Microbiota Metabolite Regulation of Host Immune Homeostasis: A Mechanistic Missing Link

S. Steinmeyer · K. Lee · A. Jayaraman · R. C. Alaniz

Abstract Metazoans predominantly co-exist with symbiotic microorganisms called the microbiota. Metagenomic surveys of the microbiota reveal a diverse ecosystem of microbes particularly in the gastrointestinal (GI) tract. Perturbations in the GI microbiota in higher mammals (i.e., humans) are linked to diseases with variegated symptomology including inflammatory bowel disease, asthma, and auto-inflammatory disorders. Indeed, studies using germ-free mice (lacking a microbiota) confirm that host development and homeostasis are dependent on the microbiota. A long-known key feature of the GI tract microbiota is metabolizing host indigestible dietary matter for maximum energy extraction; however, host signaling pathways are greatly influenced by the microbiota as well. In line with these observations, recent research has revealed that metabolites produced strictly by select microbiota members are mechanistic regulators of host cell functions. In this review, we discuss two major classes of microbiota-produced metabolites: short-chain fatty acids and tryptophan metabolites. We describe the known important roles for these metabolites in shaping host immunity and comment on the current status and future directions for microbiota metabolomics research.

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Introduction

The gastrointestinal (GI) tract is a complex, dynamic nexus for host metabolism and immunity. In concert with the abundant, varied GI tract host cells involved in metabolism and immunity are roughly 10^{14} symbiotic microorganisms that populate the luminal space; collectively known as the microbiota, these microbes are predominantly composed of bacteria but also include protozoa, fungi, viruses, helminthes, and archaea [1]. Although essential for optimal host health, the mechanisms by which the microbiota regulates host physiology and immunity are largely unknown, and since the microbiota is most abundant in the GI tract, current research has focused on this GI ecosystem of microbes. Nonetheless, other mucosal and environmentally exposed tissues have distinct compositions of microbiota [2].

Multiple microbiota factors—such as pattern recognition receptor ligands, polysaccharide A, and sphingolipid as well as diet-dependent bile acids and vitamins—influence host cell function and health and have been reviewed elsewhere [3, 4]. Beyond this, a number of groups including our own hypothesize that unique microbiota biosynthetic pathways produce metabolites that mediate many of the microbiota's important effects on host health [5–9]. This review will discuss two major classes of microbiota-derived metabolites, short-chain fatty acids (SCFAs) and tryptophan (Trp) metabolites, that are rapidly emerging as critical signals to directly influence immunity and cell function in the host and how metabolites are likely a mechanistic "missing link" in the microbiota-host communication paradigm.

The Gastrointestinal Tract Mucosal Immune System

Along with their close association with the microbiota, mucosal tissues—typified by the lungs, oral-nasal cavities, and the GI tract—are functionally and anatomically specialized regions in the host that comprise the primary barrier to the external environment. It is not surprising then that the largest collection of immune cells in the body resides in the GI tract [10]. To understand the specialized host microenvironment that interfaces with the microbiota, we briefly review major features of the GI immune system.

GI immune cells are organized as the gut-associated lymphoid tissue (GALT) that includes diffusely distributed immune cells in the lamina propria (LP), intestinal epithelial cell (IEC)-intercalating lymphocytes (IELs), secondary lymphoid tissue known as Peyer's patches (PPs) and colonic patches (CPs), and solitary isolated lymphoid tissue (SILT) in the LP. A comprehensive description of the GALT architecture and function can be found in a recent excellent review [10].

Among host lymphoid tissues, the GALT encounters the largest biomass of non-pathogenic microbes from both dietary intake and the endogenous microbiota. Consequently, the GALT—in conjunction with microbiota-dependent "colonization resistance" factors—clears most pathogenic microbes while avoiding aberrant inflammation [11, 12]. These GALT features contribute to the phenomenon of oral tolerance, whereby nominal antigen acquired orally, in contrast to that encountered systemically, is effectively ignored, which illustrates the overall modus operandi promoting GALT homeostasis [13]. Nevertheless, the GALT includes innate and adaptive immune mechanisms poised to respond to pathogenic infection.

The innate arm is composed of leukocytes such as macrophages, dendritic cells (DCs), and innate lymphoid cells (ILCs). In addition, other innate elements, including non-hematopoietic epithelial and stromal cells, produce protective cytokines and mucosal barrier components such as mucus and anti-microbial peptides [10, 14]. Macrophages and DCs possess critical antigen-presenting cell (APC) and distinct immunoregulatory roles in the GI tract [15]. ILCs—including natural killer (NK), lymphoid tissue inducer (LTi), and other effector classes [16]—support pathogen clearance and tissue repair, and the current understanding of ILCs is well summarized in two recent articles [17, 18].

The GALT adaptive arm comprises B and T cells with particularly important roles for producing microbe-neutralizing secretory IgA in the GI lumen and orchestrating the mucosal immune microclimate, respectively. Classical effector T cells such as CD8+ cytotoxic T lymphocytes (CTLs) and CD4+ T helper (Th) cells participate in mucosal immunity. Th1 (IFN-γ-producing, Tbet+), Th2 (IL-4-producing, GATA3+), Th9 (IL-9-producing, PU.1+, IRF4+), Th17 (IL-17-producing, RORγt+), Th22 (IL-22-producing), and regulatory (Treg) (transforming growth factor beta (TGF-β)- and IL-10-producing, FOXP3+) T cells reside and function in the GALT; however, Treg, Th17, and Th22 cells may have more prominent roles in the GALT. At mucosal surfaces, TGF-β is abundant and is essential for differentiation of the peripheral Treg, Th17, Th22, and Th9 lineages [19–21], and these particular lineages are strongly influenced by additional signals through the aryl hydrocarbon receptor (AhR) that predominate in the GI tract (discussed below). T cells receiving TGF-β signals plus IL-6 or tumor necrosis factor (TNF)-α differentiate into Th17 or Th22 T cells, respectively, and promote pathogen clearance and mucosal integrity [20]. On the other hand, TGF-β plus IL-4 promotes Th9 differentiation whose function is similar to antibody-promoting Th2 T cells [21]. Further, it is important to note that both peripheral (TGF-β-dependent) and thymic (TGF-β-independent) Tregs are necessary for GI tract homeostasis [19]. A more thorough discussion of general host factors involved in T cell development and function can be found here [22]. Nonetheless, what is becoming increasingly evident is that non-host-derived signals in the GI tract (i.e., from the microbiota) have profound regulatory influences on innate and adaptive cell functional fate.

Microbiota Composition in Health and Disease

The continuing advancement of metagenomic sequencing over the last 10 years has verified that the microbiota possesses greater than two orders of magnitude more genomic content than humans and that microbiota dysbiosis (perturbations in abundance or diversity) is linked to pathology in a number of complex diseases, such as inflammatory bowel disease, obesity, diabetes, asthma, and psoriasis [23, 24].

Compounding potential exogenously induced perturbations of the microbiota is the finding that microbiota variation between healthy individuals can be high, although it appears that on a population level, microbiota composition can be broadly categorized into distinct enterotypes, which correlate with a person's geographical locale, education level, and infant breastfeeding [25•]. Further, investigations of microbiome types possessing overall low or high gene content report that high gene content microbiomes have a more diverse repertoire of putative microbiota enzymatic function, and these high gene content hosts have a lower prevalence of complex disease (e.g., metabolic syndrome) [26•]. This observation supports the hypothesis that microbiota enzymatic richness and the end-product metabolites contribute important cues for optimal host immunophysiology.

Importantly, dietary components and nutrition strongly influence microbiota composition and disease. In perhaps the best direct evidence for this notion, humanized gnotobiotic mice fed a western diet (high fat, high sugar) have increased

adiposity and altered microbiota composition compared to humanized gnotobiotic mice fed a standard diet (low fat, high plant polysaccharide), and transfer of the cecal microbiota from mice fed a western diet to germ-free mice maintained on a standard diet results in increased adiposity [27]. However, a 12-week period of dietary intervention in 38 obese and 11 overweight individuals increased gene richness of the microbiota and decreased adiposity in low gene content individuals but failed to improve systemic inflammation markers to levels seen in the high gene content individuals, suggesting that dietary intervention only partially impacts microbiota composition and host inflammatory status [28•].

Given its intimate relationship with diet, it is not surprising that the microbiota regulates host metabolome status both locally in the GI tract and systemically [29]. On top of this, microbiota-specific enzymatic machinery produces unique metabolites—starting with substrates that originate in the diet—that modulate diseases with disparate pathology such as cardiovascular disease and cancer [30]. It follows that increasing our understanding of microbiota metabolite diversity and function in the host has the potential to provide new targets for treating disease, but there are considerable obstacles in identifying the universe of microbiota metabolites. For example, it is conservatively estimated that the microbiota has at least three orders of magnitude greater enzymatic/biosynthetic potential than its human host [24]; therefore, the challenges for microbiota metabolite testing are compounded by the sheer number of possible products for any given functional pathway.

To analyze the microbiota enzymatic repertoire, investigators initially focused on the abundance of different enzyme classes [31•, 32]; however, enzymatic prevalence does not account for the community-level metabolic network that enzymes may belong to within the microbiota. Recent work by our team and others has validated the utility of placing metagenomic DNA gene reads into a computational model for metabolome network comparisons between groups and for hypothesis testing [29, 31•, 33•]. For example, Greenblum et al. used the Kyoto Encyclopedia of Genes and Genomes (KEGG) to categorize metagenomic DNA sequences from the microbiota of healthy, colitic, and obese individuals. The resulting enzymatic network revealed that microbiota gene abundance in obese and colitic individuals was primarily altered in the periphery of the enzymatic network [31•]. The periphery of the network of microbiota enzymatic machinery can be thought of as representing reactions that rely on substrates from the GI lumen or that produce metabolites that are not used by other microbiota enzymes; in other words, these enzymes are likely to directly use or produce metabolites that are at the interface between host cells and microbiota. Thus, this observation suggests that complex diseases are linked with changes in microbiota metabolite signals received by host cells.

Whereas the above network approach correlates pathology to changes in the microbiota enzymatic network, our group's approach has focused on prediction of novel microbiota metabolite production in silico via network analysis of metagenomic DNA data followed by targeted in vivo mass spectrometry-based metabolomics coupled to in vitro mechanistic analysis of host cell signaling pathways [33•]. Using this functional metabolomics workflow, we have identified novel aromatic amino acid metabolites, strictly microbiota produced, that signal through the AhR (discussed below) [33•]. Thus, using a functional metabolomics workflow through the lens of network analysis, we have highlighted how select microbiota metabolites impact one critical host pathway that regulates several aspects of health and physiology. In the future, this approach can be applied to inform targeted and hypothesis-driven investigations of the universe of possible microbiota metabolites during homeostasis or dysbiosis.

Among the many potential metabolites selectively produced by the microbiota, the vast majority have not been tested or have unknown effects on homeostasis and physiology. Despite this, two major classes of metabolites have been found to have wide-ranging effects on host immunity and physiology: SCFAs which have been long recognized as important energy substrates and regulators of cell function in the gut and tryptophan (Trp) metabolites which are rapidly emerging as one of the most bioactive classes of immunomodulators.

Short-Chain Fatty Acids

Microbiota fermentation of dietary fiber, the non-starch indigestible polysaccharide and oligosaccharide portion of plants, is accomplished by microbiota encoded glucosidases to produce short-chain fatty acids (SCFAs) that have been recognized for decades as an energy source for enterocytes, thereby maximizing the total energy yield from dietary intake. Butyrate, acetate, and propionate are the most prominent SCFAs in the gut, and each can range in concentration from 1 to 20 mM (Fig. 1) [34]. However, only recently has molecular detail on the diverse regulatory effects of SCFAs, beyond simply augmenting nutritional yield, become apparent [35].

The SCFAs—acetate, propionate, and butyrate—act as histone deacetylase (HDAC) inhibitors (which regulate DNA-histone coiling) as well as ligands for certain G-protein coupled receptors (GPRs): Olfr78, GPR41, GPR43, and GPR109a [36–40, 41•]. The ubiquitous expression of HDAC enzymes in all nucleated cells, as well as the broad expression profile of these GPRs, underlies the varied physiological roles of the SCFAs, which include regulation of the nervous system [42–44], protection against colon cancer [45], and regulation of blood pressure and kidney function [41•]. However, a comprehensive understanding of the GPR- and HDAC-dependent functions of SCFAs is only beginning to be revealed.
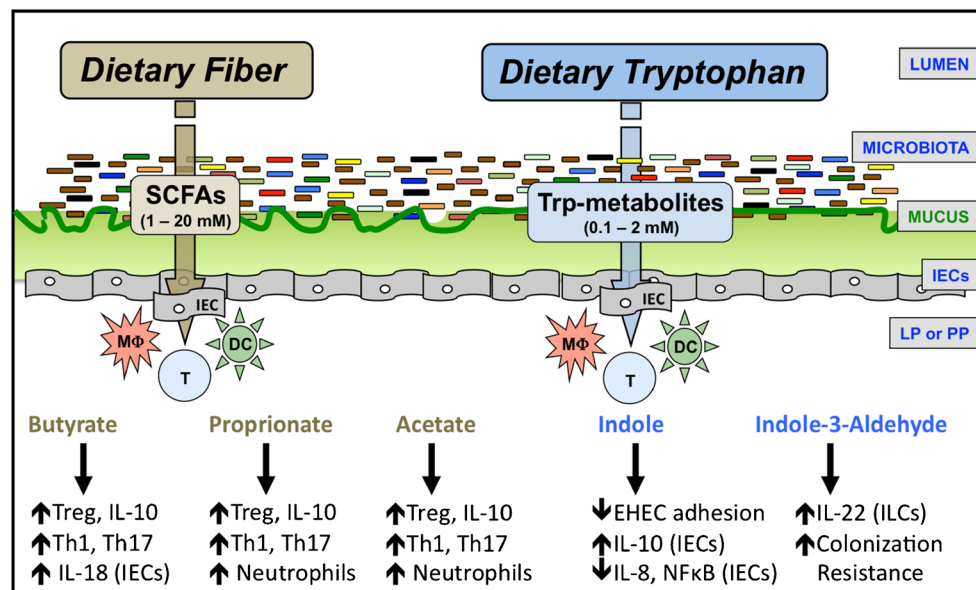
Fig. 1 On the *left*, dietary fiber is processed by microbiota to short-chain fatty acids (*SCFA*), and these compounds promote recruitment of neutrophils (*N*) while also inhibiting N production of pro-inflammatory reactive oxygen species (ROS) and TNF-α. SCFAs also promote a tolerogenic phenotype in DCs and MΦs, and they promote both effector and regulatory T cells. On the *right*, indole and Trp metabolites are produced by microbiota metabolism. These Trp metabolites block pathogenic enterohemorrhagic *E. coli* invasion, promote anti-inflammatory signaling in IECs, and promote IL-22 expression by ILCs

With the exception of GPR109a, which can be activated by either the vitamin niacin or butyrate, SCFAs appear to be necessary for the homeostatic properties of the GPRs mentioned above. Indeed, acetate supplementation in the drinking water alleviated dextran sodium sulfate (DSS) colitis in WT but not GPR43 knockout mice [46, 47]. In contrast, butyrate supplementation in the drinking water had no beneficial effect on DSS colitis [48]. Additionally, feeding mice a high-fiber diet increased serum SCFA levels and alleviated house dust mite extract-induced allergic airway inflammation, while propionate-supplemented drinking water alleviated allergic airway inflammation in WT but not GPR41 knockout mice [49•]. Using GPR43 knockout mice, Maslowski et al. found increased disease pathology in multiple disease models including DSS colitis, K/BxN serum-induced inflammatory arthritis, and OVA-induced allergic airway inflammation, and they proposed that increased inflammation in these disease models was mediated by neutrophils that lacked GPR43 signaling [47]. GPR109a-deficient mice had increased severity of DSS-induced colitis, and loss of GPR109a resulted in decreased regulatory T cells in the colon as well as decreased IL-18 production in IECs [50•]. Indeed, DSS colitis was more severe in chimeric mice that lack GPR109a in either the hematopoietic or the stromal compartment and most severe in total knockout mice [50•]. In contrast, Kim et al. found that loss of either GPR41 or GPR43 in the non-hematopoietic stromal compartment resulted in decreased inflammatory response and delayed resolution of *Citrobacter rodentium* infection [51]. These results suggest that pro- or anti-inflammatory effects of GPR activation, putatively via SCFAs, may depend on the context and/or the cell type being activated.

IECs are likely the primary cell type exposed to SCFAs, and recent work has revealed that the barrier function of IECs is directly modulated by SCFAs. For example, butyrate-treated IECs increased transcription of MUC3 and MUC5B in the presence of glucose and increased transcription of MUC2, MUC3, MUC5AC, and MUC5B in the absence of glucose [52]. In addition, a synthetic HDAC inhibitor, trichostatin A (TSA), also induced MUC3 but no other MUC genes, suggesting that HDAC inhibition may partially mediate the mucus production induced by butyrate [52]. Further, butyrate and propionate increased trans-epithelial electrical resistance in IECs, and this effect was recapitulated with TSA treatment [53]. In addition to IECs at the mucosal barrier, HDAC inhibition by SCFAs likely mediates effects on innate hematopoietic cells. For example, propionate and butyrate decrease LPS-induced nuclear factor kappa B (NF-κB) activation and pro-inflammatory ROS and TNF-α production by neutrophils, likely by HDAC inhibition [54]. Butyrate inhibits HDACs in bone-marrow-derived macrophages and inhibits secretion of IL-6, IL-12, and nitric oxide, and this correlates with decreased IL-6, IL-12, and NO synthase transcription in LP macrophages isolated from the colons of antibiotic-treated mice given butyrate in drinking water [48]. Despite the strong anti-inflammatory effects of butyrate on macrophages in vivo, butyrate did not improve inflammation for mice with DSS colitis [48].

In addition to SCFA modulation of innate immune cell types, recent work has demonstrated that SCFAs play a role

in adaptive immune cell homeostasis. Smith et al. demonstrated that acetate-, propionate-, and butyrate-supplemented drinking water augments colonic Treg numbers and the GPR43-dependent accumulation of colonic Tregs in both germ-free and specific-pathogen-free mice [55]. In contrast, Furusawa et al. found that supplementing chow with butyrylated, but not acetylated or propionylated, starch induces colonic Tregs [56•]. In agreement with these observations on the importance of the method of administration, Arpaia et al. used FOXP3 CNS1 knockout mice, which are severely limited in peripheral Treg induction, to show that acetate and propionate in the drinking water primarily promote accumulation of colonic Tregs, and butyrate induces de novo generation of colonic Tregs only when applied by liquid enema or feeding butyrylated starch [57•]. Thus, the method of administration might alter SCFA bioavailability and the resultant physiologic effects. These studies highlight the variable signaling capacity of these three SCFAs, in that acetate and propionate are most likely to activate GPR43 and promote colonic Treg migration/accumulation [55], whereas the strong HDAC inhibition of butyrate is able to promote de novo colonic Treg generation [57•].

In contrast to the homeostatic properties of SCFAs, acetate, propionate, and butyrate may have properties that are pro-inflammatory. Park et al. reported that SCFAs promote in vitro effector Th1 and Th17 differentiation [58•]. Interestingly, this study found that acetate-supplemented drinking water increases colonic inflammatory Th1 and Th17 cells during infection with pathogenic *C. rodentium*, whereas in the absence of infection, colonic IL-10+ T cells increase [58•]. However, it is not clear whether accumulation of existing T cells or de novo generation mediated the beneficial effects of acetate in this model. Further, this study found that in vitro T cells treated with acetate, propionate, or butyrate promoted phosphorylation of the ribosomal S6 protein, suggesting mammalian target of rapamycin (mTOR) activation. Additionally, S6 kinase had increased acetylation after SCFA treatment, and this may highlight the ability of HDAC inhibition to increase acetylation of histones as well as non-nuclear proteins [58•]. With growing appreciation of metabolic regulation of immunity—e.g., mTOR-mediated pathways (such as glycolysis) shape T cell differentiation lineage choice [59]—this newly revealed mechanism of SCFA signaling is intriguing and an important area for further study.

Tryptophan Metabolites

The importance of microbiota products in shaping immune cell function in the GALT (e.g., SCFAs) suggests that alternate classes of microbiota metabolites may provide the host with necessary signals for proper development and homeostasis of the immune system. In fact, emergent studies have established the importance of microbiota-derived Trp metabolites for appropriate development and function of the immune system.

Beyond being a protein building block, the essential amino acid Trp is a substrate for host-dependent metabolic biotransformation into diverse chemoeffectors, e.g., the neurotransmitters serotonin and melatonin, indoleamine-2,3-dioxygenase (IDO)-dependent kynurenines (AhR ligands [60]), and small amounts of the essential vitamin niacin. Beyond host-dependent metabolism of Trp, the microbiota performs unique catabolic biotransformations of Trp into several bioactive metabolites. Our team and others have determined that in the absence of a commensal microbiota, many notable Trp metabolites are severely limited in both the GI lumen as well as serum, while levels of Trp in the serum are roughly doubled [29, 33•]. Furthermore, during clinical diseases associated with microbiota dysbiosis (e.g., ileitis), Trp utilization in the GI tract is perturbed, causing increased luminal Trp levels and a concomitant decrease in Trp metabolites [61, 62]; this suggests that limiting concentrations of Trp metabolites may trigger or exacerbate disease. Understanding the mechanistic immunophysiology of known and yet to be discovered Trp metabolites will likely reveal new paradigms for microbiota-mediated communication with the host.

The known properties of bioactive Trp metabolites appear to have special regulatory roles for signaling pathways in host immune cells. A recent study identified that microbiota-derived niacin is an agonist (as well as butyrate) of GPR109a and promotes homeostatic IL-18 production in IECs and increases Treg prevalence in the colonic LP [50•]. However, an emerging paradigm reported by our team and others is that a number of strictly microbiota-derived Trp metabolites modulate AhR activity in immune cells [33•, 63•, 64•, 65], which may play key roles in immunohomeostasis.

The AhR belongs to the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family of proteins, and although originally identified as a receptor for the industrial toxicant dioxin [66], its physiologic role is in adapting multi-cellular organisms to the environment [67]. AhR is a ligand-inducible transcription factor that mediates cellular responses to low-molecular-weight chemicals by activating transcription of genes with promoters containing AhR-binding sites, known as xenobiotic response elements (XRE). For mammals, the importance of the AhR for homeostasis of multiple immune cell types and proper GALT structure has gained interest in the last several years, with the first clear effects of AhR immunoregulation demonstrated on the balance of anti-inflammatory Tregs and proinflammatory Th17 cells [68, 69]; a recent comprehensive review is available here [70]. Furthermore, recent research has found that AhR knockout mice have normal thymic output, but maintenance of the IEL compartment is diminished [71]. Further, lymphocyte-specific AhR knockouts (RAG1-cre × AhR-flox mice) revealed that intrinsic lymphocyte AhR activity led to a deficiency in the IEL population that exacerbated DSS colitis similar to what was seen in AhR knockout mice [71]. In a separate study, AhR knockout mice revealed a defect

in isolated lymphoid follicle formation, and this phenotype was also present in RORγT-Cre × AhR-flox mice. T cells were not necessary for follicle formation; therefore, the investigators proposed that ILC-intrinsic AhR signaling was necessary for normal GALT development. Further, a diet deficient in AhR ligands recapitulated the defect in GALT development as seen in AhR knockout mice and resulted in decreased RORγT+ ILCs, suggesting that AhR ligands may regulate optimal GALT development [72]. To understand how the AhR impacts RORγt+ ILCs requires further study, because ILCs are a critical junction between innate and adaptive immunity and necessary for physiologic tolerance of the microbiota [73].

The AhR has a number of ligands that have been identified, and although xenobiotic and exogenous natural AhR ligands may be acquired through the diet, the microbiota is likely the evolutionary and physiologically meaningful source of AhR ligands in lower and higher vertebrates [74]. Within the microbiota, the most proximal enzymatic pathway for Trp metabolites comes from species expressing Trp lyase [75, 76], which directly catabolizes Trp to produce indole, an abundant metabolite found in both human and mouse fecal samples at high concentrations [33•, 77, 78•, 79, 80].

Although indole has been known as a "by-product" of Trp catabolism, it was largely ignored as a bioactive molecule for decades [81]. However, our team recently revealed important microbiological properties for indole in decreasing bacterial pathogen chemotaxis, motility, biofilm formation, and IEC adhesion for enterohemorrhagic E. coli [82]. Subsequently, we revealed that indole promotes host IEC barrier integrity and expression of anti-inflammatory IL-10, while inhibiting inflammatory TNF-α-induced IL-8 and NF-κB signaling [77]. These protective effects of indole during inflammation were confirmed by Shimada et al. who found that oral indole therapy during DSS colitis in germ-free mice alleviated GI pathology, weight loss, and mortality [78•]. This study is notable, because it supports our own observations [77] and establishes that indole can function as a singular signal to promote homeostasis in the GI tract.

Undoubtedly, the pool of microbiota metabolites in mammals is highly complex [29]. To deconvolute this inherent complexity, we recently used a computational network and metabolomics workflow to identify functional roles for indole and a number of endogenous Trp metabolites/AhR ligands [33•]. Indeed, Venkatesh et al. observed that a novel interaction between indole and indole-3-propionate, another microbiota-derived Trp metabolite, enhances intestinal barrier integrity and inhibits inflammatory signaling in IECs through the pregnane X receptor (PXR) [83•]. This study found that indole-3-propionate plus indole activates PXR signaling in a reporter cell line, and they observed germ-free mice, and gnotobiotic mice colonized with metabolically inactive microbiota, in contrast to active, have exacerbated indomethacin-induced enteropathy. However, the investigators did not address any unique effects from indole alone, so it seems most likely that multiple signaling

mechanisms explain the protective effects of indole and indole-3-propionate in IECs. Together, these studies suggest that distinct Trp metabolites have both singular and combinatorial effects on multiple aspects of IEC signaling and physiologic responses to environmental insults.

Another microbiota-derived Trp metabolite, indole-3-aldehyde (I3Ald), promotes ILC production of IL-22 to protect against pathogenic infection, and this effect is dependent on the AhR [64•]. Zelante et al. found that when host utilization of Trp was limited (IDO knockout mice), mice have enhanced resistance to Candida albicans infection in the stomach, and this correlates with increased lactobacilli-mediated I3Ald production and ILC IL-22 production. Either increased dietary Trp or supplementation with I3Ald had a similar effect of reducing C. albicans infection load, whereas the protective effect of I3Ald supplementation was lost in AhR knockout mice [64•].

In total, there is now compelling evidence that dietary Trp is a critical substrate for microbiota-dependent production of metabolites that regulate GALT development and homeostasis-promoting properties of many GALT-resident cells, usually via AhR signaling. However, despite the impressive amount and variety of bioactive Trp metabolites produced by the microbiota [33•, 63•], it is clear that further investigation is necessary for a more comprehensive understanding of the role of Trp metabolites in shaping host immune homeostasis, particularly with respect to their properties as a consortium of metabolite signals, rather than isolated factors.

Overall, we have a limited understanding of variable microbiota compositions and the concomitant metabolome signatures in health and disease. A recent study found that feeding butyrylated starch to specific pathogen-free, but not germ free, mice increased de novo colonic Treg induction [56•]. This suggests that SCFAs alone might not be sufficient to regulate the GALT, and it is reasonable to predict that the net balance or specific interactions between SCFAs, Trp metabolites, and other microbiota metabolites are required to fully realize microbiota-based clinical regimens. Thus, we propose that the presence and interaction between SCFAs and Trp metabolites are a key paradigm for microbiota function and communication with the host.

Conclusion

Over the last 10 years and in light of the work reviewed here, microbiota metabolites will continue to develop as critical mechanistic chemoeffectors for microbiota modulation of host physiology and health. Yet, it is remarkable that SCFA and Trp microbiota metabolites have so far revealed novel mechanistic paradigms of inter-kingdom symbiosis. Nonetheless, to advance our understanding, more integrated approaches to metabolite identification and functional characterization are needed [33•]. To fully understand the link between microbiota composition and function, the field will be required to

simultaneously investigate the metabolome and microbiome during health and disease. This work will require increased resolution of microbiota composition at anatomically distinct regions along the GI tract, increased annotation of the currently sequenced microbiome databases, improved metagenomic mapping to functional metabolic networks, and coupling these multi-omic analyses to host cellular and molecular pathways. The development of deeper foundational knowledge in this field is absolutely necessary to direct informative and unbiased research on microbiota metabolite function in the host.

Even at this early stage, our understanding of the microbiota has deepened and confirmed the long-recognized importance of our symbiotic microbiota as a determinant of host physiology and immunity. The extensive initial work of cataloguing microbiota composition in health and disease has only begun to reveal the biological implications of specific microbiota consortia. Preliminary understanding of the microbiota's mechanistic effects on host immunophysiology has provided intriguing insight about how personalized and general medicine may one day be based on tailored microbiota or rationally designed microbiota-derived metabolite compositions. Nonetheless, it is highly likely that the majority of mechanistic links between microbiota and host health remain to be revealed. To realize the promise of microbiota research, the next phases will need to focus on identifying and translating the mechanisms behind host-microbiota symbiosis into refined regimens for patient care.

Compliance with Ethics Guidelines

Conflict of Interest A. Jayaraman and R.C. Alaniz are co-founders of and hold equity in Fortis Biosciences Inc. A. Jayaraman reports grants from NIH. S. Steinmeyer and K. Lee declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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