lymphoid tissues, and then migrate into effector sites. The balance of T cell subsets is critical for the maintenance of tissue homeostasis [28,29]. Largely, balance between pro-inflammatory T cells (Th1, Th2, Th17) and anti-inflammatory T cells (Treg) determine immune-homeostasis in the intestinal area. We now start to understand how gut metabolites regulate the balance of CD4+ T cell subsets. For example, short chain fatty acids (SCFAs), microbial metabolites produced in the result of carbohydrate utilization, expand Treg pool in the gut through diverse molecular

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mechanisms [30]. Large number of B cells are also found in lamina propria, however IgA expressing plasma cells are mainly located into intestinal lymphoid tissues, such as PPs and MLNs. Secretory immunoglobulin A (sIgA) plays a critical role in controlling pathogens and commensal bacteria in the lumen of intestine. It has been reported that dietary regulation of intestinal B cell responses through gut metabolites [31,32]. Collectively, innate immune cells (IECs, macrophages, DCs, ILCs and mast cells) and adaptive immune cells (IgA^+ B cells and T cells) work together to maintain immune-homeostasis at the steady state or during the healing process, and to fight against pathogens after the infection.

2. Dietary and microbial metabolites produced in the gastrointestinal tract

Various metabolites are produced via host and microbial metabolism mainly in the gastrointestinal tract. Gut metabolites participate in energy metabolism, cell-to-cell interaction, and host immune responses [33–35]. Small intestine is the most active site for nutrient digestion in the context of host metabolism. Majority of host enzymes are secreted into lumen of small intestine and therefore various metabolites such as lactate are produced and absorbed into the tissue.

The gastrointestinal tract as a digestive organ, rich in dietary materials, provides ideal niches for gut microbiota. Roughly, a thousand of microbial operational taxonomic units dominated by the bacteria kingdom are found in different anatomical locations, defined by complex gradients of chemical, physical, nutritional, and immunological factors [36]. The gut microbiota plays a pivotal role in host health through integration of host metabolism and supporting immunity [37,38]. While, many gut microbiota studies have been skewing to the regulation of host immunity, overall metabolism potential has been under-explored. In the

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gastrointestinal tract, gut microbes in metabolically active status are harvesting energy from diets including carbohydrate, protein, and lipid through microbial fermentation [33]. The gut microbiota generates metabolites by converting dietary components directly or modifying host-derived metabolites (e.g., bile acids). Also, the gut microbiota synthesizes metabolites *de novo* that the host cannot produce (e.g., SCFAs, vitamins). The microbial composition and diversity differ along the gastrointestinal tract because of different microenvironmental condition (e.g., pH, retention time, the influx of digestive enzymes and bile acids). In human, microbial density in small intestine ranges from about 1 x 10^3 CFU/g of luminal content in the duodenum to 1 x 10^8 CFU/g in the ileum [39]. Firmicutes (Streptococcaceae) and Proteobacteria (Enterobacteriaceae) are dominant phyla in small intestine. By comparison, the colon harbors about 1 x 10^{11} CFU/g of luminal content dominated by Bacterioidetes (Bacteroidaceae, Prevotellaceae, Rikenellaceae) and Firmicutes (Lachnospiraceae and Ruminococcaceae) in human [39–41]. These microbial communities' variation with physiological characteristics result in production of unique metabolites in each region of the gastrointestinal tract. Thus, abundance and nature of metabolites are the outcome of diversity and composition of the gut microbiota. Collectively, diverse metabolites are produced by host and microbial enzymatic reactions in the gastrointestinal tract.

2.1 Production of dietary metabolites in the small intestine

A. Carbohydrate metabolites

Small intestine is the major region for digestion and absorption of carbohydrates that are utilized by host tissues and gut microbes (Figure 1). Digestive enzymes such as

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disaccharidases released from enterocytes hydrolyze carbohydrates to monosaccharides (e.g., glucose, fructose, and galactose), of which undergo through glycolysis to create pyruvate for energy production [42,43]. In anaerobic condition, pyruvate converts into lactate by lactate dehydrogenase [44]. In paneth cells, lactate is a critical metabolite to maintain proliferation and differentiation of small intestinal stem cells for gut homeostasis [45]. The intestinal stem cells convert lactate into pyruvate and enhance oxidative metabolism leading to reactive oxygen species (ROS)-induced intra-cellular signaling for their differentiation [45].

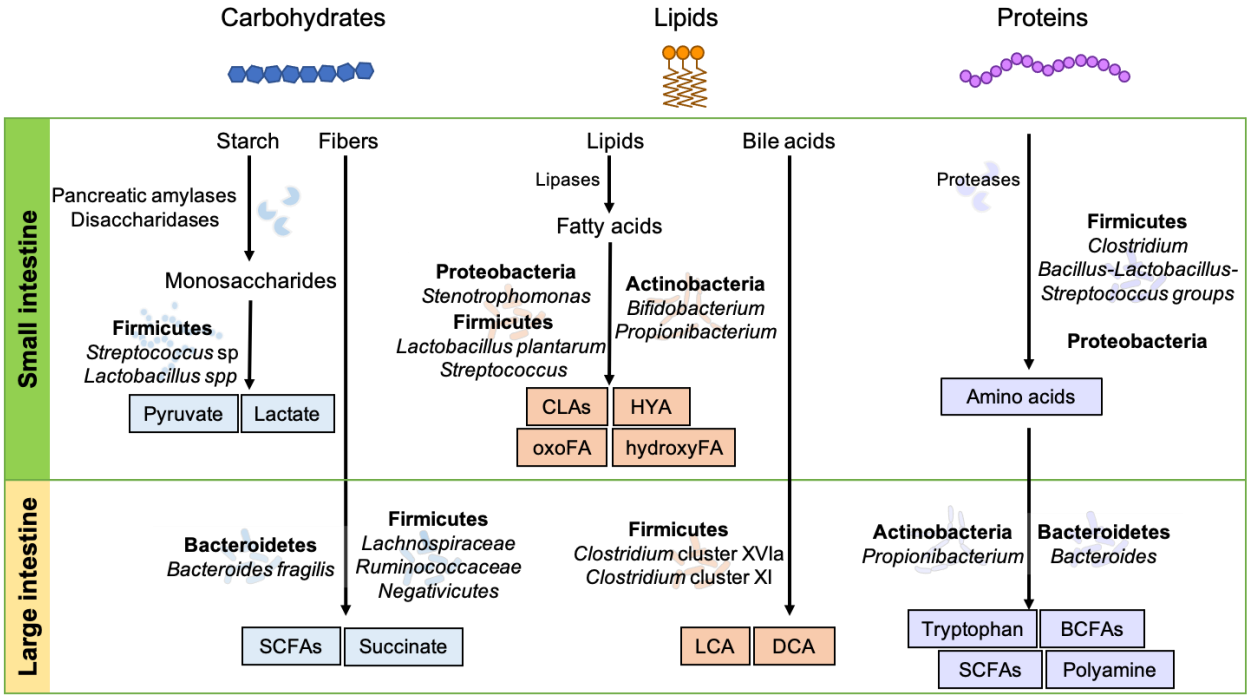

Figure 1. Dietary metabolism in the gastointestinal tract.
Dietary components, carbohydrates, lipids, and proteins, are digested into diverse metabolites via complex reactions of enzymes released from host tissues and microbial fermentation in the gut. BCFAs, branched chain fatty acids; CLAs, cojugated linoleic acids; DCA, deoxycholic acid; HYA, 10-hydroxy-cis-12-octadeccenoid acid; hydroxy FA, hydroxy fatty acid; LCA, lithocholic acid; oxoFA, oxo fatty acid; SCFAs, short chain fatty acids.

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Although, limited numbers of gut microbes exist in the small intestine, some small intestinal microbes, including *lactobacillus spp.* play a significant role in regulation of host metabolism and immunity [46]. In addition to host cells, commensal bacteria not only help carbohydrate digestion by releasing lactase that hydrolyze lactose to glucose, but also produce carbohydrate metabolites resulting in the maintenance of gut homeostasis [46]. In metagenomic and metatranscriptome analyses with human ileostomy, small intestine is enriched with *Streptococcus* sp. expressing genes for phosphotransferase systems to uptake monosaccharides, and carbohydrate metabolisms including glycolysis and pentose phosphate pathways, and consequently producing lactate (Figure 1) [41].

B. Lipid and bile acids metabolites

Lipid digestion called lipolysis and its absorption primarily occur in the duodenum and jejunum. Lipases play essential roles in digestion, transport and processing dietary lipids (i.e., triacylglycerols, fats and oils) in animals and human, and act at a specific position on the glycerol backbone of a lipid substrate [47]. For instances, gastric lipases break down triglycerides into diglycerides and fatty acids, and pancreatic lipases convert emulsified lipids into fatty acids, monoglycerides and glycerol. Phospholipases and sphingomyelinases are also seen in nature, yet they are generally treated separately from aforementioned lipases. Fatty acids are precursors for synthesis of several lipid metabolites. Fatty acids and their metabolites act as an energy source and a part of membrane components. Among four types of long chain fatty acids (saturated, monounsaturated, polyunsaturated, and trans), polyunsaturated fatty acids (PUFAs) required for variety of physiological processes and are considered mostly as essential fatty acids, because animal and human cells cannot synthesize

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them and thus must be supplied by the diet. Linoleic acid (ω6) and α-linolenic acid (ω3) are two major PUFAs. A metabolite of linoleic acid (ω6) is arachidonic acids, which are further metabolized into leukotrienes, lipoxins, prostaglandins, and thromboxane-prostanoid. The major metabolites of α-linolenic acid (ω3) are eicosapentaenoic acids and docosapentaenoic acids, which serve as precursors for specialized pro-resolving mediators: resolvins, protectins, and maresins [48]. The metabolic pathways of linoleic acid and α-linolenic acid require the same series of oxidative enzymes such as cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases, thus they are in competitive relation among themselves for immunological output [49].

Lipid metabolites are generated not only by host but also by microbiota in the intestinal tract expressing lipid metabolizing enzymes. Indeed, low microbial gene richness in human gut is related with impaired lipid metabolism and increased risk of metabolic diseases [50,51]. In animal studies, germ-free (GF) mice have higher lipid excretion and reduced lipid metabolites with an impaired lipid digestion and absorption in small intestine [52,53]. However, the impaired lipid metabolism in GF mice was recovered by transplantation of high-fat diet induced jejunal microbiota with increased abundance of *Clostridiaceae* and decrease of *Bifidobacteriaceae* and *Bacteroidaceae* [54]. *Lactobacillus plantarum* expresses polyunsaturated fatty acid-saturating enzymes are known to generate conjugated linoleic acids (CLAs), hydroxy fatty acids, and oxo fatty acids [55]. *Bifidobacterium* strains and *Propionibacterium freudenreichii* also produce CLAs with action of specific CLA-converting enzymes. 10-hydroxy-cis-12-octadeccenoid acid (HYA) is produced by the FAD-dependent myosin cross-reactive antigen protein expressing bacteria (i.e., *Lactobacillus, Bifidobacterium, Streptococcus, and Stenotrophomonas*) (Figure 1) [56–58].

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Bile is synthesized in the liver, mainly constituted by bile acids and cholesterol that emulsifies and solubilizes lipids, thereby playing a critical role in fat digestion. Cholesterol and bile acids are important for the regulation of digestive function, nutrient metabolism, and immune responses. Most cholesterol is absorbed in the duodenum and proximal jejunum by a passive diffusion process. Reabsorbed cholesterol incorporates with triglycerides and lipoproteins forming into transportable complexes called chylomicrons, which return to the liver via the enterohepatic circulation. Primary bile acids (e.g., cholic acid, chenodeoxycholic acid, hydrochloric acid) are synthesized from cholesterol in the liver and secreted as taurine or glycine-conjugated forms into the duodenum of small intestine to aid in emulsification of dietary lipids and fat-soluble vitamins. In small intestine, the secreted bile acids undergo deconjugation by microbes producing bile salt hydrolases. Most of bile acids (≥ 95%) are reabsorbed in the terminal ileum and reused through the enterohepatic circulation [59].

C. Protein metabolites

Substantial portion of amino acid metabolism is mainly occurred in the small intestine [41]. Digestion of dietary proteins starts in the stomach with pepsin and hydrochloric acid hydrolyzing the proteins into smaller polypeptides. Pancreatic proteases (trypsin, chymotrypsin, and elastase), digestive hormones (secretin and cholecystokinin), and proteases (aminopeptidase N) further digest those polypeptides to tripeptides, dipeptides, and free amino acids in small intestine. Dipeptides and tripeptides are absorbed by PepT1, the proton-dependent small oligopeptide transporter, located on apical membranes of enterocytes, and digested into amino acids by cytoplasmic peptidases [60,61].

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In addition to host-derived proteases, amino acids fermenting microbes also produce proteases to hydrolyze proteins and subsequently utilize the released amino acids for proteolytic fermentation or synthesis of microbial protein (Figure 1). The abundant amino acid fermenting microbes detected in the human small intestine are mainly *Clostridium* cluster, the *Bacillus-Lactobacillus-Streptococcus* groups, and *Proteobacteria* [62]. Studies suggested that those small intestinal microbes mainly utilize amino acids for the synthesis of bacterial protein [62]. Amino acids are absorbed into enterocytes through amino acid transporter systems such as B^0 and ASC depending on amino acid properties, and transferred into portal blood across the basolateral membrane, and then taken up into the hepatocyte in the liver to be used for protein synthesis or gluconeogenesis [63,64]. For instance, glutamine participates in many key metabolic processes, such as protein synthesis, gluconeogenesis, inter-organ nitrogen transfer, nucleic acids biosynthesis, the immune response, and regulation of cellular redox state [65].

2.2 Production of dietary metabolites in the colon

A. Carbohydrate metabolites

Carbohydrates in the diet are digested in the small intestine into glucose, galactose and fructose, and then absorbed by enterocytes. Sodium-glucose co-transporter (SGLT)1, glucose transporter (GLUT)5 and GLUT2 are responsible for transport glucose, galactose, and fructose across the brush border membrane or basolateral membrane [66]. Loss-of-function study using knock out mouse model suggests that SGLT1 is important for fast glucose absorption, however the presence of GLUT2 fails to show any role in either apical

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glucose influx or incretin secretion [67]. When dietary fiber containing indigestible carbohydrates escapes the small intestine and reaches the colon, carbohydrate targeting enzymes (e.g., glycoside hydrolases, polysaccharide lyases) released from anaerobic bacteria break down fiber into absorbable sugars. The released sugars are fermented by bacteria through metabolic pathways, the Embden-Meyerhof-Parnas pathway and the pentose-phosphate pathway, to build metabolic intermediates resulting in the production of SCFAs [68]. SCFAs are representative metabolites, synthesized *de novo* through microbial fermentation mostly in the colon. The most abundant SCFAs (≥ 95% of total) are acetate (C2), propionate (C3), and butyrate (C4) in the gut [69]. Acetate, produced from pyruvate via acetyl-CoA or the Wood-Ljungdahl pathway, can be used as a substrate for butyrate production. Propionate is produced from lactate via the acrylate pathway or from succinate via the succinate pathway. Butyrate is produced from 2 molecules of actetyl-CoA via phospho-transbutyrylase and butyrate kinase route or the butyryl-CoA:acetate CoA-transferase pathway [70]. Each enzymatic reaction for the production of each SCFA is regulated by specific microbes expressing the responsible genes for each biosynthesis pathway. Specifically, many enteric bacteria and Bacteriodetes produce acetate and propionate [35,71–74]. Firmicutes (*Lachnospiraceae* and *Ruminococcaceae*) are known to produce butyrate [71–73].

The SCFAs are absorbed by cells like colonocytes or transported to tissues via simple diffusion or active transport by using Na^+-coupled or H^+-coupled transporters such as SLC5A8 and SLC16A1 [75]. Some SCFAs (mostly acetate, but possibly some propionate) reach the circulation and can also directly affect metabolic responses in the peripheral tissues including adipose tissues, brain and liver [1,76].

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B. Lipid and bile acid metabolites

Although, the most of bile acids and cholesterol are reabsorbed in the distal small intestine, some of bile acids (approximately 5%) and cholesterol reach the colon then dynamically interact with gut microbiota [77]. The cholesterol evading re-absorption reaches the colon, at which it is metabolized by the intestinal microbiota, otherwise excreted with feces [78]. The microbial activities on cholesterol are based on its enzymatic reduction to produce coprostanone and coprostanol via two different pathways [79]. The first, direct reduction of double bond at 5–6 to give coprostanol by cholesterol reductases. The second, oxidation of the 3b-hydroxy group and isomerization of the double bond produce 4-cholesten-3-one by cholesterol oxidase (ChOx) or 3b-hydroxysteroid dehydrogenase/isomerase (HSD), respectively.

A small amount of escaped bile acids (< 10%) from ileum re-absorption, move into the colon, where bacteria transform primary bile acids to secondary and free bile acids. As shown in Figure 1, there are more than 20 different secondary bile acids produced by bacteria metabolism including lithocholic acid (LCA) and deoxycholic acid (DCA). Firmicutes, including Clostridium cluster XVIa and XI are known to produce secondary bile acids (i.e., LCA and DCA) from primary bile acids [80]. Furthermore, secondary bile acids undergo microbial isomerization resulting in unique immunomodulatory properties. For example, secondary bile acid DCA does not promote Treg, but its isomer, isoDCA promotes Treg differentiation [81]. Secondary bile acids resulting from bacterial action through various reactions as following; deconjugation by bile salt hydrolases that hydrolyze the amide bond and 7-dehydroxylation resulting transformation of the primary deconjugated bile acids into

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secondary bile acids [78]. While deconjugation reactions are mediated by various colonic bacteria, 7α-dehydroxylation is restricted to a limited number of intestinal bacteria such as *clostridium scindens* [77]. Bile salt hydrolases encoding genes have been found in gut microbes including Bacteroides, Clostridium, Lactobacillus, and Bifidobacterium [77]. The conversion of primary to secondary bile acids by 7a-dehydroxylases is probably one of the most physiologically relevant microbial transformations in human [77]. Through 7a-dehydroxylation, the primary cholic acid is transformed into the secondary deoxycholic acid, and the primary chenodeoxycholic acid is transformed into the secondary lithocholic acid. 7a-dehydroxylation activities have been characterized in the genera Eubacterium and Clostridium, including the species *Clostridium scindens* and *Clostridium hylemonae* [77]. Intestinal microbes participate in trimethylamine-N-oxide (TMAO) production, a well-known metabolite related with atherosclerosis development. Dietary sources including choline, phosphadidylcholine, L-carnithine, and other methylamine containing nutrients provide substrates for microbiota-mediated production of trymethlamine (TMA). It has been suggested that TMA is absorbed into IECs and subsequently metabolized to TMAO by flavin monooxygenase in the liver [82–84].

C. Amino acid metabolites

Although most of protein digestion and absorption occur in the small intestine, some undigested proteins and amino acids together with nitrogenous compounds move into the large intestine. The transferred proteins are degraded mostly in the descending colon, because of luminal pH difference (slightly acidic in ascending to neutral in descending) and activity of proteases produced by colonocytes and microbes [85]. Moreover, large intestinal

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microbes prefer carbohydrate metabolism rather than protein metabolism, thus protein fermentation occurs mainly in the descending colon where a large amount of carbohydrates are already depleted [85]. In the large intestine, amino acids fermenting microbes utilize amino acids intensively for proteolytic fermentation or *de novo* synthesis of amino acids rather than the synthesis of microbial cell components [86,87]. The predominant amino acid fermenting microbes detected in the human large intestine are *Bacteroides and Propionibacterium.* The abundance of *Bacteroides* is highly correlated with high protein diet such as various meats [88]. Also, other proteolytic microbes, *Streptococci, Clostridium, Baccilus, and Staphylococcus* are detected in the colon [85,89]. The microbes generate diverse metabolites including branched-chain fatty acids (BCFAs), SCFAs, essential amino acids, polyamines, and ammonia through proteolytic fermentation of amino acid precursors.

Amino acids serve as precursors for SCFAs during proteolytic fermentation similar to carbohydrate fermentation. Acetate is generated from catabolism of glycine, alanine, threonine, glutamate, lysine, and aspartate. Butyrate is produced from catabolism of glutamate and lysine, and propionate from alanine and threonine [90]. Approximately 5-10% of SCFAs is BCFAs such as isobutyrate, isovalerate, and valerate [91]. BCFAs are produced during fermentation of branched chain amino acids; leucine, valine and isoleucine.

Tryptophan is metabolized by kynurenine, serotonin and indole pathways. Approximately 95% of tryptophan is metabolized through kynurenine pathway, a cascade of enzymatic reactions [92]. Indoleamine 2,3-dioxygenase (IDO) produced by immune cells and IECs initiates degradation of tryptophan and leads to the production of metabolites including kynurenine, kynurenic acid, 3-hydroxykynurenine (3-OHKyn), 3-hydroxyanthranilic acids (3HAA), and quinolinic acid. Minor portion of tryptophan (~1%) is converted to serotonin

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by tryptophan hydroxylase enzyme expressed by enterochromaffin cells. Both kynurenine and serotonin production pathway are regulated by host cells. On the other hand, approximately 5% of tryptophan is catabolized by gut microbes (e.g. *Lactobacilli and Clostridium sporogenes*) producing tryptophanase and decarboxylase, resulting in generation of tryptamine and indole metabolites including indole-3-propionic acid, indole-3-acetic acid, and indole-3-aldehyde [93].

3. Mucosal immune regulatory mechanism of gut metabolites

The balance of intestinal immune system is controlled by various extrinsic factors. As intestinal tissues are constantly exposed to trillions of gut microbes and dietary components thus, not surprisingly, they are one of the most important regulatory factors for intestinal immunity. Accumulating studies reported that, together with dietary factors, altered gut microbiome is closely associated with gut inflammatory diseases such as IBD. While underlying mechanisms are yet to be fully understood, abnormal intestinal immune responses induced by altered gut metabolite profiles are considered as a critical reason for the development of disease in the gut. As a number of studies have unveiled mechanisms for explaining role of diet-microbiota-metabolite axis in regulation of host immunity, we summarize the emerging molecular and cellular mechanism of intestinal immunity regulation by major gut metabolites from carbohydrate, lipid, bile acids, and amino acids.

A. Carbohydrate metabolites

Lactic acid bacteria, the most popular probiotic strain, has been used for enhancing health for a long time. One of suggesting mechanisms of positive effects of lactic acid bacteria

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is through lactate production. Lactate is a major component of lactic acid bacteria fermented food and can regulate critical functions macrophages, DCs, T cells, and epithelial cells. Lactate is known to have immunomodulatory effects in the inflammatory environment [94]. GPR81, a cell-surface receptor for lactate, regulate intestinal homeostasis and protect mice from experimental colitis. A study found that GPR81 deficient mice displayed imbalanced $CD4^+$ T cell subsets, which characterized by increased Th1/Th17 and decreased Treg. GPR81 signaling in colonic DCs and macrophages are important for suppressing inflammation and restoring colonic homeostasis [95]. Lactate produced in the gut is recognized by Gpr81 expressed on Paneth cells and stromal cells and activates Wnt/β-catenin signal for gut homeostasis [8]. Microbiota-derived lactate has been suggested as a major factor inducing enterocyte hyperproliferation in starvation-refed mice [96]. Furthermore, lactate and pyruvate produced by bacteria induce dendrite protrusion of intestinal $CX3CR1^+$ macrophages in a GPR31-dependant manner, causing enhancement of local immune responses and high resistance to intestinal *Salmonella* infection [97].

SCFAs, well-known microbial metabolites, have been investigated extensively for its role on maintenance of immune homeostasis through regulation of epithelial integrity as well as innate and adaptive immunity (Figure 2). SCFAs involved in the regulation of a variety of intestinal immune cells through mainly three distinct mechanisms: 1) integration of cellular metabolism, 2) inhibition of histone deacetylases (HDAC), and 3) G-protein coupled receptors (GPCRs) activation.

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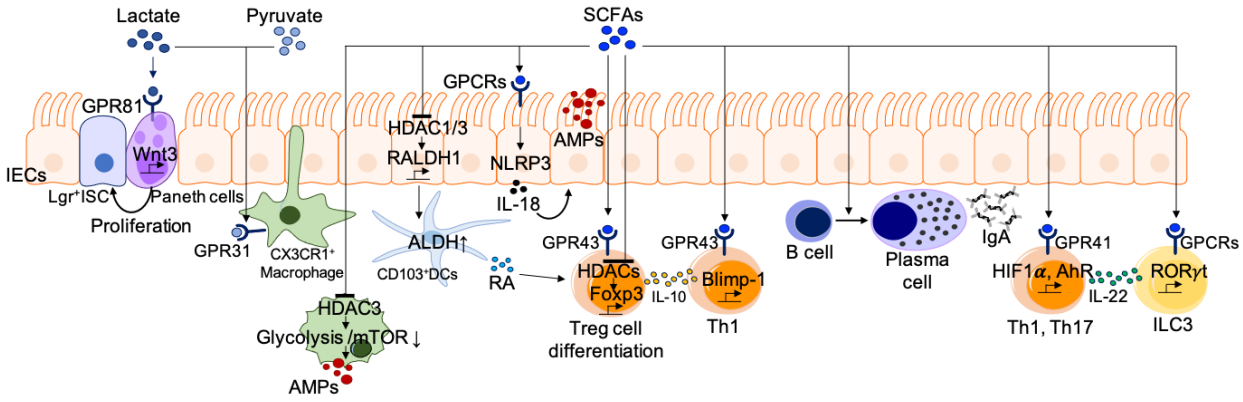

Figure 2. Immune regulation by carbohydrate metabolites.
Lactate and pyruvate induce dendrite protrusion of intestinal CX3CR1⁺ macrophages via GRP31 to enhance immune response. Lactate also maintains gut homeostasis via Wnt/β-catenin signaling activation to induce intestinal stem cell proliferation. SCFAs act as HDAC inhibitors or stimulator for GPCRs to regulate intestinal immune homeostasis. SCFAs induce Treg cell differentiation, plasma B cell differentiation, and IL-22 production from ILC3 as well as Th1 and Th17 cells. Also, SCFAs regulate activity of certain macrophages and DCs. AhR, aryl hydrocarbon receptor; ALDH, aldehyde dehydrogenase; AMPs, anti-microbial peptides; GPCRs, G protein coupled receptor; HDAC, histone deacetylase; HIF1α, hypoxia-inducible factor 1-alpha; ISC, intestinal stem cell; RA, retinoic acid.

1) Integration of cellular metabolism. As SCFAs are small, they can be absorbed into various cells and then integrate into cellular metabolism. The most prominent role of SCFAs is energy source for colonocytes and immune cells as well [69]. It is well known that dietary fiber and SCFAs support intestinal epithelial proliferation. SCFAs are converted to acetyl-CoA for energy production through the tricarboxylic acid cycle and lipid synthesis [69]. Immune cells need a significant portion of metabolic building blocks during the activation. For instance, tey utilize available nutrients to produce energy (e.g., adenosine triphosphate, ATP), biogenesis of cellular component and production of effector molecules such as antibodies and cytokines. Moreover, SCFAs integrate cellular metabolism to support functional changes in adaptive immune cells. Both glycolysis and oxidative phosphorylation for ATP production is prerequisite step for B cell activation and plasma B cell differentiation [98]. Kim and

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colleagues reported that dietary fiber and SCFAs support plasma B cell differentiation through metabolic integration [99]. In fact, SCFA (C3 or SCFA mixture) administration rescued impaired germinal center formation and IgA expression in mice fed with low-fiber diet. SCFAs promote metabolic process such as fatty acid oxidation and mitochondrial respiration in B cells during the activation. These results suggest that SCFAs partially mediate positive effects of dietary fiber intake on gut IgA responses by supporting metabolic process [99].

2) Act as HDAC inhibitors. Histone acetylation is an epigenetic modification of histone, which promotes open chromatin by adding acetyl groups onto the lysine residues and activates the transcription. On the contrary, HDACs remove acetyl groups and repress gene transcription. SCFAs inhibit HDACs to promote gene expression in epithelial cells, T cells, and macrophages. SCFAs promote gut barrier integrity through induction of AMPs such as RegIIIγ and β-defensin in epithelial cells via activation of mammalian target of rapamycin signaling pathway (mTOR) and STAT3 [100,101]. Such functional regulation is mediated by HDAC inhibition together with AMP-activated protein kinase (AMPK) activation. Diet-derived SCFAs are also known to stimulate IECs to induce mucosal tolerogenic DCs through HDAC inhibitory action [102]. SCFAs induced increase of RALDH1 by inhibiting HDAC1 and HDAC3 in IECs. The increased RALDH1 expression in IECs correlates with ALDH activity of $CD103^+$ tolerogenic DCs, along with increased numbers of intestinal Treg and high luminal IgA [102]. Among SCFA species, butyrate has a strong HDAC-inhibitory activity. HDCA3 inhibition by butyrate induces differentiation of macrophages possessing antimicrobial activity. The inhibition of metabolic program in macrophages by glycolysis and mTOR inhibition results in enhancing AMPs, such as S100A8/A9/A12 and lysozyme [103]. SCFAs

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as HDAC inhibitors also affect T cell differentiation through the regulation of metabolic sensors [104]. For instance, HDAC inhibition by SCFAs enhances acetylation of key metabolic sensors for mTOR pathway (p70S6 kinase and phosphorylation rS6) to support IL-10 expressing Treg differentiation. Butyrate directly induce Treg generation for immune tolerance in response to commensal bacteria by enhancing acetylation of genetic locus for *Foxp3* [30,105,106]. In addition to Treg, SCFAs can directly promote naïve $CD4^+$ T-cell differentiation into Th1 or Th17 cells [104].

3) GPCRs activation. SCFAs can activate many cell types via GPCRs expressed on diverse cells including IECs, neutrophils, macrophages, DCs, B and T cells, and ILCs. Acetate is known to be a ligand of GPR43 and propionate is of GPR43 and GPR41. Butyrate induces the activation through GPR41 and GPR109A [107]. SCFAs facilitate the production of inflammatory effector molecules by IECs in response to immune challenges including ethanol-induced breach, TNBS, and *C. rodentium* infection [108]. GPR41 and GPR43 deficient animals displayed abnormally low level of inflammatory responses in the gut [108]. SCFAs recognized by GPR43 and GRP109A on IECs activate NLRP3 inflammasome leading to IL-18 secretion for maintenance of gut barrier integrity in chronic inflammation and colorectal cancer cases [109–112]. SCFAs activate antigen specific Th2 cells to produce immunosuppressive cytokines IL-10 [113] and IL-22 [114,115] via GPCRs. Butyrate binding to GPR41 promotes IL-22 production in Th1 and Th17 as well as ILC3 by upregulation of hypoxia-inducible factor 1α and AhR [115]. Propionate binding to GPR43 regulate colonic IL-22 expression in ILC3 via AKT and STAT3 signaling. GPR43-deficient ILC3s enhance susceptibility to colonic inflammation and *C. rodentium* infection [114]. Neutrophils express SCFA receptor GPR43, and its activation induces chemotaxis and functional activation that

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could have a role on intestinal homeostasis. SCFAs also indirectly regulate Treg generation by inducing expression of anti-inflammatory molecules (IL-10 and Aldh1a) on DCs and macrophage [112]. Propionate also induces increase of GPR15 expression, which regulates homing of Treg to the large intestine [30,116].

B. Lipid and bile acid metabolites

A variety of microbial metabolites are produced from lipids that have a significant impact on immune system through regulation of functional activities of immune cells (Figrue 3). PUFAs, ω-3 and ω-6, are essential fatty acids metabolized into bioactive lipid mediators through the reactions that are mediated by several oxidative enzymes. It is thought that ω-3 PUFAs dampen inflamatory reactions, whereas ω-6 PUFAs have pro-inflammatory properties [117]. It has been reported that the resolution of inflammation by ω-3 PUFAs plays a role on recovery of intestinal immune homeostasis via GPR120 [118]. The resolution process includes the termination of neutrophil recruitment, suppression of pro-inflammatory responses, stimulation of cell debris clearance by macrophages, and tissue remodeling. As aforementioned, commensal bacteria metabolize PUFA to produce microbial metabolites such as CLA, HYA, oxo fatty acids, and hydroxy fatty acids. Administration of HYA ameliorates experimental colitis by enhancing the expression of tight junction (TJ) proteins on epithelial cells. The TJ constitutes the barrier to the passage of ions and molecules through the paracellular pathway and to the movement molecules including, but not limited to, proteins and lipids between the apical and the basolateral side of the plasma membrane. TJ proteins are mutliprotein junctional complex that observed at the tight junctions of epithelial, endothelial and myelinated cells. Occludin and claudins are integral TJ proteins capable of

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interacting adhesively with complementary molecules on adjacent cells, working together with another class of TJ protein, Zonula occludens that are required for the coordination of signals coming from the plasma membrane. The barrier integrity regulation by HYA initiates through the interaction with GPR40, which suppresses TNFR2 expression via NF-kB and MEK-ERK pathways [119].

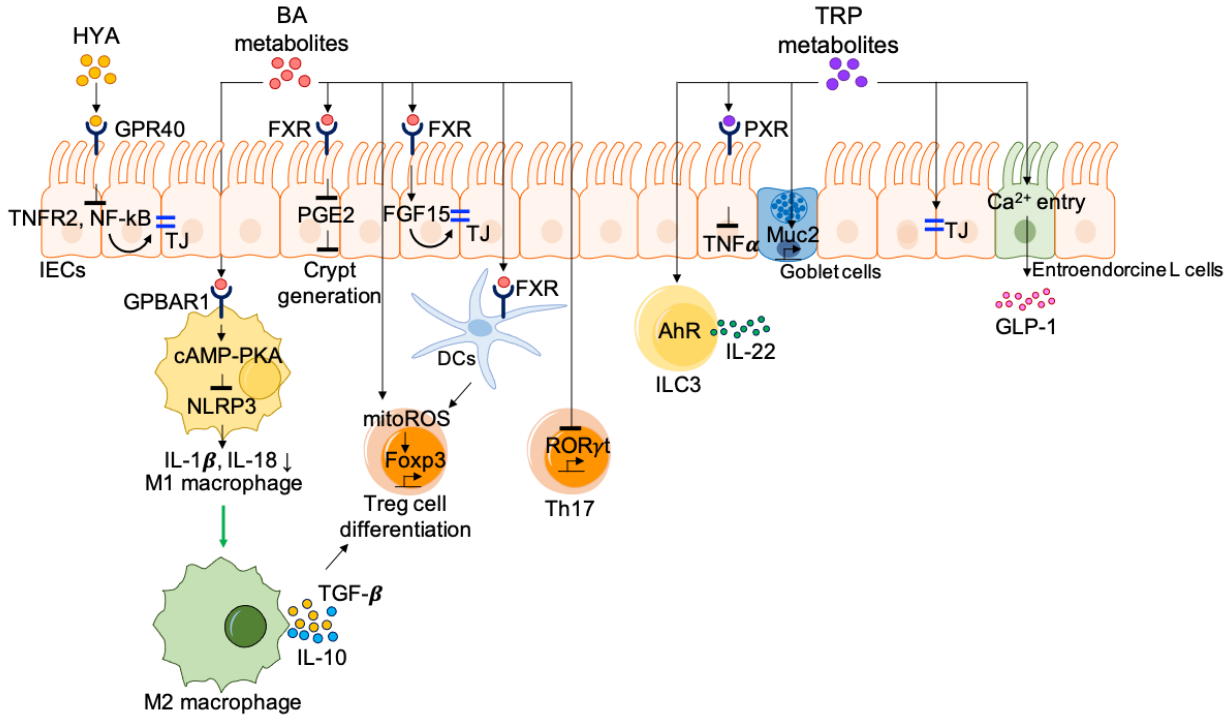

Figure 3. Immune regulation by metabolites derived from lipid and amino acids. Microbial metabolites produced from lipids and amino acids regulate intestinal homeostasis. HYA from lipids and tryptophan metabolites induce the expression of TJ proteins on epithelial cells to fortify intestinal barrier integrity. Bile acid metabolites inhibit differentiation of Th17 cells, but promote Treg cell differentiation through production of mitoROS, macrophage conversion into M2 macrophage, and FXR signaling on DCs. Also, bile acids help to maintain gut barrier integrity through accelerating crypt regeneration and induction of TJ proteins. Tryptophan metabolites enhance intestinal homeostatis through AhR activation to promote IL-22 production in ILC3 and stimulation of enteroendocrine L cells to produce GLP-1. BA, bile acdis; EFG15, epidermal growth factor 15; FoxP3, forkhead box P3; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; GPBAR1, G protein-coupled bile acid receptor 1; HYA, 10-hydroxy-cis-12-octadeccenoid acid; IECs, Intestinal Epithelial Cells; mitoROS, mitochondrial reactive oxygen species; MUC2, mucin 2; PGE2, prostaglandin E2; PXR, pregnane X receptor; RORγt, RAR-related orphan receptor gamma; TJ, tight junction; TNFα, tumor necrosis factor alpha; TRP, tryptophan.

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Host enzymatic reaction produce primary bile acids and gut microbiota further produce various secondary bile acids that work as signaling molecules via interaction with receptors; the farnesoid X receptor, the vitamin D receptor, G protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5, GPCR19, or M-BAR), and the pregnane X receptor [120–123]. Ablation of GPBAR1, farnesoid X receptor (FXR), or pregnane X receptor (PXR) causes increased susceptibility to chemically induced intestinal inflammation [124–127]. Specifically, bile acids activate GPBAR1 or FXR in macrophages, resulting in differentiation of anti-inflammatory properties and reduction of pro-inflammatory cytokine production via inhibition of NLRP3-dependent inflammasome and NF-κB-dependent signaling pathways [128]. Exposure of GPBAR1 agonist shifts inflammatory M1 to anti-inflammatory M2 macrophages, consequently increasing Treg via expression of IL-10 and TGF-β [126,129]. In terms of Treg differentiation, multiple groups reported that significant role of bile acids on induction of intestinal Treg via activation of bile acid receptors. IsoDCA, the secondary bile acid generated via microbial epimerization of cholic acid, promotes Treg differentiation through FXR signaling on DCs [81]. Interestingly, in this study, researchers utilized synthetic biology approach and designed minimal microbial consortia containing IsoDCA-producing bacteria that promotes RORγt$^+$ Treg pool in the gut [81]. Intriguingly, the homeostasis of RORγt-expressing Treg is regulated by the gut bile acids pool rather than single type of primary or secondary bile acids [130]. The mixture of certain primary bile acids (e.g., cholic acid / ursodeoxycholic acid mix, chenodeoxycholic acid / ursodeoxycholic acid mix, cholic acid / chenodeoxycholic acid / ursodeoxycholic acid mix) and secondary bile acids generated from bacterial oxidation and dihydroxylation pathway preferentially maintain colonic frequencies of RORγt$^+$ Treg and Foxp3$^+$ Treg. It points out the importance of host-

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microorganism biliary network on maintenance of immune homeostasis in the gut [130]. In addition to effect on Treg differentiation, bile acids metabolites are able to directly regulate balance between Th17 and Treg cells. The 3-oxoLCA signlaling through RORγt inhibits differentiation of Th17 cells. Another bile acid metabolites, isoalloLCA also increases Treg differentiation by enhancement of mitochondrial reactive oxygen species (mitoROS)-dependent Foxp3 expression [131]. One of important regulatory functions of secondary bile acids is to fortify gut barrier function through multiple mechanisms, including maintaining intestinal barrier integrity and inhibiting pathogen colonization. DCA downregulates prostaglandin E2 synthesis in an FXR-dependent manner, thereby accelerating intestinal crypt regeneration and wound repair [132]. Administration of a mixture of LCA and ursodeoxycholic acid helps to maintain gut barrier integrity through the activation of the FXR-FGF15 pathway [133].

C. Amino acid metabolites

The absorbed amino acids are important on the maintenance of IEC integrity (Figure 3). The protective functions of amino acids in the intestine is closely associated with the apoptosis and proliferation of IECs, expression of TJ proteins, alleviation of intestinal inflammation and oxidative stress by inhibiting NF-κB signaling pathway, and activating nuclear erythroid-related factor 2 (Nrf2) signaling pathway. Catabolism of glutamate, glutamine, and aspartate provides ATP required for metabolic processes of IECs [134]. L-glutamine enhances intestinal enterocytes growth in porcine epithelial cells, IPEC-1 cultured in glutamine-free media. L-glutamine treatment activated mTOR, independently of AMPK [135]. Also, some amino acids are essential for limiting cellular stress on epithelial cells. For

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instance, glutathione synthesized from glutamate, glycine, and cysteine is a powerful antioxidant, which protects IECs from oxidative damage [136]. Furthermore, amino acids act as essential precursors for synthesis of important proteins and peptides such as mucins, immunoglobulins, defensins for maintaining normal gut structure and function.

Among amino acid metabolism in relation to gut physiology, tryptophan metabolism is the most widely reported as tryptophan metabolites play an important role in regulation of intestinal immune homeostasis. Lacking tryptophan in diet or its amino acid transporter, B^0AT1 (SLC6A19) decreases the expression of anti-microbial peptides in enterocytes with alteration of the gut microbiome, which increases susceptibility to intestinal inflammation [137]. Metabolites derived from tryptophan catabolism regulated by host or microbiota act as aryl hydrocarbon receptor ligands, which control transcription of a wide variety of target genes such as IL-22 and IL-10 [138–140]. Indole metabolites derived from microbial tryptophan metabolism such as indole 3-aldehyde and indole 3-propionic acid activate AhR to promote IL-22 production in ILC3, which increases the expression of anti-microbial peptides and consequently enhances intestinal homeostasis [138]. Deficiency of caspase recruitment domain 9, an IBD susceptibility gene, alters gut microbiota that fails to produce AhR ligands because of impaired tryptophan metabolism. It leads to the reduction of IL-22 and thus higher susceptibility to colitis [141]. Indole 3-propionic acid, a symbiotic bacterial indole metabolite, activates PXR on IECs, which regulates TLR4-mediated control of the intestinal barrier function [142]. Oral administration of indole 3-propionic acid ameliorates DSS-induced colitis with increased colonic epithelial IL-10R1 expression [143]. Indole acrylic acid produced by commensal *Peptostreptococcus* species has anti-inflammatory effects detected by enhanced IL-10 production and increased Muc2 expression [144]. Indole

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promotes intestinal epithelial barrier function by inducing expression of tight junction associated genes such as *Cldn7*, *Ocln*, and *Tjp1* [144]. Even brief exposure of indole enhances Ca^{2+} entry into enteroendocrine L cells leading to glucagon-like peptide-1 production [145]. In addition to indole pathway, kynurenine pathway participates in regulation of immune homeostasis. Considering IDO1 initiates kynurenine pathway, IDO1 activity and its regulatory function in association with kynurenine pathway have been well studied. Activation of IDO1 in DCs and macrophages results in increased production of kynurenine, which induces Treg generation by activating AhR and ultimately promotes homeostasis [146–148]. Kynurenine generated by IFNγ-stimulated IDO1 induction increases IL-10R1 expression on intestinal epithelia cells resulting in mitigation of colitis [149]. Furthermore, overexpression of IDO1 in the intestinal epithelium augments resistance to colitis via promotion of secretory cell differentiation and mucus production [150].

3. Summary

The intestinal homeostasis requires stringent regulation of the immune responses. Multiple types of innate and adaptive immune cells that maintain the balance between tolerance and activation in intestinal tissues facing potential antigens, including dietary materials, commensal bacteria, and sometimes unexpected pathogens. These signals derived from antigenic materials in the lumen of intestine act as key regulatory factors to regulate intestinal inflammation.

Through both host and microbial enzymatic activities, diet can be converted into various gut metabolites, which have unique functional activities. From recent progress of

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field of nutritional immunology, we start to understand molecular and cellular mechanism by which gut metabolites regulate intestinal immune cells. Unveiled mechanisms includes activation of specific cell surface receptors, controlling of gene expression by epigenetic regulation, and integration of cellular metabolism. These mechanistic studies help to develop new strategy to control intestinal diseases such as IBD and colorectal cancer.

To develop effective strategy, we need to focus on raising issues from recent progress on metabolite studies as followed. 1) The first, differential effects of metabolites on intestinal inflammation. Several contradict reports suggested that metabolites impact intestinal inflamatory reaction differently. For example, SCFAs have a dual role for T cell differentiation and intestinal inflammation. The explanation for this issue is probable that the role of gut metabolites in the regulation of intestinal inflammation could be switched by immune tone; inflammatory disease or cancer vs. homeostatic condition. Thus, we need to put more efforts on understanding how gut metabolites differentially affect immune cell functions in the context of intestinal immunity. 2) The second, balance of metabolites in the gastrointestinal tract. Intestinal immune cells exposing diverse gut metabolite pool. Thus, changes of intestinal immunity can be explained by actions of multiple metabolites, not just single type of metabolite. We need to have an integrated approach to understand complex metabolite signals. For example, we may need to determine whether certain metabolite affect functional activities of other metabolite on intestinal immunity. 3) The third, precision strategy to manipulate diet-metabolite-microbiota axis. To induce production of particular metabolites for controlling intestinal immunity, it could be necessary to directly admnister the diet or intervene microbiota communities. Practical strategy to control intestinal environment need to be developed based on physiological and immunological conditions required in the

gastrointestinal tract. Collectively, further integrative mechanistic studies for gut metabolites will proceed the development of effective dietary strategy to prevent or cure the intestinal diseases.

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References

1. Zheng, D.; Liwinski, T.; Elinav, E. Interaction between microbiota and immunity in health and disease. *Cell Res.* **2020**, *30*, 492–506, doi:10.1038/s41422-020-0332-7.
2. Rooks, M.G.; Garrett, W.S. Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol.* **2016**, *16*, 341–352, doi:10.1038/nri.2016.42.
3. Mowat, A.M.; Agace, W.W. Regional specialization within the intestinal immune system. *Nat. Rev. Immunol.* **2014**, *14*, 667–85, doi:10.1038/nri3738.
4. Ohno, H. Intestinal M cells. *J. Biochem.* **2016**, *159*, 151–160, doi:10.1093/jb/mvv121.
5. Reboldi, A.; Cyster, J.G. Peyer's patches: organizing B-cell responses at the intestinal frontier. *Immunol. Rev.* **2016**, *271*, 230–245, doi:10.1111/imr.12400.
6. Randall, T.D.; Mebius, R.E. The development and function of mucosal lymphoid tissues: a balancing act with micro-organisms. *Mucosal Immunol.* **2014**, *7*, 455–66, doi:10.1038/mi.2014.11.
7. Houston, S.A.; Cerovic, V.; Thomson, C.; Brewer, J.; Mowat, A.M.; Milling, S. The lymph nodes draining the small intestine and colon are anatomically separate and immunologically distinct. *Mucosal Immunol.* **2016**, *9*, 468–478, doi:10.1038/mi.2015.77.
8. Lee, Y.-S.; Kim, T.-Y.; Kim, Y.; Lee, S.-H.; Kim, S.; Kang, S.W.; Yang, J.-Y.; Baek, I.-J.; Sung, Y.H.; Park, Y.-Y.; et al. Microbiota-Derived Lactate Accelerates Intestinal Stem-Cell-Mediated Epithelial Development. *Cell Host Microbe* **2018**, *24*, 833-846.e6, doi:10.1016/j.chom.2018.11.002.
9. Parada Venegas, D.; De la Fuente, M.K.; Landskron, G.; González, M.J.; Quera, R.; Dijkstra, G.; Harmsen, H.J.M.; Faber, K.N.; Hermoso, M.A. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front. Immunol.* **2019**, *10*, doi:10.3389/fimmu.2019.00277.

10. Levy, M.; Thaiss, C.A.; Zeevi, D.; Dohnalová, L.; Zilberman-Schapira, G.; Mahdi, J.A.; David, E.; Savidor, A.; Korem, T.; Herzig, Y.; et al. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. *Cell* **2015**, *163*, 1428–1443, doi:10.1016/j.cell.2015.10.048.

11. Allaire, J.M.; Crowley, S.M.; Law, H.T.; Chang, S.-Y.; Ko, H.-J.; Vallance, B.A. The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. *Trends Immunol.* **2018**, *39*, 677–696, doi:10.1016/j.it.2018.04.002.

12. Alam, A.; Neish, A. Role of gut microbiota in intestinal wound healing and barrier function. *Tissue Barriers* **2018**, *6*, 1539595, doi:10.1080/21688370.2018.1539595.

13. Lebedev, K.A.; Poniakina, I.D. [Immunophysiology of epithelial cells and pattern-recognition receptors]. *Fiziol. Cheloveka 32*, 114–26.

14. Gourbeyre, P.; Berri, M.; Lippi, Y.; Meurens, F.; Vincent-Naulleau, S.; Laffitte, J.; Rogel-Gaillard, C.; Pinton, P.; Oswald, I.P. Pattern recognition receptors in the gut: analysis of their expression along the intestinal tract and the crypt/villus axis. *Physiol. Rep.* **2015**, *3*, e12225, doi:10.14814/phy2.12225.

15. Schneider, C.; O'Leary, C.E.; von Moltke, J.; Liang, H.-E.; Ang, Q.Y.; Turnbaugh, P.J.; Radhakrishnan, S.; Pellizzon, M.; Ma, A.; Locksley, R.M. A Metabolite-Triggered Tuft Cell-ILC2 Circuit Drives Small Intestinal Remodeling. *Cell* **2018**, *174*, 271-284.e14, doi:10.1016/j.cell.2018.05.014.

16. Burrows, K.; Ngai, L.; Wong, F.; Won, D.; Mortha, A. ILC2 Activation by Protozoan Commensal Microbes. *Int. J. Mol. Sci.* **2019**, *20*, 4865, doi:10.3390/ijms20194865.

17. Gause, W.C.; Rothlin, C.; Loke, P. Heterogeneity in the initiation, development and function of type 2 immunity. *Nat. Rev. Immunol.* **2020**, *20*, 603–614, doi:10.1038/s41577-020-0301-x.

18. Keir, M.E.; Yi, T.; Lu, T.T.; Ghilardi, N. The role of IL-22 in intestinal health and disease. *J. Exp. Med.* **2020**, *217*, doi:10.1084/jem.20192195.

19. Zhen, Y.; Zhang, H. NLRP3 Inflammasome and Inflammatory Bowel Disease. *Front. Immunol.* **2019**, *10*, doi:10.3389/fimmu.2019.00276.

20. Elinav, E.; Strowig, T.; Kau, A.L.; Henao-Mejia, J.; Thaiss, C.A.; Booth, C.J.; Peaper, D.R.; Bertin, J.; Eisenbarth, S.C.; Gordon, J.I.; et al. NLRP6 Inflammasome Regulates Colonic Microbial Ecology and Risk for Colitis. *Cell* **2011**, *145*, 745–757, doi:10.1016/j.cell.2011.04.022.

21. Merad, M.; Sathe, P.; Helft, J.; Miller, J.; Mortha, A. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. *Annu. Rev. Immunol.* **2013**, *31*, 563–604, doi:10.1146/annurev-immunol-020711-074950.

22. Sawai, C.M.; Serpas, L.; Neto, A.G.; Jang, G.; Rashidfarrokhi, A.; Kolbeck, R.; Sanjuan, M.A.; Reizis, B.; Sisirak, V. Plasmacytoid Dendritic Cells Are Largely Dispensable for the Pathogenesis of Experimental Inflammatory Bowel Disease. *Front. Immunol.* **2018**, *9*, doi:10.3389/fimmu.2018.02475.

23. Arimura, K.; Takagi, H.; Uto, T.; Fukaya, T.; Nakamura, T.; Choijookhuu, N.; Hishikawa,

Y.; Yamashita, Y.; Sato, K. Crucial role of plasmacytoid dendritic cells in the development of acute colitis through the regulation of intestinal inflammation. *Mucosal Immunol.* **2017**, *10*, 957–970, doi:10.1038/mi.2016.96.

24. Dasgupta, S.; Erturk-Hasdemir, D.; Ochoa-Reparaz, J.; Reinecker, H.-C.; Kasper, D.L. Plasmacytoid Dendritic Cells Mediate Anti-inflammatory Responses to a Gut Commensal Molecule via Both Innate and Adaptive Mechanisms. *Cell Host Microbe* **2014**, *15*, 413–423, doi:10.1016/j.chom.2014.03.006.
25. Shimokawa, C.; Kanaya, T.; Hachisuka, M.; Ishiwata, K.; Hisaeda, H.; Kurashima, Y.; Kiyono, H.; Yoshimoto, T.; Kaisho, T.; Ohno, H. Mast Cells Are Crucial for Induction of Group 2 Innate Lymphoid Cells and Clearance of Helminth Infections. *Immunity* **2017**, *46*, 863-874.e4, doi:10.1016/j.immuni.2017.04.017.
26. Gieseck, R.L.; Wilson, M.S.; Wynn, T.A. Type 2 immunity in tissue repair and fibrosis. *Nat. Rev. Immunol.* **2018**, *18*, 62–76, doi:10.1038/nri.2017.90.
27. Olivares-Villagómez, D.; Van Kaer, L. Intestinal Intraepithelial Lymphocytes: Sentinels of the Mucosal Barrier. *Trends Immunol.* **2018**, *39*, 264–275, doi:10.1016/j.it.2017.11.003.
28. Ai, T.L.; Solomon, B.D.; Hsieh, C.-S. T-cell selection and intestinal homeostasis. *Immunol. Rev.* **2014**, *259*, 60–74, doi:10.1111/imr.12171.
29. van Wijk, F.; Cheroutre, H. Mucosal T cells in gut homeostasis and inflammation. *Expert Rev. Clin. Immunol.* **2010**, *6*, 559–566, doi:10.1586/eci.10.34.
30. Smith, P.M.; Howitt, M.R.; Panikov, N.; Michaud, M.; Gallini, C.A.; Bohlooly-Y, M.; Glickman, J.N.; Garrett, W.S. The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis. *Science (80-.).* **2013**, *341*, 569–573, doi:10.1126/science.1241165.
31. Kim, M.; Qie, Y.; Park, J.; Kim, C.H. Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host Microbe* **2016**, *20*, 202–214, doi:10.1016/j.chom.2016.07.001.
32. Sanchez, H.N.; Moroney, J.B.; Gan, H.; Shen, T.; Im, J.L.; Li, T.; Taylor, J.R.; Zan, H.; Casali, P. B cell-intrinsic epigenetic modulation of antibody responses by dietary fiber-derived short-chain fatty acids. *Nat. Commun.* **2020**, *11*, 60, doi:10.1038/s41467-019-13603-6.
33. Cani, P.D.; Van Hul, M.; Lefort, C.; Depommier, C.; Rastelli, M.; Everard, A. Microbial regulation of organismal energy homeostasis. *Nat. Metab.* **2019**, *1*, 34–46, doi:10.1038/s42255-018-0017-4.
34. Cani, P.; Delzenne, N. The Role of the Gut Microbiota in Energy Metabolism and Metabolic Disease. *Curr. Pharm. Des.* **2009**, *15*, 1546–1558, doi:10.2174/138161209788168164.
35. Levy, M.; Thaiss, C.A.; Elinav, E. Metabolites: messengers between the microbiota and the immune system. *Genes Dev.* **2016**, *30*, 1589–1597, doi:10.1101/gad.284091.116.
36. Donaldson, G.P.; Lee, S.M.; Mazmanian, S.K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **2016**, *14*, 20–32, doi:10.1038/nrmicro3552.
37. Marchesi, J.R.; Adams, D.H.; Fava, F.; Hermes, G.D.A.; Hirschfield, G.M.; Hold, G.;

Quraishi, M.N.; Kinross, J.; Smidt, H.; Tuohy, K.M.; et al. The gut microbiota and host health: a new clinical frontier. *Gut* **2016**, *65*, 330–339, doi:10.1136/gutjnl-2015-309990.

38. Britton, G.J.; Contijoch, E.J.; Mogno, I.; Vennaro, O.H.; Llewellyn, S.R.; Ng, R.; Li, Z.; Mortha, A.; Merad, M.; Das, A.; et al. Microbiotas from Humans with Inflammatory Bowel Disease Alter the Balance of Gut Th17 and RORγt+ Regulatory T Cells and Exacerbate Colitis in Mice. *Immunity* **2019**, *50*, 212-224.e4, doi:10.1016/j.immuni.2018.12.015.

39. Eckburg, P.B. Diversity of the Human Intestinal Microbial Flora. *Science (80-.).* **2005**, *308*, 1635–1638, doi:10.1126/science.1110591.

40. Flint, H.J.; Scott, K.P.; Louis, P.; Duncan, S.H. The role of the gut microbiota in nutrition and health. *Nat. Rev. Gastroenterol. Hepatol.* **2012**, *9*, 577–589, doi:10.1038/nrgastro.2012.156.

41. Zoetendal, E.G.; Raes, J.; van den Bogert, B.; Arumugam, M.; Booijink, C.C.; Troost, F.J.; Bork, P.; Wels, M.; de Vos, W.M.; Kleerebezem, M. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J.* **2012**, *6*, 1415–1426, doi:10.1038/ismej.2011.212.

42. Jang, C.; Hui, S.; Lu, W.; Cowan, A.J.; Morscher, R.J.; Lee, G.; Liu, W.; Tesz, G.J.; Birnbaum, M.J.; Rabinowitz, J.D. The Small Intestine Converts Dietary Fructose into Glucose and Organic Acids. *Cell Metab.* **2018**, *27*, 351-361.e3, doi:10.1016/j.cmet.2017.12.016.

43. Horecker, B.L.; Mehler, A.H. Carbohydrate Metabolism. *Annu. Rev. Biochem.* **1955**, *24*, 207–274, doi:10.1146/annurev.bi.24.070155.001231.

44. Rogatzki, M.J.; Ferguson, B.S.; Goodwin, M.L.; Gladden, L.B. Lactate is always the end product of glycolysis. *Front. Neurosci.* **2015**, *9*, doi:10.3389/fnins.2015.00022.

45. Rodríguez-Colman, M.J.; Schewe, M.; Meerlo, M.; Stigter, E.; Gerrits, J.; Pras-Raves, M.; Sacchetti, A.; Hornsveld, M.; Oost, K.C.; Snippert, H.J.; et al. Interplay between metabolic identities in the intestinal crypt supports stem cell function. *Nature* **2017**, *543*, 424–427, doi:10.1038/nature21673.

46. Hove, H.; Nørgaard, H.; Brøbech Mortensen, P. Lactic acid bacteria and the human gastrointestinal tract. *Eur. J. Clin. Nutr.* **1999**, *53*, 339–350, doi:10.1038/sj.ejcn.1600773.

47. Carey, M.C.; Small, D.M.; Bliss, C.M. Lipid Digestion and Absorption. *Annu. Rev. Physiol.* **1983**, *45*, 651–677, doi:10.1146/annurev.ph.45.030183.003251.

48. Epstein, F.H.; Lewis, R.A.; Austen, K.F.; Soberman, R.J. Leukotrienes and Other Products of the 5-Lipoxygenase Pathway. *N. Engl. J. Med.* **1990**, *323*, 645–655, doi:10.1056/NEJM199009063231006.

49. Schmitz, G.; Ecker, J. The opposing effects of n−3 and n−6 fatty acids. *Prog. Lipid Res.* **2008**, *47*, 147–155, doi:10.1016/j.plipres.2007.12.004.

50. Le Chatelier, E.; Nielsen, T.; Qin, J.; Prifti, E.; Hildebrand, F.; Falony, G.; Almeida, M.; Arumugam, M.; Batto, J.-M.; Kennedy, S.; et al. Richness of human gut microbiome

correlates with metabolic markers. *Nature* **2013**, *500*, 541–546, doi:10.1038/nature12506.

51. Cotillard, A.; Kennedy, S.P.; Kong, L.C.; Prifti, E.; Pons, N.; Le Chatelier, E.; Almeida, M.; Quinquis, B.; Levenez, F.; Galleron, N.; et al. Dietary intervention impact on gut microbial gene richness. *Nature* **2013**, *500*, 585–588, doi:10.1038/nature12480.
52. Rabot, S.; Membrez, M.; Bruneau, A.; Gérard, P.; Harach, T.; Moser, M.; Raymond, F.; Mansourian, R.; Chou, C.J. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J.* **2010**, *24*, 4948–4959, doi:10.1096/fj.10-164921.
53. Velagapudi, V.R.; Hezaveh, R.; Reigstad, C.S.; Gopalacharyulu, P.; Yetukuri, L.; Islam, S.; Felin, J.; Perkins, R.; Borén, J.; Orešič, M.; et al. The gut microbiota modulates host energy and lipid metabolism in mice. *J. Lipid Res.* **2010**, *51*, 1101–1112, doi:10.1194/jlr.M002774.
54. Martinez-Guryn, K.; Hubert, N.; Frazier, K.; Urlass, S.; Musch, M.W.; Ojeda, P.; Pierre, J.F.; Miyoshi, J.; Sontag, T.J.; Cham, C.M.; et al. Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe* **2018**, *23*, 458-469.e5, doi:10.1016/j.chom.2018.03.011.
55. Kishino, S.; Takeuchi, M.; Park, S.-B.; Hirata, A.; Kitamura, N.; Kunisawa, J.; Kiyono, H.; Iwamoto, R.; Isobe, Y.; Arita, M.; et al. Polyunsaturated fatty acid saturation by gut lactic acid bacteria affecting host lipid composition. *Proc. Natl. Acad. Sci.* **2013**, *110*, 17808–17813, doi:10.1073/pnas.1312937110.
56. Hirata, A.; Kishino, S.; Park, S.-B.; Takeuchi, M.; Kitamura, N.; Ogawa, J. A novel unsaturated fatty acid hydratase toward C16 to C22 fatty acids from Lactobacillus acidophilus. *J. Lipid Res.* **2015**, *56*, 1340–1350, doi:10.1194/jlr.M059444.
57. Chen, Y.Y.; Liang, N.Y.; Curtis, J.M.; Gänzle, M.G. Characterization of Linoleate 10-Hydratase of Lactobacillus plantarum and Novel Antifungal Metabolites. *Front. Microbiol.* **2016**, *7*, doi:10.3389/fmicb.2016.01561.
58. Kang, W.-R.; Seo, M.-J.; Shin, K.-C.; Park, J.-B.; Oh, D.-K. Comparison of Biochemical Properties of the Original and Newly Identified Oleate Hydratases from Stenotrophomonas maltophilia. *Appl. Environ. Microbiol.* **2017**, *83*, doi:10.1128/AEM.03351-16.
59. Chiang, J.Y.L. Bile Acid Metabolism and Signaling. In *Comprehensive Physiology*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2013.
60. Wang, C.-Y.; Liu, S.; Xie, X.-N.; Tan, Z.-R. Regulation profile of the intestinal peptide transporter 1 (PepT1). *Drug Des. Devel. Ther.* **2017**, *Volume 11*, 3511–3517, doi:10.2147/DDDT.S151725.
61. McConnell, R.E.; Benesh, A.E.; Mao, S.; Tabb, D.L.; Tyska, M.J. Proteomic analysis of the enterocyte brush border. *Am. J. Physiol. Liver Physiol.* **2011**, *300*, G914–G926, doi:10.1152/ajpgi.00005.2011.
62. Dai, Z.-L. Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. *Front. Biosci.* **2011**, *16*, 1768, doi:10.2741/3820.

63. Bröer, S. Amino Acid Transport Across Mammalian Intestinal and Renal Epithelia. *Physiol. Rev.* **2008**, *88*, 249–286, doi:10.1152/physrev.00018.2006.

64. Stevens, B.R.; Kaunitz, J.D.; Wright, E.M. Intestinal Transport of Amino Acids and Sugars: Advances Using Membrane Vesicles. *Annu. Rev. Physiol.* **1984**, *46*, 417–433, doi:10.1146/annurev.ph.46.030184.002221.

65. Cruzat, V.; Macedo Rogero, M.; Noel Keane, K.; Curi, R.; Newsholme, P. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients* **2018**, *10*, 1564, doi:10.3390/nu10111564.

66. Koepsell, H. Glucose transporters in the small intestine in health and disease. *Pflügers Arch. - Eur. J. Physiol.* **2020**, *472*, 1207–1248, doi:10.1007/s00424-020-02439-5.

67. Röder, P. V.; Geillinger, K.E.; Zietek, T.S.; Thorens, B.; Koepsell, H.; Daniel, H. The Role of SGLT1 and GLUT2 in Intestinal Glucose Transport and Sensing. *PLoS One* **2014**, *9*, e89977, doi:10.1371/journal.pone.0089977.

68. Krautkramer, K.A.; Fan, J.; Bäckhed, F. Gut microbial metabolites as multi-kingdom intermediates. *Nat. Rev. Microbiol.* **2020**, doi:10.1038/s41579-020-0438-4.

69. den Besten, G.; van Eunen, K.; Groen, A.K.; Venema, K.; Reijngoud, D.-J.; Bakker, B.M. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **2013**, *54*, 2325–2340, doi:10.1194/jlr.R036012.

70. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332–1345, doi:10.1016/j.cell.2016.05.041.

71. Zhernakova, A.; Kurilshikov, A.; Bonder, M.J.; Tigchelaar, E.F.; Schirmer, M.; Vatanen, T.; Mujagic, Z.; Vila, A.V.; Falony, G.; Vieira-Silva, S.; et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science (80-.).* **2016**, *352*, 565–569, doi:10.1126/science.aad3369.

72. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K.S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **2010**, *464*, 59–65, doi:10.1038/nature08821.

73. Louis, P.; Flint, H.J. Formation of propionate and butyrate by the human colonic microbiota. *Environ. Microbiol.* **2017**, *19*, 29–41, doi:10.1111/1462-2920.13589.

74. Louis, P.; Hold, G.L.; Flint, H.J. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* **2014**, *12*, 661–672, doi:10.1038/nrmicro3344.

75. Sivaprakasam, S.; Bhutia, Y.D.; Yang, S.; Ganapathy, V. Short-Chain Fatty Acid Transporters: Role in Colonic Homeostasis. In *Comprehensive Physiology*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2017; pp. 299–314.

76. Cummings, J.H.; Pomare, E.W.; Branch, W.J.; Naylor, C.P.; Macfarlane, G.T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **1987**, *28*, 1221–1227, doi:10.1136/gut.28.10.1221.

77. Ridlon, J.M.; Kang, D.-J.; Hylemon, P.B. Bile salt biotransformations by human intestinal bacteria. *J. Lipid Res.* **2006**, *47*, 241–259, doi:10.1194/jlr.R500013-JLR200.

78. Gérard, P. Metabolism of Cholesterol and Bile Acids by the Gut Microbiota. *Pathogens* **2013**, *3*, 14–24, doi:10.3390/pathogens3010014.

79. Molinero, N.; Ruiz, L.; Sánchez, B.; Margolles, A.; Delgado, S. Intestinal Bacteria Interplay With Bile and Cholesterol Metabolism: Implications on Host Physiology. *Front. Physiol.* **2019**, *10*, doi:10.3389/fphys.2019.00185.

80. Ridlon, J.M.; Kang, D.J.; Hylemon, P.B.; Bajaj, J.S. Bile acids and the gut microbiome. *Curr. Opin. Gastroenterol.* **2014**, *30*, 332–338, doi:10.1097/MOG.0000000000000057.

81. Campbell, C.; McKenney, P.T.; Konstantinovsky, D.; Isaeva, O.I.; Schizas, M.; Verter, J.; Mai, C.; Jin, W.-B.; Guo, C.-J.; Violante, S.; et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* **2020**, *581*, 475–479, doi:10.1038/s41586-020-2193-0.

82. Koeth, R.A.; Wang, Z.; Levison, B.S.; Buffa, J.A.; Org, E.; Sheehy, B.T.; Britt, E.B.; Fu, X.; Wu, Y.; Li, L.; et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* **2013**, *19*, 576–585, doi:10.1038/nm.3145.

83. Wang, Z.; Klipfell, E.; Bennett, B.J.; Koeth, R.; Levison, B.S.; DuGar, B.; Feldstein, A.E.; Britt, E.B.; Fu, X.; Chung, Y.-M.; et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **2011**, *472*, 57–63, doi:10.1038/nature09922.

84. Koeth, R.A.; Lam-Galvez, B.R.; Kirsop, J.; Wang, Z.; Levison, B.S.; Gu, X.; Copeland, M.F.; Bartlett, D.; Cody, D.B.; Dai, H.J.; et al. l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J. Clin. Invest.* **2018**, *129*, 373–387, doi:10.1172/JCI94601.

85. Korpela, K. Diet, Microbiota, and Metabolic Health: Trade-Off Between Saccharolytic and Proteolytic Fermentation. *Annu. Rev. Food Sci. Technol.* **2018**, *9*, 65–84, doi:10.1146/annurev-food-030117-012830.

86. Blachier, F.; Mariotti, F.; Huneau, J.F.; Tomé, D. Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. *Amino Acids* **2007**, *33*, 547–562, doi:10.1007/s00726-006-0477-9.

87. Smith, E.A.; Macfarlane, G.T. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. *J. Appl. Bacteriol.* **1996**, *81*, 288–302, doi:10.1111/j.1365-2672.1996.tb04331.x.

88. Wu, G.D.; Chen, J.; Hoffmann, C.; Bittinger, K.; Chen, Y.-Y.; Keilbaugh, S.A.; Bewtra, M.; Knights, D.; Walters, W.A.; Knight, R.; et al. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science (80-.).* **2011**, *334*, 105–108, doi:10.1126/science.1208344.

89. Macfarlane, G.T.; Cummings, J.H.; Allison, C. Protein Degradation by Human Intestinal Bacteria. *Microbiology* **1986**, *132*, 1647–1656, doi:10.1099/00221287-132-6-1647.

90. Sayers, G.; Seelig, S.; Kumar, S. Isolated adrenal cortex cells in suspension: Stimulation and inhibition of steroidogenesis by analogues of ACTH. *J. Steroid*

Biochem. **1975**, *6*, 371–375, doi:10.1016/0022-4731(75)90159-4.

91. Mortensen, P.B.; Clausen, M.R. Short-Chain Fatty Acids in the Human Colon: Relation to Gastrointestinal Health and Disease. *Scand. J. Gastroenterol.* **1996**, *31*, 132–148, doi:10.3109/00365529609094568.
92. Badawy, A.A.-B. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *Int. J. Tryptophan Res.* **2017**, *10*, 117864691769193, doi:10.1177/1178646917691938.
93. Roager, H.M.; Licht, T.R. Microbial tryptophan catabolites in health and disease. *Nat. Commun.* **2018**, *9*, 3294, doi:10.1038/s41467-018-05470-4.
94. Certo, M.; Tsai, C.-H.; Pucino, V.; Ho, P.-C.; Mauro, C. Lactate modulation of immune responses in inflammatory versus tumour microenvironments. *Nat. Rev. Immunol.* **2020**, doi:10.1038/s41577-020-0406-2.
95. Ranganathan, P.; Shanmugam, A.; Swafford, D.; Suryawanshi, A.; Bhattacharjee, P.; Hussein, M.S.; Koni, P.A.; Prasad, P.D.; Kurago, Z.B.; Thangaraju, M.; et al. GPR81, a Cell-Surface Receptor for Lactate, Regulates Intestinal Homeostasis and Protects Mice from Experimental Colitis. *J. Immunol.* **2018**, ji1700604, doi:10.4049/jimmunol.1700604.
96. Okada, T.; Fukuda, S.; Hase, K.; Nishiumi, S.; Izumi, Y.; Yoshida, M.; Hagiwara, T.; Kawashima, R.; Yamazaki, M.; Oshio, T.; et al. Microbiota-derived lactate accelerates colon epithelial cell turnover in starvation-refed mice. *Nat. Commun.* **2013**, *4*, 1654, doi:10.1038/ncomms2668.
97. Morita, N.; Umemoto, E.; Fujita, S.; Hayashi, A.; Kikuta, J.; Kimura, I.; Haneda, T.; Imai, T.; Inoue, A.; Mimuro, H.; et al. GPR31-dependent dendrite protrusion of intestinal CX3CR1+ cells by bacterial metabolites. *Nature* **2019**, *566*, 110–114, doi:10.1038/s41586-019-0884-1.
98. Price, M.J.; Patterson, D.G.; Scharer, C.D.; Boss, J.M. Progressive Upregulation of Oxidative Metabolism Facilitates Plasmablast Differentiation to a T-Independent Antigen. *Cell Rep.* **2018**, *23*, 3152–3159, doi:10.1016/j.celrep.2018.05.053.
99. Kim, M.; Qie, Y.; Park, J.; Kim, C.H. Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host Microbe* **2016**, *20*, 202–14, doi:10.1016/j.chom.2016.07.001.
100. Zhao, Y.; Chen, F.; Wu, W.; Sun, M.; Bilotta, A.J.; Yao, S.; Xiao, Y.; Huang, X.; Eaves-Pyles, T.D.; Golovko, G.; et al. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunol.* **2018**, *11*, 752–762, doi:10.1038/mi.2017.118.
101. Chang, P. V.; Hao, L.; Offermanns, S.; Medzhitov, R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc. Natl. Acad. Sci.* **2014**, *111*, 2247–2252, doi:10.1073/pnas.1322269111.
102. Goverse, G.; Molenaar, R.; Macia, L.; Tan, J.; Erkelens, M.N.; Konijn, T.; Knippenberg, M.; Cook, E.C.L.; Hanekamp, D.; Veldhoen, M.; et al. Diet-Derived Short Chain Fatty Acids Stimulate Intestinal Epithelial Cells To Induce Mucosal Tolerogenic Dendritic Cells. *J. Immunol.* **2017**, *198*, 2172–2181, doi:10.4049/jimmunol.1600165.

103. Schulthess, J.; Pandey, S.; Capitani, M.; Rue-Albrecht, K.C.; Arnold, I.; Franchini, F.; Chomka, A.; Ilott, N.E.; Johnston, D.G.W.; Pires, E.; et al. The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages. *Immunity* **2019**, *50*, 432-445.e7, doi:10.1016/j.immuni.2018.12.018.

104. Park, J.; Kim, M.; Kang, S.G.; Jannasch, A.H.; Cooper, B.; Patterson, J.; Kim, C.H. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol.* **2015**, *8*, 80–93, doi:10.1038/mi.2014.44.

105. Arpaia, N.; Campbell, C.; Fan, X.; Dikiy, S.; van der Veeken, J.; DeRoos, P.; Liu, H.; Cross, J.R.; Pfeffer, K.; Coffer, P.J.; et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **2013**, *504*, 451–455, doi:10.1038/nature12726.

106. Furusawa, Y.; Obata, Y.; Fukuda, S.; Endo, T.A.; Nakato, G.; Takahashi, D.; Nakanishi, Y.; Uetake, C.; Kato, K.; Kato, T.; et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **2013**, *504*, 446–450, doi:10.1038/nature12721.

107. Singh, N.; Gurav, A.; Sivaprakasam, S.; Brady, E.; Padia, R.; Shi, H.; Thangaraju, M.; Prasad, P.D.; Manicassamy, S.; Munn, D.H.; et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* **2014**, *40*, 128–39, doi:10.1016/j.immuni.2013.12.007.

108. Kim, M.H.; Kang, S.G.; Park, J.H.; Yanagisawa, M.; Kim, C.H. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **2013**, *145*, 396-406.e1–10, doi:10.1053/j.gastro.2013.04.056.

109. Kim, M.; Friesen, L.; Park, J.; Kim, H.M.; Kim, C.H. Microbial metabolites, short-chain fatty acids, restrain tissue bacterial load, chronic inflammation, and associated cancer in the colon of mice. *Eur. J. Immunol.* **2018**, *48*, 1235–1247, doi:10.1002/eji.201747122.

110. Macia, L.; Tan, J.; Vieira, A.T.; Leach, K.; Stanley, D.; Luong, S.; Maruya, M.; Ian McKenzie, C.; Hijikata, A.; Wong, C.; et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat. Commun.* **2015**, *6*, 6734, doi:10.1038/ncomms7734.

111. Zaki, M.H.; Boyd, K.L.; Vogel, P.; Kastan, M.B.; Lamkanfi, M.; Kanneganti, T.-D. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* **2010**, *32*, 379–91, doi:10.1016/j.immuni.2010.03.003.

112. Singh, N.; Gurav, A.; Sivaprakasam, S.; Brady, E.; Padia, R.; Shi, H.; Thangaraju, M.; Prasad, P.D.; Manicassamy, S.; Munn, D.H.; et al. Activation of Gpr109a, Receptor for Niacin and the Commensal Metabolite Butyrate, Suppresses Colonic Inflammation and Carcinogenesis. *Immunity* **2014**, *40*, 128–139, doi:10.1016/j.immuni.2013.12.007.

113. Sun, M.; Wu, W.; Chen, L.; Yang, W.; Huang, X.; Ma, C.; Chen, F.; Xiao, Y.; Zhao, Y.; Ma,

C.; et al. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. *Nat. Commun.* **2018**, *9*, 3555, doi:10.1038/s41467-018-05901-2.

114. Chun, E.; Lavoie, S.; Fonseca-Pereira, D.; Bae, S.; Michaud, M.; Hoveyda, H.R.; Fraser, G.L.; Gallini Comeau, C.A.; Glickman, J.N.; Fuller, M.H.; et al. Metabolite-Sensing Receptor Ffar2 Regulates Colonic Group 3 Innate Lymphoid Cells and Gut Immunity. *Immunity* **2019**, *51*, 871-884.e6, doi:10.1016/j.immuni.2019.09.014.
115. Yang, W.; Yu, T.; Huang, X.; Bilotta, A.J.; Xu, L.; Lu, Y.; Sun, J.; Pan, F.; Zhou, J.; Zhang, W.; et al. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat. Commun.* **2020**, *11*, 4457, doi:10.1038/s41467-020-18262-6.
116. Kim, S. V.; Xiang, W. V.; Kwak, C.; Yang, Y.; Lin, X.W.; Ota, M.; Sarpel, U.; Rifkin, D.B.; Xu, R.; Littman, D.R. GPR15-Mediated Homing Controls Immune Homeostasis in the Large Intestine Mucosa. *Science (80-.).* **2013**, *340*, 1456–1459, doi:10.1126/science.1237013.
117. Ungaro, F.; Rubbino, F.; Danese, S.; D'Alessio, S. Actors and Factors in the Resolution of Intestinal Inflammation: Lipid Mediators As a New Approach to Therapy in Inflammatory Bowel Diseases. *Front. Immunol.* **2017**, *8*, doi:10.3389/fimmu.2017.01331.
118. Oh, D.Y.; Talukdar, S.; Bae, E.J.; Imamura, T.; Morinaga, H.; Fan, W.; Li, P.; Lu, W.J.; Watkins, S.M.; Olefsky, J.M. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects. *Cell* **2010**, *142*, 687–698, doi:10.1016/j.cell.2010.07.041.
119. Miyamoto, J.; Mizukure, T.; Park, S.-B.; Kishino, S.; Kimura, I.; Hirano, K.; Bergamo, P.; Rossi, M.; Suzuki, T.; Arita, M.; et al. A Gut Microbial Metabolite of Linoleic Acid, 10-Hydroxy- cis -12-octadecenoic Acid, Ameliorates Intestinal Epithelial Barrier Impairment Partially via GPR40-MEK-ERK Pathway. *J. Biol. Chem.* **2015**, *290*, 2902–2918, doi:10.1074/jbc.M114.610733.
120. Staudinger, J.L.; Goodwin, B.; Jones, S.A.; Hawkins-Brown, D.; MacKenzie, K.I.; LaTour, A.; Liu, Y.; Klaassen, C.D.; Brown, K.K.; Reinhard, J.; et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci.* **2001**, *98*, 3369–3374, doi:10.1073/pnas.051551698.
121. Makishima, M. Vitamin D Receptor As an Intestinal Bile Acid Sensor. *Science (80-.).* **2002**, *296*, 1313–1316, doi:10.1126/science.1070477.
122. Yu, J.; Lo, J.-L.; Huang, L.; Zhao, A.; Metzger, E.; Adams, A.; Meinke, P.T.; Wright, S.D.; Cui, J. Lithocholic Acid Decreases Expression of Bile Salt Export Pump through Farnesoid X Receptor Antagonist Activity. *J. Biol. Chem.* **2002**, *277*, 31441–31447, doi:10.1074/jbc.M200474200.
123. Maruyama, T.; Miyamoto, Y.; Nakamura, T.; Tamai, Y.; Okada, H.; Sugiyama, E.; Nakamura, T.; Itadani, H.; Tanaka, K. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem. Biophys. Res. Commun.* **2002**, *298*, 714–719, doi:10.1016/S0006-291X(02)02550-0.

124. Shah, Y.M.; Ma, X.; Morimura, K.; Kim, I.; Gonzalez, F.J. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-κB target gene expression. *Am. J. Physiol. Liver Physiol.* **2007**, *292*, G1114–G1122, doi:10.1152/ajpgi.00528.2006.

125. Renga, B.; Mencarelli, A.; Cipriani, S.; D'Amore, C.; Carino, A.; Bruno, A.; Francisci, D.; Zampella, A.; Distrutti, E.; Fiorucci, S. The Bile Acid Sensor FXR Is Required for Immune-Regulatory Activities of TLR-9 in Intestinal Inflammation. *PLoS One* **2013**, *8*, e54472, doi:10.1371/journal.pone.0054472.

126. Biagioli, M.; Carino, A.; Cipriani, S.; Francisci, D.; Marchianò, S.; Scarpelli, P.; Sorcini, D.; Zampella, A.; Fiorucci, S. The Bile Acid Receptor GPBAR1 Regulates the M1/M2 Phenotype of Intestinal Macrophages and Activation of GPBAR1 Rescues Mice from Murine Colitis. *J. Immunol.* **2017**, *199*, 718–733, doi:10.4049/jimmunol.1700183.

127. Cipriani, S.; Mencarelli, A.; Chini, M.G.; Distrutti, E.; Renga, B.; Bifulco, G.; Baldelli, F.; Donini, A.; Fiorucci, S. The Bile Acid Receptor GPBAR-1 (TGR5) Modulates Integrity of Intestinal Barrier and Immune Response to Experimental Colitis. *PLoS One* **2011**, *6*, e25637, doi:10.1371/journal.pone.0025637.

128. Guo, C.; Xie, S.; Chi, Z.; Zhang, J.; Liu, Y.; Zhang, L.; Zheng, M.; Zhang, X.; Xia, D.; Ke, Y.; et al. Bile Acids Control Inflammation and Metabolic Disorder through Inhibition of NLRP3 Inflammasome. *Immunity* **2016**, *45*, 802–816, doi:10.1016/j.immuni.2016.09.008.

129. Vavassori, P.; Mencarelli, A.; Renga, B.; Distrutti, E.; Fiorucci, S. The Bile Acid Receptor FXR Is a Modulator of Intestinal Innate Immunity. *J. Immunol.* **2009**, *183*, 6251–6261, doi:10.4049/jimmunol.0803978.

130. Song, X.; Sun, X.; Oh, S.F.; Wu, M.; Zhang, Y.; Zheng, W.; Geva-Zatorsky, N.; Jupp, R.; Mathis, D.; Benoist, C.; et al. Microbial bile acid metabolites modulate gut RORγ+ regulatory T cell homeostasis. *Nature* **2020**, *577*, 410–415, doi:10.1038/s41586-019-1865-0.

131. Hang, S.; Paik, D.; Yao, L.; Kim, E.; Trinath, J.; Lu, J.; Ha, S.; Nelson, B.N.; Kelly, S.P.; Wu, L.; et al. Bile acid metabolites control TH17 and Treg cell differentiation. *Nature* **2019**, *576*, 143–148, doi:10.1038/s41586-019-1785-z.

132. Jain, U.; Lai, C.-W.; Xiong, S.; Goodwin, V.M.; Lu, Q.; Muegge, B.D.; Christophi, G.P.; VanDussen, K.L.; Cummings, B.P.; Young, E.; et al. Temporal Regulation of the Bacterial Metabolite Deoxycholate during Colonic Repair Is Critical for Crypt Regeneration. *Cell Host Microbe* **2018**, *24*, 353-363.e5, doi:10.1016/j.chom.2018.07.019.

133. Wang, K.; Liao, M.; Zhou, N.; Bao, L.; Ma, K.; Zheng, Z.; Wang, Y.; Liu, C.; Wang, W.; Wang, J.; et al. Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep.* **2019**, *26*, 222-235.e5, doi:10.1016/j.celrep.2018.12.028.

134. Blachier, F.; Boutry, C.; Bos, C.; Tomé, D. Metabolism and functions of l-glutamate in the epithelial cells of the small and large intestines. *Am. J. Clin. Nutr.* **2009**, *90*, 814S-821S, doi:10.3945/ajcn.2009.27462S.

135. Yi, D.; Hou, Y.; Wang, L.; Ouyang, W.; Long, M.; Zhao, D.; Ding, B.; Liu, Y.; Wu, G. l-Glutamine enhances enterocyte growth via activation of the mTOR signaling pathway independently of AMPK. *Amino Acids* **2015**, *47*, 65–78, doi:10.1007/s00726-014-1842-8.

136. Gould, R.L.; Pazdro, R. Impact of Supplementary Amino Acids, Micronutrients, and Overall Diet on Glutathione Homeostasis. *Nutrients* **2019**, *11*, 1056, doi:10.3390/nu11051056.

137. Hashimoto, T.; Perlot, T.; Rehman, A.; Trichereau, J.; Ishiguro, H.; Paolino, M.; Sigl, V.; Hanada, T.; Hanada, R.; Lipinski, S.; et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* **2012**, *487*, 477–481, doi:10.1038/nature11228.

138. Zelante, T.; Iannitti, R.G.; Cunha, C.; De Luca, A.; Giovannini, G.; Pieraccini, G.; Zecchi, R.; D'Angelo, C.; Massi-Benedetti, C.; Fallarino, F.; et al. Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22. *Immunity* **2013**, *39*, 372–385, doi:10.1016/j.immuni.2013.08.003.

139. Gutiérrez-Vázquez, C.; Quintana, F.J. Regulation of the Immune Response by the Aryl Hydrocarbon Receptor. *Immunity* **2018**, *48*, 19–33, doi:10.1016/j.immuni.2017.12.012.

140. Wikoff, W.R.; Anfora, A.T.; Liu, J.; Schultz, P.G.; Lesley, S.A.; Peters, E.C.; Siuzdak, G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl. Acad. Sci.* **2009**, *106*, 3698–3703, doi:10.1073/pnas.0812874106.

141. Lamas, B.; Richard, M.L.; Leducq, V.; Pham, H.-P.; Michel, M.-L.; Da Costa, G.; Bridonneau, C.; Jegou, S.; Hoffmann, T.W.; Natividad, J.M.; et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat. Med.* **2016**, *22*, 598–605, doi:10.1038/nm.4102.

142. Venkatesh, M.; Mukherjee, S.; Wang, H.; Li, H.; Sun, K.; Benechet, A.P.; Qiu, Z.; Maher, L.; Redinbo, M.R.; Phillips, R.S.; et al. Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-like Receptor 4. *Immunity* **2014**, *41*, 296–310, doi:10.1016/j.immuni.2014.06.014.

143. Alexeev, E.E.; Lanis, J.M.; Kao, D.J.; Campbell, E.L.; Kelly, C.J.; Battista, K.D.; Gerich, M.E.; Jenkins, B.R.; Walk, S.T.; Kominsky, D.J.; et al. Microbiota-Derived Indole Metabolites Promote Human and Murine Intestinal Homeostasis through Regulation of Interleukin-10 Receptor. *Am. J. Pathol.* **2018**, *188*, 1183–1194, doi:10.1016/j.ajpath.2018.01.011.

144. Wlodarska, M.; Luo, C.; Kolde, R.; D'Hennezel, E.; Annand, J.W.; Heim, C.E.; Krastel, P.; Schmitt, E.K.; Omar, A.S.; Creasey, E.A.; et al. Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. *Cell Host Microbe* **2017**, *22*, 25-37.e6, doi:10.1016/j.chom.2017.06.007.

145. Chimerel, C.; Emery, E.; Summers, D.K.; Keyser, U.; Gribble, F.M.; Reimann, F. Bacterial Metabolite Indole Modulates Incretin Secretion from Intestinal Enteroendocrine L Cells. *Cell Rep.* **2014**, *9*, 1202–1208, doi:10.1016/j.celrep.2014.10.032.

146. Matteoli, G.; Mazzini, E.; Iliev, I.D.; Mileti, E.; Fallarino, F.; Puccetti, P.; Chieppa, M.; Rescigno, M. Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* **2010**, *59*, 595–604, doi:10.1136/gut.2009.185108.

147. Opitz, C.A.; Litzenburger, U.M.; Sahm, F.; Ott, M.; Tritschler, I.; Trump, S.; Schumacher, T.; Jestaedt, L.; Schrenk, D.; Weller, M.; et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* **2011**, *478*, 197–203, doi:10.1038/nature10491.

148. Bessede, A.; Gargaro, M.; Pallotta, M.T.; Matino, D.; Servillo, G.; Brunacci, C.; Bicciato, S.; Mazza, E.M.C.; Macchiarulo, A.; Vacca, C.; et al. Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature* **2014**, *511*, 184–190, doi:10.1038/nature13323.

149. Lanis, J.M.; Alexeev, E.E.; Curtis, V.F.; Kitzenberg, D.A.; Kao, D.J.; Battista, K.D.; Gerich, M.E.; Glover, L.E.; Kominsky, D.J.; Colgan, S.P. Tryptophan metabolite activation of the aryl hydrocarbon receptor regulates IL-10 receptor expression on intestinal epithelia. *Mucosal Immunol.* **2017**, *10*, 1133–1144, doi:10.1038/mi.2016.133.

150. Alvarado, D.M.; Chen, B.; Iticovici, M.; Thaker, A.I.; Dai, N.; VanDussen, K.L.; Shaikh, N.; Lim, C.K.; Guillemin, G.J.; Tarr, P.I.; et al. Epithelial Indoleamine 2,3-Dioxygenase 1 Modulates Aryl Hydrocarbon Receptor and Notch Signaling to Increase Differentiation of Secretory Cells and Alter Mucus-Associated Microbiota. *Gastroenterology* **2019**, *157*, 1093-1108.e11, doi:10.1053/j.gastro.2019.07.013.