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The cross talk between microbiota and the immune system: metabolites take center stage

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The human meta-organism consists of more than 90% of microbial cells. The gastrointestinal tract harbors trillions of commensal microorganisms that influence the development and homeostasis of the host. Alterations in composition and function of the microbiota, termed dysbiosis, have been implicated in a multitude of metabolic and inflammatory diseases in humans. Thus, understanding the molecular underpinnings the cross talk between commensal bacteria and their host during homeostasis and dysbiosis may hold the key to understanding many idiopathic diseases. While most attention has focused on the innate recognition of immune-stimulatory bacterial molecules, such as cell wall components and nucleic acids, we emphasize here the impact of diet-dependent microbial metabolites on the development and function of the immune system.

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Introduction

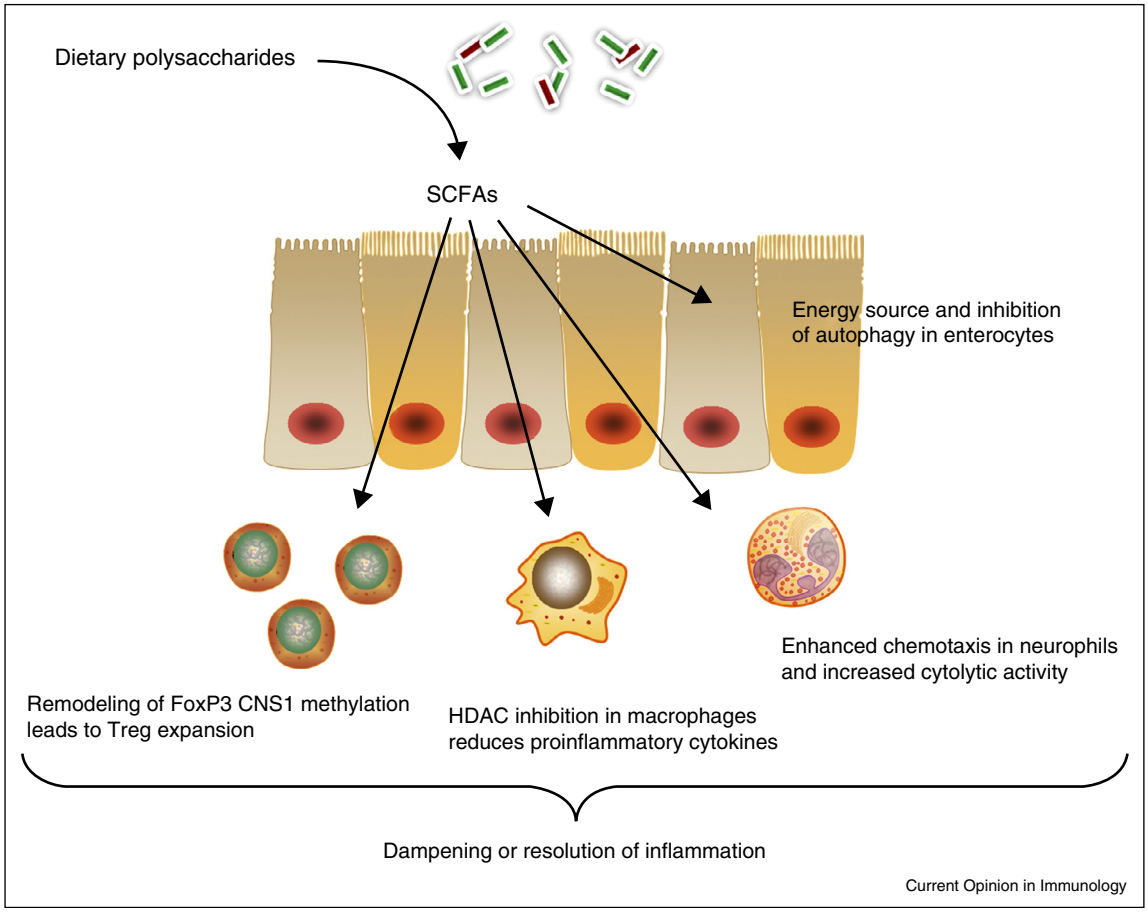
The mammalian gastrointestinal tract harbors one of the highest microbial densities on Earth, a population comprising around 1000–5000 species from all domains of life. The recent recognition that intestinal microbiota exerts profound effects on many aspects of human health and disease, including the metabolic, immune, and nervous system, has led to the concept of a human meta-organism that integrates the communication between both prokaryotic and eukaryotic parts to achieve homeostasis [1]. Because of its capability for direct microbial recognition and microbial community shaping through anti-microbial pathways, the host immune system plays a key role in this communication. So far, most research has focused on the recognition of microbial surface molecules and nucleic acids by the innate immune system, and a large number of reciprocal feedback loops between the microbiota and the innate immune system has been uncovered [2,3]. However, relatively little attention has been given to another means of communication between commensal bacteria and the immune system, namely the immunomodulatory effects of microbiota metabolites. These small molecules are intermediates and end products of diet-dependent commensal bacterial metabolism. Many of them serve as functional complementation to the metabolic capacities of the host, providing an example for bona fide mutualistic co-evolution with the mammalian part of the super-organism reducing its genomic capabilities to the extent that can be complemented by the microbiota. Other metabolites may serve as signaling molecules for inter-bacterial communication and quorum sensing. Since similar functional states of the microbiota can be reached by distinct taxonomic compositions, immune sensing of metabolites, rather than surface molecules, might allow more meaningful evaluation of microbiota function and consequences for the immune system. Here, we provide an overview about the most prominent examples of how microbiota metabolism products contribute to health and disease of the meta-organism by shaping the development and function of the immune system. As such, these metabolites integrate the functional states of food intake, microbiota ecology, and accordingly fine-tuning the host response.

Short-chain fatty acids

Diet dramatically influences the composition and function of the microbiota, and dietary changes can influence intestinal microbial ecology within the time scale of days [4]. Plant-derived non-digestible polysaccharides, such as cellulose, are an integral component of human diet. Bacterial fermentation of these polysaccharides produces short-chain fatty acids (SCFA) of 1–6 carbon length, including acetate, propionate, and butyrate. SCFAs have recently emerged as pivotal regulators of host metabolism and immunity (Figure 1). Receptors for SCFAs include the G-protein coupled receptors GPR41, GPR43, and GPR109A. Upon receptor binding, SCFAs modulate intracellular cAMP and calcium levels, and activate ERK1/2.

SCFAs products of commensal microbiota dampen inflammatory responses through GPR43, and mice

Figure 1



Immunomodulatory role of short-chain fatty acids. SCFAs are microbial fermentation products of dietary polysaccharides. They may function directly on intestinal epithelial cells, where they serve as an energy source and modulate autophagosome formation. In addition, SCFAs act directly on leukocytes. SCFAs enhance neutrophil recruitment and cytolytic activity, reduce proinflammatory cytokine production in macrophages by inhibition of histone deacetylases, and epigenetically remodel the FoxP3 locus in regulatory T cells to expand their population. These multifaceted actions promote the resolution of intestinal inflammation.

deficient in GPR43 develop colitis, arthritis, and asthma [5••]. Recently, three studies demonstrated a direct role for gut microbiota-derived SCFAs in the regulation of colonic regulatory T cells (Treg) homeostasis. SCFAs were shown to increase the number and suppressive function of colonic FoxP3$^+$ Tregs through Treg-intrinsic expression of GPR43 [6••]. Another study found that specifically butyrate is able to potentiate extrathymic generation of Tregs dependent on the intronic enhancer region conserved non-coding sequence 1 (CNS1) of the FoxP3 locus. Propionate, but not acetate, was similarly able to enhance peripheral Treg generation [7••]. Butyrate also ameliorated inflammation in a T cell-dependent model of colitis. Mechanistically, butyrate enhances histone H3 acetylation in the non-coding sequences of the FoxP3 locus, thereby driving the expression of the Treg lineage regulator [6••]. Butyrate's role as a histone deacetylase (HDAC) inhibitor also dampens the production of pro-inflammatory cytokines from intestinal mononuclear phagocytes (MNPs) [8].

Furthermore, butyrate was recently shown to exert anti-inflammatory activity through a different receptor, GPR109A, which also functions as a receptor for microbiota-derived niacin [9]. The effects of GPR109A signaling in the gut are manifold: on one hand, it reduces pro-inflammatory activities in colonic MNPs and leads to enhanced Treg differentiation and IL-10 production, suggesting that the effects of butyrate as an HDAC inhibitor might be connected to its activity as a GPR109A agonist. On the other hand, deficiency in GPR109A reduces the colonic epithelial secretion of IL-18, a cytokine that function as a major regulator of microbiota ecology and colonic homeostasis [10–13].

Apart from its major effect on colonic Treg and MNP populations, butyrate can be utilized as an energy source by colonic enterocytes, thereby driving mitochondrial respiration and decreasing autophagy [14]. The SCFA propionate signals through GPR41 to modify the MNP compartment that is released by the bone marrow and seeds the lung. As a result, dendritic cells (DCs) with higher phagocytic but decreased T cell priming capacity promote airway homeostasis [15].

Taken together, the abundance and localization of SCFAs in the intestine might be reflective of the functional state of the microbiota and thus represent a very fundamental way of host–microbiota communication that shapes the responsiveness of the intestinal immune system to bacterial sensing.

Long chain fatty acids

Long chain fatty acids (LCFAs) are an integral part of our diet. Various biochemical forms of LCFAs have been associated with health risks and benefits [16]. Like SCFA, some of the LCFA quantity and composition is modulated by the microbiota, and in turn participates in microbial-induced signaling in host cells. Dietary poly-unsaturated fatty acids (PUFA), such as various linoleic acids, are transformed into conjugated linoleic acids (CLA) and trans fatty acids [17,18,19]. Accordingly, mice fed with linoleic acid-supplemented diet with addition of *Bifidobacterium breve* exhibited an increase in liver CLA [20•]. In germ-free (GF) mice, CLA and hydroxy-fatty acid levels were undetectable in the colon, small intestine and plasma, suggesting that the microbiota is pivotal for PUFA metabolism [21•]. The physiological functions of CLA generation in host metabolism include reduction of hepatic triacylglycerol content [22] and inhibition of atherosclerosis [23]. Modified PUFA are potent agonists for peroxisome proliferator-activated receptor (PPAR)γ [24] and PPARα [25]. Several studies indicated an important role for the gut microbiota in regulating PPARγ, leading to attenuated inflammation [26,27]. However, the mechanistic links between gut microbiota production of conjugated PUFA and modulation of immunue responses need further invesigation.

Bile acids

A further important element of intestinal fatty acid metabolism are bile acids, that are continuously secreted into the proximal intestinal where they have multiple physiological roles in facilitation of digestion and absorption of multiple essential food-derived particles. Bile acids are derived from cholesterol catabolism in the liver. Newly synthesized bile acids are conjugated to glycine (in humans) or taurine (in mice), and conjugated bile acids are transported into the gallbladder. Postprandial contraction of the gallbladder empties bile acids into the intestinal lumen [28–30], where microbial enzymes can modify the primary bile acids into secondary bile acids by promoting dehydroxylation, dehydrogenation and deconjugation [28,31]. In antibiotics-treated animals or GF mice and rats, bile acid levels were reduced in the gallbladder and small intestine and elevated in the cecum, colon and feces [32,33,34••,35]. GF mice lack secondary bile acids and show reduced bile acid diversity compared to conventional mice.

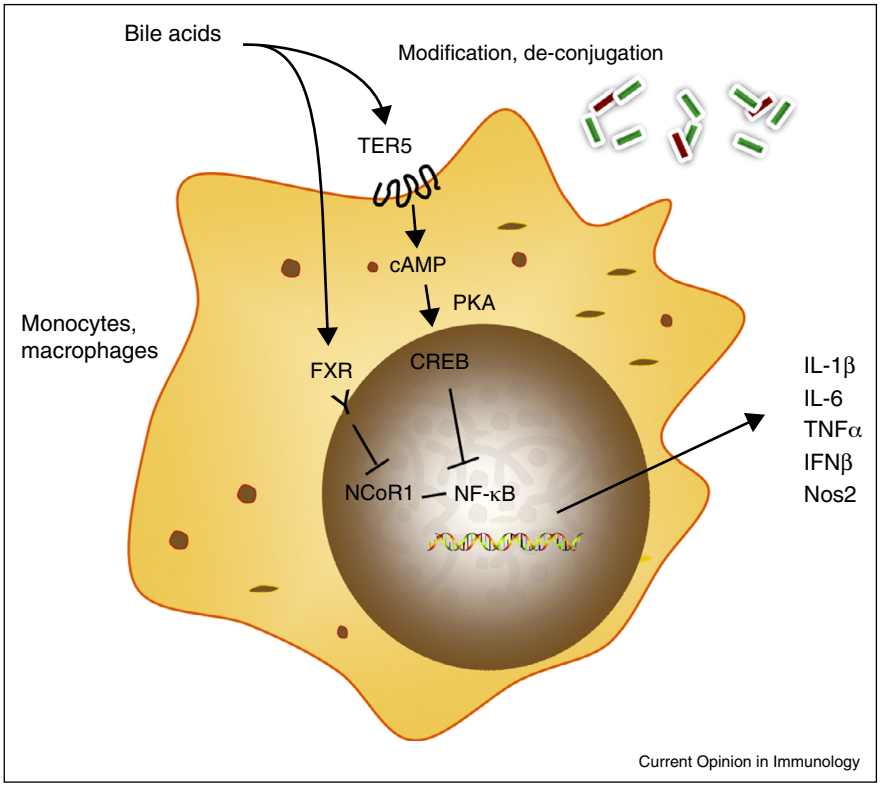
Sayin *et al.* [34••] demonstrated that the gut microbiota controls bile acid signaling via two main bile acid receptors: G protein-coupled receptor TGR5 (also known as GPBAR1) as well as the nuclear receptor farnesoid X receptor (FXR, also known as NR1H4). Fibroblast grown factor 15 (FGF15), which is the FXR target gene in the ileum, leads to suppression of liver CYP7A1, a rate-limiting enzyme in bile acid synthesis. In turn, FXR may have a protective role in maintenance of bacterial growth and epithelial barrier in the small intestine as demonstrated by mice lacking FXR which displayed bacterial overgrowth in the ileum, increased infiltrating neutrophils and disruption of the epithelial barrier [36•]. Other studies suggest that alterations in microbial transformation of bile acids and FXR-FGF15 signaling may contribute to the effects of the microbiota on obesity and metabolism [37,38]. Together, these studies propose that gut bacteria play a dominant role in regulating bile acid diversity and biological functions via FXR and TGR5 signaling.

The effect of commensal bacteria on bile acid diversity also modulates the host immune response. Bile acid signaling via both FXR and FGR5 results in inhibition of NF-κB mediated pro-inflammatory gene induction through two different mechanisms (Figure 2). Activation of FXR in lamina propria monocytes and macrophages results in stabilization of the NF-κB–NcoR complex and blocking of NF-κB binding to its responsive elements [39]. The second mechanism induced by bile acid-TGR5 signaling in macrophages also involves suppression of NF-κB activity [40,41•], possibly by induction of cAMP signaling leading to CREB activation that interacts with RelA (p65) [42] (Figure 2). The beneficial effect of bile acid signaling via FXR and TGR5 comprising attenuation of pro-inflammatory innate immune response has been demonstrated in several diseases, such as non-alcoholic fatty liver disease (NAFLD) [43], LPS-induced hepatic damage and inflammation [40], atherosclerosis [41•] and inflammatory bowel disease (IBD) [44].

In addition, FXR signaling may control expression of osteopontin in liver NKT cells in hepatitis [45]. The effect of bile acid composition on adaptive immune cells response has been demonstrated by administration of saturated fat, which altered conjugated bile acids and caused microbial dysbiosis associated with enhanced T_H1 response and increased colitis in IL-10 deficient mice [46••].

Together, the microbiota is intimately involved in host bile acid metabolism. Metabolic sensors of the immune

Figure 2



Bile acids, microbiota and innate immune response. Bile acids are synthesized to compound bile acids by the microbiota in the Ileum. In turn, the different bile acids bind the G protein coupled receptor TGR5 and the nuclear receptor FXR. In innate immune cells, such as monocytes and macrophages, bile acid-FXR signaling leads to attenuated inflammation by FXR stabilization of NF-κB-NcoR complex leading to suppression of NF-κB binding to its responsive element and thus inhibition of pro-inflammatory gene transcription such as IL-1β, IL-6, TNFα, IFNγ and Nos2. Likewise, TGR5-bile acid signaling leads to suppression of NF-κB activation and diminished inflammation, but via a different mechanism: TGR5 activates cAMP-PKA signaling and CREB activation, leading to inhibition of NF-κB.

system serve as guardians of this process and might thus evaluate the functional state of the microbiota.

Polysaccharides

In addition to intermediates of primary microbial metabolism, structural components of commensal microbes may be involved in the cross talk between the microbiota and the host immune system. Of all bacterial carbohydrates, the capsular polysaccharide A (PSA) of the commensal *Bacteroides fragilis* and its impact on the host immune system has received most attention. Initially, monocolonization of GF mice with *B. fragilis* modulates $CD4^+$ T cell homeostasis and cytokine production in a PSA-dependent manner [47], thereby ameliorating intestinal inflammation [48]. Recognition of *B. fragilis* PSA by TLR2 on Tregs mediates immune regulation and bacterial niche colonization [49]. This intestinal recognition of a bacterial polysaccharide can also influence systemic immunoregulation, as *B. fragilis* colonization potentially ameliorates CNS inflammation [50,51] and neurodegeneration [52]. Whether other commensal carbohydrates exert similar immunoregulatory effects on T cells remains to be investigated.

Amino acids

Only 10 amino acids can be *de novo* synthesized by human metabolic pathways, the rest are taken up through dietary proteins subjected to microbiota metabolism. In addition to this complementation of host metabolism [53–56], some amino acids have a role in regulation of the immune response. One prominent such amino acid is tryptophan, which is degraded by macrophages, leading to suppression of T cell proliferation [57]. Hashimoto *et al.* [58••] linked intestinal amino acid homeostasis and epithelial immunity by showing that mice deficient of Angiotensin I converting enzyme 2 (ACE2) exhibited increased susceptibility to intestinal inflammation caused by epithelial damage. The effect was transferable by microbiota transplantation into GF mice, and tryptophan-rich diet reverted microbial composition. The importance of tryptophan is further supported by the demonstration that mice lacking indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolizing enzyme [59], display alterations in both the composition

and metabolic pathways of the microbiota as well as immune homeostasis [60•]. Specifically, Ido1$^{-/-}$ mice showed outgrowth of *Lactobacilli*, similar to mice fed with tryptophan-rich diet, leading to production of the aryl hydrocarbon receptor (AhR) ligand indole-3-aledhyde. AhR activation, in turn, induces mucosal IL-22 transcription by innate lymphoid cells (ILCs) [61,62]. In addition, the differentiation of IL-17-secreting CD4$^+$ T cells is affected by tryptophan catabolism through IDO1 and modulated by the microbiota [63,64], affecting epithelial barrier integrity [65,66]. IDO1 activity is induced during the course of pathologic HIV infection [67], and untreated HIV-infected subjects that are likely to harbor enteropathogenic bacteria catabolizing tryptophan into immunomodulatory kynurenine derivatives known to correlate with disease progression and mucosal immune disruption [63].

The amino acid arginine is a substrate for protein synthesis and a precursor to nitric oxide (NO) [68,69] and arginase. Besides its fundamental role in the hepatic urea cycle, arginase is expressed in the immune system and arginine modulates the immune response during infection. The arginase pathway can support the growth of bacterial and parasitic pathogens. Mature myeloid cells can produce arginase I, which inhibits T-cell receptor expression and antigen-specific T-cell responses and thus promotes tumor evasion [70,71]. Granulocytes-associated arginase accounts for the suppression of immune reactivity in various models of tumor growth and chronic infections through the suppression of T-cell proliferation and cytokine synthesis [72].

L-Carnitine is an ammonium compound synthesized from lysine and methionine and is a common food supplement in red meat. L-Carnitine is metabolized by the gut bacteria to trimethylamineoxide (TMAO) and TMAO levels are highly associated with cardiovascular diseases (CVD). Mice fed with L-carnitine had elevated TMAO and showed reduced bile acid synthesis and reverse cholesterol transport and increased atherosclerosis, which was abrogated in GF and antibiotics-treated mice, demonstrating a link between gut microbiota, red meat consumption and CVD risk [73].

Vitamins

Vitamins are nutrients that are vital for multiple cellular and organ functions. Vitamin deficiency has been associated with well-defined clinical entities, and growing numbers of individuals, healthy and diseased alike, have adopted an empiric multi-vitamin usage. It has long been realized that vitamins biosynthesis can be performed or modulated by some members of the commensal microbiota [74–76]. For instance, gut *Bifidobacterium* and *Lactobacilli* synthesize the B9 vitamin folate [77,78,79,80••]. Vitamins A, C, and some of the B members influence host defense mechanisms. Vitamin A and its metabolite retinoic acid (RA) are the most prominent examples. Vitamin A deficiency results in increased susceptibility to infections [81] and mortality [82–85], through a significant modulation of the immune system [86] impairing both humoral and cellular immunity in the mucosa [87]. Vitamin A-deficient children have lower circulating T cells [88]. Vitamin A is required for antibody responses [89,90], prevention of activation-induced T cell apoptosis [91,92] and normal phagocytosis [93,94]. Importantly, vitamin A has also been reported to play a role in integrity of mucus-secreting goblet cells [95], goblet cell hyperplasia, and gut microbiota diversity [96]. RA can promote both Treg and Th17 lineages [83,96], through induction of histone acetylation of FoxP3 promoter [97]. RA is also necessary to elicit pro-inflammatory CD4$^+$ T cell responses to infection, mucosal vaccination [98•] and rejection of allogeneic skin grafts. RA signaling is required for T cell polarity affecting the Th1/Th17 to Th2 shift [99] and for B cell Ig switch to IgA [100]. Many intestinal cells are capable of synthesizing RA, including epithelial, stromal and migratory DCs [101]. Moreover, DCs from MyD88$^{-/-}$ mice expressed low levels of a critical enzyme for RA biosynthesis and were significantly impaired in induction of gut-homing T cells [94,102,103].

Vitamin B12 acts as an immunomodulator for cellular immunity, and B12 deficiency results in decreased numbers of CD8$^+$ and CD4$^+$ cells, as well as suppression of NK cell activity [104]. In addition to vitamin B12, vitamin B9 contributes to Treg cells levels in the small intestine [105•]. The monomorphic major histocompatibility complex class I-related protein (known as MR1) was shown to bind and present intermediates of the riboflavin (vitamin B2) biosynthetic pathway promoting mucosa-associated invariant T cell activation [106,107]. Vitamin D was recently shown to have profound and direct effects on T cell activation [108]. Several studies have linked vitamin D deficiency to IBD susceptibility [109] and colitis-associated colon cancer [110,111].

These studies suggest that we are starting to unravel the mechanistic underpinnings of what were merely empirical beneficial effects of vitamins on the immune system. These newly established insights might form the basis for a more evidence-based therapeutic administration of vitamins, as means of modulating diet, microbiota, and the immune response.

Perspective

The study of microbiota metabolites and their interactions with the immune system has brought our understanding of host–microbiota interactions to a new mechanistic level. One may argue that sensing of bacterial metabolites by the host is much more informative about the state of microbial colonization than recognition of microbial surface molecules, since metabolites provide information about the activity and function of microorganisms, rather than mere presence or absence. It remains to be determined the abundance and localization of microbiota metabolites that can be interpreted by the host. In this regard, despite the enormous progress made

in our understanding of feedback loops between innate immune function and the microbiota [3], one fundamental question remains insufficiently answered, namely how immune sensing of intestinal bacteria can distinguish between commensal bacteria with beneficial functions for the host and invasive pathogens despite their similarity in surface molecule expression. Interestingly, patterns that distinguish live from dead bacteria seem to provoke more potent immune responses [112], so the study of context and localization-dependent metabolite production might yield interesting insights into this question.

The recent progress made in studying the bacteria–metabolite–immune system axis also opens two further areas of investigation. First, the metabolites studied so far represent merely a fraction of all diet-dependent small molecules produced by commensal bacteria. The impact of entire pathways of bacterial metabolism on host–microbiota interactions remains almost completely unknown, including but not limited to metal and hydrogen metabolism. Interestingly, it has recently been found that such metabolic pathways play a role in microbiota competition with enteric pathogens [113,114]. In addition, small intermediates of metabolic pathways, such as the ones described here, might only represent a small minority of the potential range of host–microbial interactions involved in the maintenance of mutualistic homeostasis. Direct cellular contact, recognition of large protein or lipid complexes, exchange of ions, and the regulation of quorum sensing signals are all potential ways of host–microbiota communication to be studied in future research.

Finally, given the large potential of microbial metabolites as modulators of the immune response, it will pose a major challenge for future research to develop dietary interventions of immunity and inflammation. Targeted inference with microbiota metabolite production and downstream immunomodulation presents a very attractive and non-invasive avenue for future therapy design.

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