Immunity
Perspective

Diet, Metabolites, and "Western-Lifestyle" Inflammatory Diseases

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One explanation for the increased incidence of allergies, asthma, and even some autoimmune diseases has been the hygiene hypothesis. However, recent studies also highlight an important role for diet and bacterial metabolites in controlling various immune pathways, including gut and immune homeostasis, regulatory T cell biology, and inflammation. Dietary-related metabolites engage "metabolite-sensing" G-protein-coupled receptors, such as GPR43, GPR41, GPR109A, GPR120, and GPR35. These receptors are expressed on immune cells and some gut epithelial cells and generally mediate a direct anti-inflammatory effect. Insufficient intake of "healthy foodstuffs" adversely affects the production of bacterial metabolites. These metabolites and those derived directly from food drive beneficial downstream effects on immune pathways. We propose that insufficient exposure to dietary and bacterial metabolites might underlie the development of inflammatory disorders in Western countries. This review highlights what is currently known about diet, metabolites, and their associated immune pathways in relation to the development of inflammatory disease.

Introduction

The incidence of many inflammatory conditions, such as asthma and allergies, has increased dramatically in Western countries over the past few decades. Although less dramatic, the incidence of some autoimmune diseases, particularly type 1 diabetes, has also increased. The prevailing hypothesis for the increased incidence of asthma and allergies has been the hygiene hypothesis (Strachan, 1989; Strachan, 2000), which initially proposed that declining family size and improvements in personal hygiene reduce opportunities for cross-infections in families and that this leads to dysregulation in the immune system. As this hypothesis has evolved, it has incorporated the disruption of the beneficial gut commensal flora, which might be the real driver of inflammatory disease incidence in the Western world (Noverr and Huffnagle, 2004; Rook et al., 2003). A number of recent studies highlight the profound effect of diet on gut microbiota composition and the connection to immunological pathways (see also reviews by Ahern et al., 2014; Dorrestein et al., 2014; and Huttenhower et al., 2014, in this issue of *Immunity*). Mechanisms by which dietary, bacterial, and primary metabolites interact with the immune system have been identified and are outlined in this review. Many of the foodstuffs that have traditionally been considered healthy, such as dietary fiber, fish, and elements of the Mediterranean diet, can now be connected to molecular pathways that promote gut health and immune tolerance. We will provide a brief outline of the epidemiological evidence that supports a dietary basis for the emergence of inflammatory diseases, outline the connection to the gut microbiota, and then explain mechanisms by which metabolites intersect with the immune system.

Epidemiological Evidence that Supports a Dietary Basis for Inflammatory Diseases

In addition to improved hygiene, the nutritional change that has occurred in the Western world over the past few decades and the more recent "Westernization" of many countries coincide with the rise in the prevalence of asthma, allergy (Eder et al., 2006), and certain autoimmune diseases. Indeed, the change in dietary foodstuffs and the correlation with disease are particularly evident in epidemiological studies correlating fiber and fat intake. The average American consumes ~16 g of fiber per day, which is well below the recommended 25–38 g per day, and individuals in lower socioeconomic groups consume even less fiber (King et al., 2012). Notably, rural Africans consume substantially more fiber than Western individuals and rarely suffer from allergies, asthma, or colon cancer. Indeed, high intake of dietary fiber correlates with a lower risk of death from a range of conditions (Park et al., 2011), including cardiovascular disease and cancers but also infectious and respiratory diseases. Western individuals are more likely to eat an "obesogenic" diet, characterized by increased consumption of energy-dense, processed foods and reduced consumption of nutrient-rich foods, such as fruits and vegetables. A number of studies have discerned a correlation between asthma incidence and obesity (Boulet, 2013; Dixon et al., 2010; Jensen et al., 2012; Sin and Sutherland, 2008). Furthermore, interventional studies have shown that high fat (Wood et al., 2011) and low fruit and vegetable (Wood et al., 2012) consumption is linked to worse asthma outcomes. Conversely, the Mediterranean diet, which is based on high consumption of vegetables, fruits, olive oil, and fish, has now gained scientific credibility, at least in the prevention of cardiovascular diseases and asthma (Berthon et al., 2013; Castro-Rodriguez et al., 2008; Estruch et al., 2013; Nagel et al., 2010). Others have also proposed a dietary basis for asthma and allergies on the basis of convincing epidemiological studies (Devereux, 2006; Eder et al., 2006). A dietary basis for inflammatory diseases is most likely explained by interactions between dietary or bacterial metabolites and immune cells, or pathways for gut homeostasis. The current leading metabolites that play protective roles are short-chain fatty acids (SCFAs), ω-3 fatty acids,

and those derived from tryptophan catabolism; however, this is an emerging field, and there might be many more. Regardless, there is clear epidemiological evidence supporting a dietary basis for inflammatory disease. The molecular mechanisms that link diet, the gut microbiota, metabolites, immune responses, and inflammatory diseases will be discussed.

Dietary Influences on Gut Microbiota Composition and Metabolite Production

Humans and other vertebrates have coevolved over millennia with gut bacteria to the point of reliance on "commensal" bacteria, which might include symbiotic bacteria, for fiber digestion and the production of metabolites and certain vitamins. Indeed, diet shapes gut bacterial ecology and diversity (De Filippo et al., 2010; Le Chatelier et al., 2013; Ou et al., 2013; Turnbaugh et al., 2008). Furthermore, the composition of the gut microbiota relates to human disease (Clemente et al., 2012; Kau et al., 2011; Round and Mazmanian, 2009); however, the exact features of a healthy microbiota have not been fully elucidated. The diversity of the microbiota can be considered in terms of both richness (the number of species per sample) and evenness (the relative abundance of species). In one recent study, individuals with low bacterial richness (~23% of the Danish population) tended toward adiposity, insulin resistance, and dyslipidemia and a more pronounced inflammatory phenotype in comparison to individuals with high bacterial richness (Le Chatelier et al., 2013). Low bacterial diversity has also been associated with inflammatory bowel disease (IBD) (Lepage et al., 2011; Manichanh et al., 2006); however, it is unknown whether this is a cause or a consequence of the disease. Interestingly, in some studies, only a few bacterial species are necessary for distinguishing between individuals with high and low bacterial richness (Le Chatelier et al., 2013). One such species associated with bacterial richness is *Faecalibacterium prausnitzii*, which is well characterized for its anti-inflammatory effects (Sokol et al., 2008), including the ability of its metabolite(s) (e.g., butyrate) to inhibit NF-κB.

The major metabolites produced by bacteria in the gut are SCFAs. As more dietary fiber is ingested, SCFA production increases. In one often-quoted study (De Filippo et al., 2010), the fecal microbiota of European children and rural African children (from Burkina Faso) were compared. African children showed higher bacterial richness and a significant enrichment of bacteria from the genera *Prevotella* and *Xylanibacter* (efficient at digesting fiber and producing SCFAs), whereas these bacteria were completely absent from the microbiota of the European children. This correlation extended to significantly more SCFAs in the feces of African children than in the feces of European children because of their higher consumption of fiber. Notably, the microbiota composition of rural Africans is also different from that of African Americans in that it has a higher proportion of Prevotella and a lower proportion of *Bacteroides*, associated with a higher production of SCFAs, such as butyrate (Ou et al., 2013). In addition, other important metabolites, such as those related to tryptophan catabolism (see below), profoundly affect gut homeostasis. To date, there is little information on the relative ability of different bacterial species to produce various metabolites. Despite the intense interest in the topic of which features of commensal bacteria promote gut or immune health, this field is in its infancy. However, as we assert throughout this article, metabolites such as SCFAs, long-chain fatty acids, and tryptophan metabolites play a major role in the prevention of inflammatory disease and are highlighted for their interaction with the immune system, which might explain at least some of the dietary and microbiota-related associations with human disease. The next phase of research will involve the identification of specific blood and/or fecal metabolites that associate with or protect against human disease. The coming years should see vastly improved bioinformatics tools that equate the presence of different bacterial species with enzymatic machinery and the actual capacity for the production of various metabolites.

Major Points where Dietary or Bacterial Metabolites Intersect with the Immune System

It is important to consider where and when dietary metabolites influence the immune response. Figure 1 illustrates some of the major points where dietary metabolites intersect with the immune system, i.e., the gastrointestinal (GI) tract, blood, and fetal environment. Originally, the gut was considered the primary site where dietary metabolites mediated their effects, through either gut epithelial integrity or mucosal immunity. Indeed, the distal colon is where fiber is fermented by commensal bacteria to produce large quantities of acetate, propionate, and butyrate (~40, 20, and 20 mM, respectively) (Tan et al., 2014). However, several papers have shown that metabolites (particularly acetate) distribute systemically. Indeed, the exacerbated inflammatory reactions observed in germ-free mouse models of disease (Herbst et al., 2011; Maslowski et al., 2009) are likely to relate in part to the absence of SCFAs in the gut, blood, or tissues. In one recent study, the SCFA propionate was shown to affect dendritic cell (DC) and macrophage biology in the bone marrow and affect T helper 2 (Th2) cell responses in the airways (Trompette et al., 2014). That circulating SCFAs can have such a profound effect on systemic macrophage and DC biology illustrates the strong connection between dietary fiber intake and many types of immune responses under the control of DCs or macrophages. Another major point at which metabolites can intersect the immune system is through their transport across the placenta to the developing fetus, although the full effects of this process have yet to be determined. Interestingly, the placenta has recently been shown to contain commensal microbes (Aagaard et al., 2014). This implicates a potential role for maternal diet and the commensal microbes in directing immune pathways during fetal development. Similarly, metabolites such as SCFAs are present in breast milk, and this might be an important point of interaction between metabolites and the immune system. To date, the role of breast milk in protecting (or promoting) allergies and asthma is inconclusive. However, high-fat feeding of mice during lactation predisposes adult offspring to obesity and metabolic syndrome diseases (Vogt et al., 2014). Further research is required for determining the relative contribution to the development of inflammatory disease at the points where dietary and bacterial metabolites intersect the immune system.

SCFAs, Major Mediators of Gut Homeostasis

There is a major role for SCFAs in promoting gut homeostasis. As we proposed previously, deficiency of dietary fiber might underlie poor gut homeostasis, and this somehow contributes to the development of asthma, allergies, and certain autoimmune

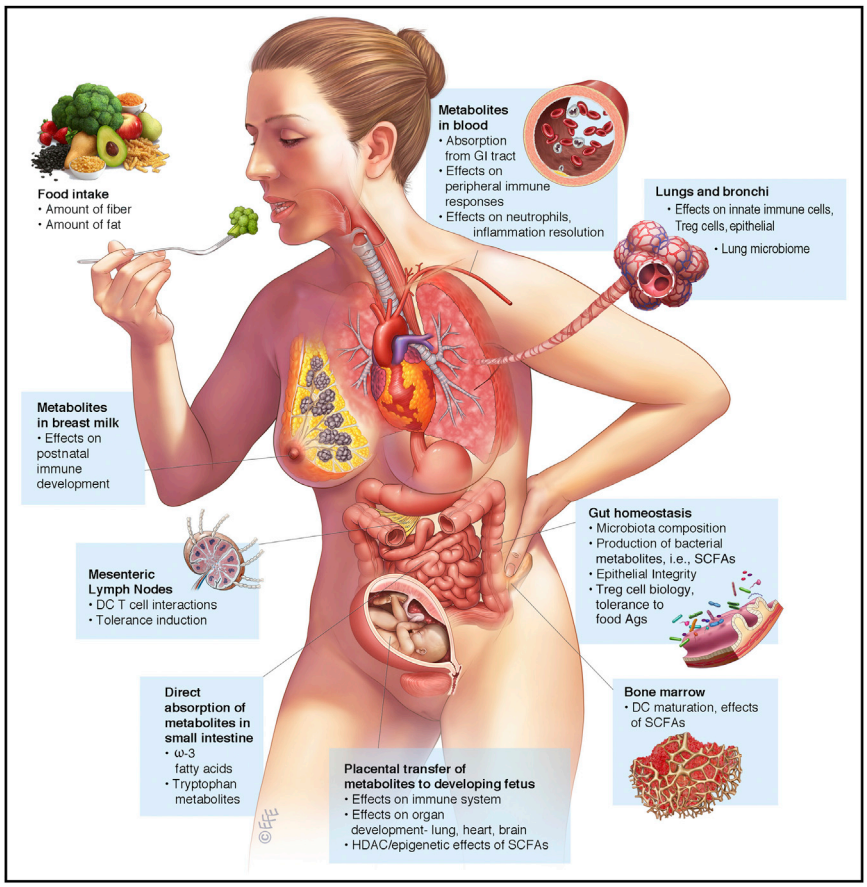

Figure 1. Major Points where Dietary or Bacterial Metabolites Intersect with the Immune System

In the GI tract, dietary fiber is primarily digested by commensal bacteria in the colon, which produces high concentrations of SCFAs, such as acetate, propionate, and butyrate. Other metabolites, such as ω-3 fatty acids, succinate, or kynurenic acid, are directly consumed and absorbed throughout the GI tract. In addition, metabolites can be directly absorbed in the small intestine. SCFAs (mainly acetate) are transported from the gut to the blood, where they can influence bone marrow and many cell types throughout the body. Another major point of intersection is the transfer of metabolites to the developing fetus. SCFAs are able to cross the placenta or be delivered via breast milk, where they can influence gene expression and the development of the immune system.

diseases (Maslowski and Mackay, 2011). Over the past several years, support for this concept has grown, and new mechanisms have come to light. Compromised epithelial integrity allows translocation of bacteria and/or dissemination of their products, such as food antigens and lipopolysaccharides, from the gut lumen to tissues, which might potentiate immune cell stimulation. There is considerable evidence that poor GI tract integrity plays a role in promoting the pathogenesis of autoimmune type 1 diabetes (Vaarala et al., 2008). Moreover, obese mice display increased intestinal permeability and endotoxemia (Cani et al., 2009). The aforementioned obesogenic diet disrupts gut flora ecology and is likely to result in lower production of metabolites such as SCFAs, which maintain epithelial integrity. Certain commensal bacteria, such as the acetate-producing *Bifidobacterium* species, have been shown to promote gut epithelial integrity (Fukuda et al., 2011). It is therefore likely that disrupted gut homeostasis and disrupted immunological tolerance are a central mechanism that precedes the development of numerous inflammatory diseases.

Some of the major mechanisms whereby commensal bacteria and SCFAs facilitate gut homeostasis are illustrated in Figure 2. The first relevant mechanism is "competitive exclusion," whereby high-fiber diets expand commensal bacteria and thus limit access of pathogenic bacteria to the gut epithelium. In addition to the commensals, the mucus barrier is an important element for physical separation of bacteria from the epithelial surface and is a critical contributor to both gut homeostasis and immune tolerance (Shan et al., 2013). One of the known effects of SCFAs is promoting the secretion of mucus by gut epithelial cells (Willemsen et al., 2003). More specifically, the high-acetate-producing species *Bacteroides thetaiotaomicron* has been shown to promote goblet cell differentiation and expression of genes for mucus production (Wrzosek et al., 2013). Interestingly, NALP6 is necessary for proper mucus secretion in the gut (Wlodarska et al., 2014), and we suspect that SCFA signaling through metabolite-sensing G-protein-coupled receptors (GPCRs) activates NALP6 (data not shown). Furthermore, immunoglobulin A (IgA) plays a key role in maintaining a noninflammatory relationship between the host and the gut microbiota (Peterson et al., 2007), as well as microbiota composition (Round and Mazmanian, 2009). Indirect evidence suggests that SCFAs might also promote the secretion of IgA by B cells (Ishikawa and Nanjo, 2009). Another point at which SCFAs operate in the gut is in tissue repair. The gut mucosa is prone to ulceration, and like any other tissue, relies on tissue repair processes. This might be of particular importance in the GI tract given that ulcers, physical damage, or actions of parasites rely on the capacity and efficiency of the repair process.

Point 5 of Figure 2 highlights the role of SCFAs in promoting regulatory T (Treg) cell responses in the gut (Atarashi et al., 2011; Atarashi et al., 2013; Geuking et al., 2011; Geuking et al., 2013). This presumably facilitates immunological tolerance to food antigens. In one of the first studies to investigate the role of the gut microbiota in initiating a Treg cell response, Atarashi et al. colonized germ-free mice with human fecal samples and then applied a sequential methodology to identify bacterial species capable of inducing Treg cells (Atarashi et al., 2013). The most potent inducers of Treg cells fell within clusters IV, XIVa, and XVIII of Clostridia. Another study showed that oral inoculation of Clostridia to mice during early life resulted in resistance to colitis and a reduction in IgE responses (Atarashi et al., 2011). Although Clostridia species are one of the highest producers of the SCFA butyrate, which could explain the ability of

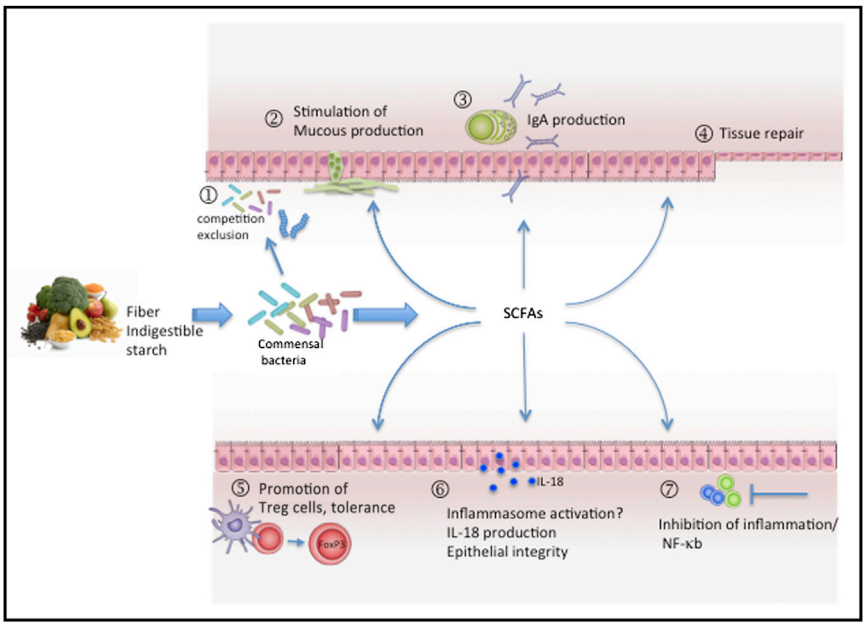

Figure 2. Dietary Fiber, SCFAs, and Mechanisms of Gut Homeostasis

There is now overwhelming evidence of the positive health benefits of high consumption of dietary fiber and the associated high concentrations of SCFAs in the gut (acetate, ~40 mM; propionate, ~20 mM; and butyrate, ~20 mM). The seven major actions for fiber and SCFAs can be summarized as follows: (1) "competitive exclusion," whereby a high-fiber diet expands commensal bacteria and limits pathogenic bacteria access to the gut epithelium; (2) SCFA-induced promotion of mucus by gut epithelial cells; (3) SCFA-induced secretion of IgA by B cells; (4) SCFA-induced promotion of tissue repair and wound healing; (5) SCFA-induced promotion of Treg cell development in the gut in a process that presumably facilitates immunological tolerance; (6) SCFA (particularly acetate)-mediated enhancement of epithelial integrity in a process dependent on inflammasome activation and IL-18 production; and (7) anti-inflammatory effects, particularly inhibition of NF-κb.

Clostridia to promote Treg cell numbers, the exact properties that Clostridia use to induce Treg cells are unclear. Indeed, recent reports have shown that SCFAs, particularly butyrate, directly influence numbers and function of inducible Treg cells (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013). Furthermore, Smith et al. showed that when SCFAs (particularly acetate and propionate) were fed to germ-free mice, they regulated the size and function of the colonic Treg cell pool (Smith et al., 2013).

Point 6 in Figure 2 depicts the effects of SCFAs on epithelial integrity. The best-characterized molecular pathway for maintenance of epithelial integrity involves the inflammasome pathway and production of the inflammasome-related cytokine interleukin-18 (IL-18) (Dupaul-Chicoine et al., 2010; Elinav et al., 2011; Normand et al., 2011; Zaki et al., 2010). Recently, membrane hyperpolarization, especially mediated by K^+ efflux, or Ca^{2+} flux, was found to trigger NALP3 inflammasome activation (Muñoz-Planillo et al., 2013). GPCR signaling is a common pathway for K^+ efflux, or Ca^{2+} flux, and it is possible that this is central to the activation of the NALP3 inflammasome and production of IL-18. This might be a key mechanism underlying diet-induced epithelial integrity and requires further investigation.

Point 7 in Figure 2 depicts the anti-inflammatory effects of SCFAs, for instance, on inflammatory cytokine production or on leukocyte recruitment. The ability of SCFAs to inhibit NF-κB is well known. More specifically, SCFAs have been reported to reduce expression of vascular cell adhesion protein and intracellular adhesion molecule, production of inflammatory chemokines, and production of inflammatory cytokines tumor necrosis factor (TNF), IL-6, and interferon-γ (Vinolo et al., 2011). The anti-inflammatory effects of SCFAs on chemotaxis and leukocyte recruitment have been documented in a number of in vitro studies, as well as in animal models of inflammation (Maslowski et al., 2009; Tan et al., 2014).

The actions of SCFAs on the gut, as shown in Figure 2, align with the results from decades of research showing the health beneficial effects of either SCFAs or dietary fiber, particularly in gut health (Harig et al., 1989; Kanauchi et al., 2001; Kanauchi et al., 2002; Scheppach, 1996; Topping and Clifton, 2001; Treem et al., 1994). In addition to SCFAs, other metabolites—such as ω-3 fatty acids, which inhibit TNF or IL-6 production from macrophages—show anti-inflammatory properties (Oh et al., 2010; Yan et al., 2013). The anti-inflammatory properties of many metabolites presumably relate to the need for constrained and well-regulated immune responses to bacterial or food antigens in the gut. The following sections outline the main molecular mechanisms whereby metabolites influence biological outcomes; these mechanisms include "metabolite-sensing" GPCRs and the effects on gene transcription through histone deacetylase (HDAC) inhibition or agonism of specific transcription factors.

Metabolite-Sensing GPCRs

Vertebrates have evolved several mechanisms to respond to dietary and bacterial metabolites. One mechanism is via metabolite-sensing GPCRs, which produce immediate biological responses to specific metabolites. Many of the common dietary and bacterial metabolites have GPCR sensors (Figure 3 and Table 1). The reason that body cells, including immune cells, use these receptors to modify their function is still uncertain but presumably relates to a need to sense the availability of nutrients.

Many of the metabolite-sensing GPCRs listed in Table 1 were identified as late as the 1990s through large-scale sequencing efforts, and these sensors were deorphaned in the 2000s (reviewed in Macia et al., 2012; Tan et al., 2014). Probably the best-characterized metabolite-sensing GPCRs are GPR43, GPR41, and GPR109A, which bind SCFAs. GPR43 and GPR109A appear to be important for gut homeostasis, and both are expressed by the colonic epithelium, by inflammatory leukocytes (such as neutrophils and macrophages), and by Treg cells. Regulation of colonic and peripheral Treg cell numbers relates to these metabolite-sensing receptors (Singh et al., 2014; Smith et al., 2013). Lack of GPR109A, a metabolite

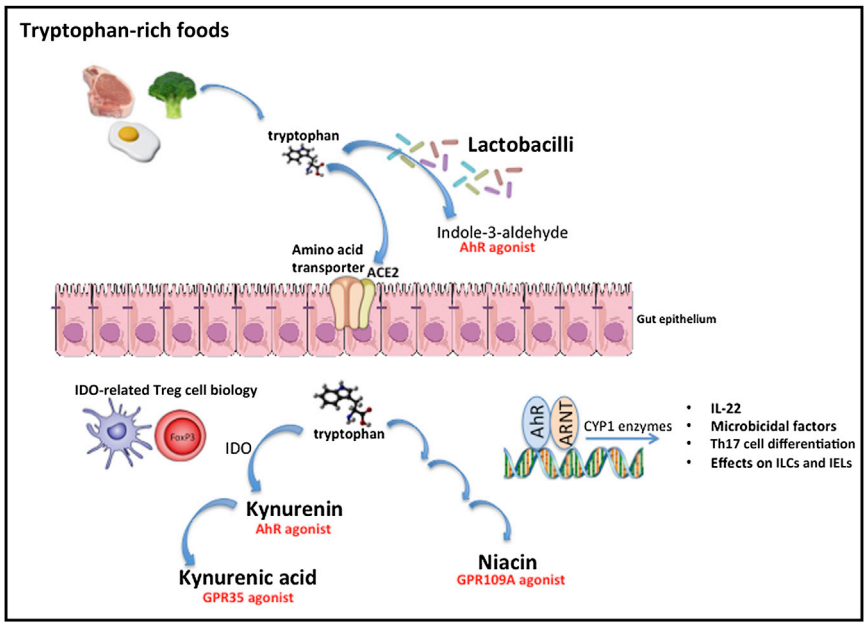

Figure 3. Tryptophan Catabolites, Agonism of AhR, or Stimulation of Metabolite-Sensing GPCRs

Tryptophan, an essential amino acid, is found in foodstuffs such as red meat, fish, eggs, and many vegetables. Tryptophan can be catabolized by microbial species, such as lactobacilli, to yield indole-3-aldehyde, an aryl hydrocarbon receptor (AhR) agonist. Tryptophan can also be transported across the epithelium by transport machinery comprising Ace2. Tryptophan is degraded to kynurenin (an AhR agonist) by the immune-regulatory enzyme indoleamine 2,3-dioxygenase (IDO). After agonist binding, AhR-dependent gene expression includes genes involved in the production of mediators important for gut homeostasis; such mediators include IL-22, antimicrobicidal factors, increased Th17 cell activity, and the maintenance of intraepithelial lymphocytes (IELs) and RORγt^{+} innate lymphoid cells (ILCs). A number of tryptophan metabolites, including kynurenic acid and niacin, agonize metabolite-sensing GPCRs, such as GPR35 and GPR109A.

sensor for butyrate, is associated with fewer colonic Treg cells as a result of the reduced ability of colonic macrophages and DCs to promote their development (Singh et al., 2014). Deletion of either receptor in mice exacerbates dextran sulphate sodium (DSS)-induced colitis (Maslowski et al., 2009; Singh et al., 2014). Hence, many of the actions described above for SCFAs in gut homeostasis can be ascribed to GPR43 and GPR109A. Even though these recent papers show some discrepancies relating to the relative roles of acetate, propionate, and butyrate, SCFAs (the main metabolic products of gut bacteria) are used as a mechanism for tolerance induction in the gut.

Other important metabolite-sensing GPCRs include GPR120, which recognizes long-chain fatty acids, such as ω-3 fatty acids (Oh et al., 2010), derived from foodstuffs (including fish and olive oil); GPR91, a receptor for succinate (a product of the citric acid cycle); GPR84, a receptor for medium-chain fatty acids, derived from milk in certain species; and GPR35, a receptor for metabolites of tryptophan catabolism, e.g., kynurenic acid (Table 1). Some of the most relevant publications associated with these receptors are referenced in Table 1. All of the metabolite-sensing GPCRs are expressed by immune cells, particularly by innate-type cells, although this knowledge has mostly been gleaned from transcript expression analyses. They are also expressed on the intestinal epithelium, which probably relates to roles in maintenance of epithelial integrity. Another common and defining feature of metabolite-sensing GPCRs is their expression on tissues or cells relevant to metabolism, for instance, pancreatic islets or white adipose tissue. The role of many of these receptors is poorly defined, and tools for studying their expression and function await development.

Metabolite-sensing GPCRs have a large bearing on the control of inflammatory responses, particularly IBD (Maslowski et al., 2009; Oh et al., 2010; Singh et al., 2014; Trompette et al., 2014). Indeed, most metabolite-sensing GPCRs and their ligands (including GPR43 and SCFAs, GPR120 and ω-3 fatty acids, GPR109A and its ligands, and possibly GPR35 and its ligands) facilitate anti-inflammatory effects. Concerning the SCFA receptors, GPR43-deficient (*Ffar2*$^{-/-}$) mice show exacerbated inflammation in models of airway hypersensitivity, DSS-induced colitis, and rheumatoid arthritis (Maslowski et al., 2009). Administration of acetate to mice in drinking water protects against colitis in wild-type mice, but not in *Ffar2*$^{-/-}$ mice, suggesting that acetate mediates its anti-inflammatory effects through GPR43. The anti-inflammatory effects of SCFAs are not limited to GPR43, given that a recent study (Trompette et al., 2014) established a role for propionate and its receptor (GPR41) in the generation of macrophage and DC precursors and in the seeding of the lungs by DCs that have high phagocytic capacity but an impaired ability to promote Th2 cell responses. This and other studies from our laboratory clearly establish a role for fiber intake as protective against allergic airway disease in mice. Furthermore, it is likely that SCFA-mediated inhibition of HDAC activity (see below), in addition to SCFA actions on metabolite-sensing receptors, contributes to the beneficial role of fiber in diseases such as asthma.

GPR109A has recently emerged as a major regulator of gut homeostasis. GPR109A binds the SCFA butyrate but also the tryptophan metabolite nicotinic acid. Nicotinic acid is known to have anti-inflammatory properties, such as inhibition of inflammatory cytokine secretion by monocytes, macrophages, adipocytes, and epithelial cells (Digby et al., 2010; Digby et al., 2012; Gambhir et al., 2012; Zandi-Nejad et al., 2013). Nicotinic acid has been used since the 1950s to reduce progression of atherosclerosis. More recently, in a mouse model of atherosclerosis, nicotinic acid was shown to inhibit disease progression in wild-type mice, but not in mice lacking GPR109A (Lukasova et al., 2011). This effect was not related to any metabolic changes but instead depended on GPR109A expression by bone-marrow-derived cells, such as macrophages.

Table 1. Summary of Currently Recognized Metabolite-Sensing GPCRs and Their Ligands, Signaling Molecules, Effector Mechanisms, Expression, and Function

GPCR	Ligands	Signaling	Effector	Main Expression	Function in Immunity	Function in Metabolism
GPR41 (FFAR3)	SCFAs (C3–C7): formate, acetate, propionate, butyrate, pentanoate	$G_{i/o}$, β-arrestin-2	↓ cAMP	adipocytes, enteroendocrine L cells	DC maturation, anti-inflammatory (asthma model)	leptin production, regulation of energy balance
GPR43 (FFAR2)	SCFAs (C2–C7): formate, acetate, propionate, butyrate, pentanoate	$G_{i/o}$, G_q, β-arrestin-2	↓ cAMP, ↑ Ca^{2+}	innate immune cells, enteroendocrine L cells, gut epithelium white adipose tissue	anti-inflammatory, gut homeostasis, tumor suppressor	insulin-mediated fat accumulation, control of body energy
GPR109A (NIACR1, HM74)	SCFAs (C4-C8): particularly butyrate and nicotinic acid (niacin)	$G_{i/o}$, β-arrestin-2	↓ cAMP, ↑ Ca^{2+}	adipocytes, neutrophils, macrophages, intestinal epithelial cells	DC trafficking, gut homeostasis, anti-inflammatory, tumor suppressor	intracellular triglyceride lipolysis in adipocytes
GPR120 (FFAR4)	long-chain fatty acids (C14–1C8), ω-3 fatty acids, i.e., docosahexaeonic acid	$G_{q/11}$, β-arrestin-2	↑ Ca^{2+}	enteroendocrine cells in the colon, macrophages	anti-inflammatory, inhibition of TNF and IL-6	regulation of insulin secretion by GLP-1 (role in diabetes?), *FFAR4* SNPs associate with obesity
GPR40	medium- to long-chain fatty acids (C12–C18)	$G_{q/11}$	↑ Ca^{2+}	pancreatic β cells, enteroendocrine K cells	anti-inflammatory	insulin secretion from pancreatic β cells
GPR84	medium-chain fatty acids (C9–C14), i.e., capric acid	$G_{i/o}$	↓ cAMP	immune cells	proinflammatory? (role unclear)	role in adiposity?
GPR35	kynurenic acid, kysophosphatidic acid, pamoic acid	$G_{i/o}$, G16, β-arrestin-2	↓ cAMP, ↑ Ca^{2+}	monocytes, neutrophils, iNKT cells, GI tract, peripheral nervous tissues, mast cells	*GPR35* SNPs implicated in human IBD	unstudied
GPR91 (SUCNR1)	Succinate	$G_{i/o}$, G_q	↓ cAMP, ↑ Ca^{2+}	high levels in adipose tissue, kidney, nervous system, DCs; lower levels in liver, spleen	proinflammatory, migration of Langerhans cells, hematopoiesis, angiogenesis	hypertensive effects, activation of renin-angiotensin system

The precise expression and function of many of these metabolite-sensing receptors are still evolving. The information in this table was based in part on the following studies (only major publications are cited): GPR41 (Samuel et al., 2008; Trompette et al., 2014; Xiong et al., 2004), GPR43 (Kimura et al., 2013; Maslowski et al., 2009), GPR109A (Lukasova et al., 2011; Singh et al., 2014; Thangaraju et al., 2009), GPR40 (Fujita et al., 2011; Itoh et al., 2003), GPR120 (Hirasawa et al., 2005; Ichimura et al., 2012; Oh et al., 2010), GPR84 (Suzuki et al., 2013), and GPR35 and GPR91 (Sapieha et al., 2008; Toma et al., 2008). Metabolite-sensing GPCRs, such as GPR41, GPR120, GPR109A, and GPR43, also play fundamental roles in the regulation of metabolism and are usually expressed on tissues such as pancreatic islets, adipose tissue, or enteroendocrine cells in the gut. Abbreviations are as follows: cAMP, cyclic AMP; DC, dendritic cell; GI, gastrointestinal; GLP-1, glucagon-like peptide 1; GPCR, G-protein-coupled receptor; IBD, inflammatory bowel disease; IL-6, interleukin-6; iNKT, invariant natural killer T cell; SCFA, short-chain fatty acid; and TNF, tumor necrosis factor.

It is important to define how GPCR signaling initiated by metabolites produces the aforementioned anti-inflammatory effects. Several possible outcomes follow the engagement of a metabolite sensor with its agonist; one example is signaling through regular G-proteins, such as Gαi or Gq, which usually leads to activation of MAP kinases, PI3 kinases, or mTOR. Interestingly, many metabolite-sensing GPCRs engage an alternative signaling pathway mediated by β-arrestin-2 (Oh et al., 2010), which generally produces anti-inflammatory effects, some of which relate to inhibition of NF-κB. All of the major metabolite-sensing GPCRs signal through β-arrestin-2, but the precise circumstances or cell types that preferentially use β-arrestin-2 have not been determined. β-arrestin-2 directly interacts with IκBα (an inhibitor of NF-κB), thereby preventing the phosphorylation and degradation of IκBα (Gao et al., 2004). It is therefore predicted that agonism of these metabolite-sensing GPCRs triggers an immediate and direct biological response.

Transcriptional and Epigenetic Effects of Dietary and Bacterial Metabolites

In addition to signaling via GPCRs, some metabolites are also implicated in regulating transcription through epigenetic pathways. Acetylation and deacetylation of histones, as well as nonhistone proteins (such as transcription factors), are major mechanisms for epigenetic regulation of gene expression. Acetylation of histones, by histone acetyl transferases (HATs) or inhibition of HDACs, results in negatively charged histones, which, when interacting with negatively charged DNA, loosen the chromatin structure and result in a transcriptionally active conformation. Acetylation also promotes the activation, nuclear translocation, and DNA binding of transcription factors, such as STAT3, NF-κB, FoxP3, N-FAT, and RUNX1. This leads to the expression of multiple genes, including those for proinflammatory cytokine production (Wang et al., 2009). Acetylation of transcription factors can also lead to structural changes that alter

transcription factor binding to the promoter region, thereby initiating or preventing gene expression. In the case of NF-κB, acetylation of the RelA subunit prevents interaction with IκBα and results in poor formation and reduced transcriptional interaction (Chen et al., 2001). Notably, a total of ~1,750 acetylated proteins have the potential to be affected by HDAC activity (Choudhary et al., 2009).

Synthetic small-molecule inhibitors of HDACs have shown the capacity to attenuate inflammation in animal models of arthritis, IBD, asthma, diabetes, cardiovascular disease, and multiple sclerosis (Wang et al., 2009). In addition, the broad HDAC inhibitor trichostatin-A increases Treg cell numbers and suppressive capacity (Tao et al., 2007), illustrating the role of HDAC inhibition in promoting immunological tolerance in addition to anti-inflammatory responses. SCFAs are natural inhibitors of HDAC enzymatic activity. Of the SCFAs, butyrate is the most potent HDAC inhibitor, and acetate is the least potent. Butyrate-induced HDAC inhibition has been shown to regulate macrophage function in the intestine and to promote Treg cells (Arpaia et al., 2013; Chang et al., 2014). Deletion of HDAC3 from intestinal epithelial cells severely disrupts gut homeostasis in the DSS model (Alenghat et al., 2013), indicating that HDAC3 might play an important regulatory role in gut homeostasis. Of note, in addition to inhibiting the enzyme activity, SCFAs might also affect HDAC gene transcription directly, thereby indirectly influencing HDAC activity. Therefore, the implications of SCFAs in regulating transcription through epigenetic pathways must be considered in future research.

Other Important Metabolites: Tryptophan and ω-3 Fatty Acids

We must consider the independent contribution of gut-microbiota-derived metabolites versus metabolites derived directly from food, such as tryptophan metabolites and ω-3 fatty acids. Tryptophan, an essential amino acid, is found in foodstuffs such as red meat, fish, eggs, yogurt, and many vegetables. Tryptophan can be catabolized by microbial species, such as lactobacilli (Figure 3). Indole-3-aldehyde, one tryptophan metabolite produced by lactobacilli, is an aryl hydrocarbon receptor (AhR) agonist (Zelante et al., 2013). After agonist binding, AhR translocates to the nucleus, where it forms a heterodimer with AhR nuclear translocator (ARNT). AhR-dependent gene expression includes genes involved in the production of mediators important for gut homeostasis; these mediators include IL-22, antimicrobicidal factors, increased Th17 cell activity, and the maintenance of intraepithelial lymphocytes and RORγt$^+$ innate lymphoid cells (Li et al., 2011; Veldhoen and Brucklacher-Waldert, 2012). The absence of AhR in mice has been shown to increase the severity of DSS colitis (Li et al., 2011) and *Citrobacter rodentium* infection (Kiss et al., 2011). However, various other metabolites, including flavonoids and glucosinolates, which are abundant in plants, bind AhR. Tryptophan might also be transported across the epithelium by transport machinery comprising angiotensin I converting enzyme 2 (ACE2). Deficiency of ACE2 in mice also exacerbates DSS colitis (Hashimoto et al., 2012), indicating that tryptophan catabolism in vertebrate tissues is also necessary for proper gut homeostasis. Tryptophan is converted to kynurenin (also an AhR agonist) by the immune-regulatory enzyme indoleamine 2,3-dioxygenase (IDO), and IDO activity is linked to suppression of T cell responses, promotion of Treg cells, and immune tolerance (King and Thomas, 2007). Moreover, a number of tryptophan metabolites, including kynurenic acid and niacin, agonize metabolite-sensing GPCRs, such as GPR35 and GPR109A (Figure 3). Of note, *Gpr35* polymorphisms are a genetic risk factor for IBD (Jostins et al., 2012), and thus multiple elements of tryptophan catabolism facilitate gut homeostasis.

ω-3 fatty acids might be a major contributing component of the beneficial effects of a Mediterranean diet. Indeed, ω-3 fatty acids show anti-inflammatory effects through their interaction with the metabolite sensor GPR120 (Oh et al., 2010). ω-3 fatty acids also show anti-inflammatory effects in animal models and in clinical intervention studies (Simopoulos, 2002). ω-3 fatty acids have long been suspected as a protective metabolite for asthma, in part because of observations on Greenland Eskimos, who consume very high levels of ω-3 fatty acids and have extremely low rates of heart disease and chronic inflammatory diseases, including asthma (Dyerberg and Bang, 1979). For this population, fiber and associated microbiota-derived metabolites might be less important. The mere existence of metabolite-sensing GPCRs for tryptophan metabolites and other dietary factors such as ω-3 fatty acids (Oh et al., 2010; Toma et al., 2008) suggests that nonbacterial metabolites are important for regulating immunity. In all likelihood, the combined effects of both microbiota-derived and dietary metabolites operate together.

Concluding Remarks

New understanding of gut microbial ecology, metabolite biology, metabolite-sensing GPCRs, and transcriptional regulation by metabolites opens up attractive possibilities for prevention and treatment of "Western-lifestyle" inflammatory diseases.

Why the mammalian immune system has adopted molecules traditionally associated with metabolism is intriguing. It is also unclear why there appears to be a high degree of redundancy in molecules that regulate gut and immune homeostasis and why gut homeostasis can be affected by the deletion of any one of numerous molecules. Determining the relative roles of receptors such as GPR43, GPR41, and GPR109A versus HDACs is a necessary task. A confusing aspect of this field is signaling by metabolite-sensing GPCRs—do receptors signal via β-arrestin-2, which typically produces anti-inflammatory effects, or through conventional G-proteins? Irrespective of these unanswered questions, there exists an opportunity to manipulate immunity and gut health through metabolite-sensing GPCRs, HDACs, or transcription factors, such as AhR. One simple approach is through consumption of healthy foodstuffs, perhaps in combination with the manipulation of the gut microbiome via probiotics. This should affect the incidence of many inflammatory diseases in human populations.

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