JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Effects of the Modulation of Microbiota on the Gastrointestinal Immune System and Bowel Function

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ABSTRACT: The gastrointestinal tract harbors a tremendous number and variety of commensal microbiota. The intestinal mucosa simultaneously absorbs essential nutrients and protects against detrimental antigens or pathogenic microbiota as the first line of defense. Beneficial interactions between the host and microbiota are key requirements for host health. Although the gut microbiota has been previously studied in the context of inflammatory diseases, it has recently become clear that this microbial environment has a beneficial role during normal homeostasis, by modulating the immune system or bowel motor function. Recent studies revealed that microbiota, including their metabolites, modulate key signaling pathways involved in the inflammation of the mucosa or the neurotransmitter system in the gut−brain axis. The underlying molecular mechanisms of host−microbiota interactions are still unclear; however, manipulation of microbiota by probiotics or prebiotics is becoming increasingly recognized as an important therapeutic option, especially for the treatment of the dysfunction or inflammation of the intestinal tract.

KEYWORDS: *microbiota, inflammation, dysfunction, short-chain fatty acids, dietary fiber, inflammatory bowel disease, irritable bowel syndrome, stress, serotonin, cognition*

INTRODUCTION

During a normal lifetime, a human ingests about 60 tons of food, and this food all passes through the intestinal tract. Although food is indispensable, its passage also constitutes a threat to health, because food provides exposure to dietary antigens, viable microorganisms, and bacterial products. In the intestinal tract, the mucosa is the first line of defense to protect against pathogenic products (e.g., endotoxins, hydrogen sulfide, phenols, ammonia, and indoles) or microbiota and also absorbs essential nutrients. At the same time, the intestinal tract acts as an ideal fermentor for microbiota.[1] The microbiota in the gastrointestinal tract represents an ecosystem of the highest complexity. A comprehensive culture-independent phylogenetic analysis of microbes showed that at least 2000 bacterial species are harbored in the gastrointestinal tract of a single individual.[2,3] The microflora plays many critical roles in the body; thus, there are many areas of host health that can be compromised when the microflora is drastically altered.[4] Dysbiosis, which is defined as a condition with microbial imbalances in the body, has been associated with various illnesses (e.g., inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS)).

In addition, metagenomic approaches have provided new insights into the microbiota and the relationship between the microbiota and host.[5−7] Although metagenomic carriage of metabolic pathways was relatively stable among individuals in different community structures, the human microbiome project consortium showed that racial background had a strong association with the microbiota.[5] It has been reported that the early stage of life, within 3 years after birth, strongly affects the maturation of the microbiome in individuals.[7] Humans share at least 160 species of microbiota, which have the potential to affect the fundamental uptake of energy from the gastrointestinal tract; however, there are significant differences in the microbial clusters between healthy volunteers and patients with various diseases, such as IBD.[8]

Recently, many reviews have been published regarding the relationship between dysbiosis and the mucosal immune system or human health. In developing countries, it has been reported that the nutritional value of food is influenced by the status of the gut microbial community.[9] As is well-known, malnutrition causes serious problems, and nutrient deficiencies are reported to cause impaired intestinal mucosal barrier function, which is associated with several defects in the innate and adaptive immune systems, which can lead to lethal diarrheal infectious illnesses, especially for children and infants. It is speculated that dysbiosis contributes to disease risk and pathogenesis, and the diet may shape the gut microbial community and its functions.[9]

On the other hand, the "hygiene hypothesis" is also known well in Western countries and posits that individuals raised in a sanitary environment are more likely to develop IBD. In a population-based study, it was reported that the status of hygiene in childhood was associated with the risk for IBD.[10] Interestingly, exposure to a variety of microorganisms early in life could result in the colonization of the intestine with

Received: June 6, 2013
Revised: September 26, 2013
Accepted: September 27, 2013
Published: September 27, 2013

dx.doi.org/10.1021/jf402441f | J. Agric. Food Chem. 2013, 61, 9977−9983

microbiota that can respond more efficiently to an episode of gastroenteritis and postinfectious IBS.[11] In this review, we focus on the role of microbiota in gastrointestinal tract diseases, especially IBD and IBS.

RELATIONSHIP BETWEEN GASTROINTESTINAL DYSFUNCTION AND MICROBIOTA

The diversity of the colonic microbiota in IBD patients is distinct from that of healthy subjects,[12] consisting of fewer sequences of the phylum Bacteroidetes and the family Lachnospiraceae of the phylum Firmicutes, and more sequences are representatives of the phylum Proteobacteria and the *Bacillus* subgroup. In addition, Spiller noted that, in comparison to control subjects, the microbiota in IBS patients displayed abnormalities related to malfermentation in the lower intestine and a change in transit time.[13] Furthermore, the intestinal microbiota and their products have been found to play an increasingly important role in the mucosal immune function (inflammation), motor function, and mucosal barrier function in both IBD and IBS.[14] Immune cells recognize microbiota through the identification of unique prokaryotic molecular motifs to prevent the invasion of pathogenic microbiota and to regulate the immune response to commensal bacteria.[15] It is well-known that the mucosal immune cells recognize the pathogen-associated molecular patterns (PAMPs) by their respective Toll-like receptors (TLRs) and then initiate protective sequences including an inflammatory response via the rapid induction of myeloid-differentiation factor 88 (MyD88) and nuclear factor-kappa B (NF-κB)-dependent signaling pathways. This results in the expression of a broad array of pro-inflammatory cytokines, adhesion molecules, and chemokines. In intestinal epithelial cells, PAMPs have also been shown to lead to the promotion of barrier enhancement, epithelial repair, and the secretion of antimicrobial peptides, rather than overt inflammatory responses.[16]

It is beyond a shadow of a doubt that microbiota play an important role in the pathogenesis of gastrointestinal diseases, because the colitis in transgenic and knockout mutant murine models cannot occur when these lines are maintained in a germ-free environment.[17,18] Germ-free mice are also reported to display alterations in their stress response and central neurochemistry in comparison to conventional mice,[19] in addition to altered gastrointestinal transit time.[20] These data may indicate that specific control of the microbiota may be a useful strategy for modulating the comorbid aspects of gastrointestinal disorders.

ROLE OF MICROBIOTA AND RELATED METABOLITES IN IBD AND POTENTIAL THERAPEUTIC STRATEGIES

Probiotics are nonpathogenic live microorganisms that are administered to alter the intestinal microbiota and confer a beneficial effect on health.[21] It has been reported that probiotics have protective effects against intestinal inflammation, including enhancement of the epithelial barrier function, modulation of the mucosal immune system, and alteration of the intestinal microbiota. Because exogenous probiotics are distinguished from the intestinal tract immediately, it is speculated that the effects are transient due to their almost immediate exclusion.[22] *Lactobacilli* and *Bifidobacteria* have traditionally been the most common candidate probiotics, and nonpathogenic *Escherichia coli*, *Saccharomyces boulardii*, and their mixture (e.g., VSL#3) have also been used to produce probiotic effects.[23] However, there is still a discrepancy between the therapeutic potential and actual clinical outcomes of probiotic treatment in IBD.[24]

Because the pathogenesis of IBD includes genetic, microbial, and environmental factors and because this leads to heterogeneous phenotypes in patient subsets that are uniquely responsive to specific microbial manipulations, the clinical outcome of probiotic treatment has shown relatively large variations.[24] Although some clinical trials of probiotics in IBD patients showed significant efficacy,[25] it was also reported that a meta-analysis of IBD treatment by probiotics did not show any significant therapeutic merit.[26,27]

Prebiotics are nondigestible food constituents given to benefit the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon.[28] Prebiotics alter the composition or balance of intestinal microbiota in the host. The beneficial microbiota are unique to each host, and prebiotics can help provide increased resistance to gut infections and may also have immunomodulatory effects. It has been reported that prebiotics could not be absorbed directly into the epithelium and were instead converted to other metabolites, including short-chain fatty acids (SCFAs). Prebiotics also act as carbon and energy sources for bacteria growing in the colon, where they are fermented to form SCFAs. In addition to their trophic and protective functions, SCFAs, including butyrate, have been reported to attenuate inflammation in the colonic epithelium.[29]

Manichanh et al. previously indicated that there was a decrease in the abundance and biodiversity of intestinal bacteria within the dominant phylum Firmicutes (especially *Faecalibacterium prausnitzii*) in patients with recurrent Crohn's disease (CD).[30] Recently, Sokol et al. reported a unique therapeutic concept for CD using a commensal bacterial product. Counterbalancing the dysbiosis using this deficient commensal bacterium as a probiotic in CD patients could be beneficial.[31] Interestingly, the prophylactic effect on colitis was stronger when the supernatant of *F. prausnitzii* was used than when *F. prausnitzii* itself was used. Although the detailed mechanism(s) underlying the effects of this microbe is (are) still unclear, the supernatant potently down-regulated NF-κB activation.[31]

Recently, β2-1 fructans from chicory root were shown to have immunomodulatory capacity, mainly occurring through TLR2 and its adaptor, MyD88, via the NF-κB pathway. Although the detailed mechanism is still unclear, it is speculated that the β2-1 fructans directly stimulate TLR2 ligation due to its similarity to β-glucan (the normal ligand of TLR2). As a result, these fructans significantly increased the IL-10/IL-12 ratio (anti-inflammatory).[32]

IBD is a form of chronic intestinal inflammation that arises due to overly aggressive cellular immune responses to a subset of luminal bacteria, most likely commensal bacteria. Therefore, therapies targeted at restoring balance to the intestinal microenvironment are becoming rational options. Microbial therapies, including probiotics and/or prebiotics, are now considered "nutraceuticals", which are defined as foods or food components that provide medical or health benefits, including the prevention and/or treatment of disease.[33,34] Due to their low risk of adverse effects and the desire of patients wishing to use more natural physiological approaches to treating IBD, interest in such treatments has been increasing.[35,36]

In addition, unique approaches to IBD therapy via microbiota have been reported.[37,38] Those studies focused on the role of specific amino acids (Thr, Ser, Pro, and Cys) or

peptides (Pro-Glu-Leu) in the colonic mucosa, which were shown to increase mucin production and mucosal wound healing and to modulate the microbiota by producing epithelial antibacterial peptides, including defensin.

Finally, antibiotics have proven to be efficacious for inducing and maintaining IBD remission, because antibiotics alter the pattern of intestinal bacteria.[39−41] However, it is difficult to use antibiotics for a long period of time due to risk of developing microbial resistance and because of the side effects. Therefore, therapy targeted at restoring balance to the intestinal microenvironment using probiotics, prebiotics, and synbiotics is an extension of this concept. These approaches are appealing due to their lack of toxicity, and patients who wish to use more natural physiological approaches to treating IBD have expressed enthusiasm toward this approach.

ROLE OF MICROBIOTA IN IBS AND RELATED THERAPEUTIC STRATEGIES

IBS is a common functional bowel disorder, which is characterized by recurrent abdominal pain and altered bowel habits, without structural abnormalities of the bowel.[42,43] Although the pathogenesis of IBS remains unclear, food-related risk factors (including microbiota and allergic substances), psychiatric disorders, and gastroenteritis (very mild inflammation) are frequently suggested as the causes of disease in IBS patients.[44] Currently, cognitive−behavioral therapy and antidepressants are available, but are not commonly used for treatment due to their low cost-effectiveness in routine practice. Because the gastrointestinal tract contains massive amounts of 5-hydroxytryptamine (5HT) and this neurotransmitter is known to be a modulator of the gastrointestinal physiological function and gut−brain axis that is recognized by several receptors, serotonergic agents (5HT-3 antagonists or 5HT-4 agonists) can be used for the treatment of IBD.[45]

In Japan, the major medical therapies used for IBS are conventional bowel movement modulators (laxatives for constipation-dominant IBS, antidiarrheal agents for diarrhea-dominant IBS, polycarbophil Ca for alternative-type IBS). A high incidence of IBS after infective gastroenteritis has been reported, which is logical, because infective gastroenteritis generally reduces the commensal microbiota, which produce metabolites such as SCFA, and dysbiosis may be caused by the use of antibiotics.[46,47] In addition, recent findings suggest that changes in the microbiota in IBS models due to probiotics and/or prebiotics are related to improvements in the colonic motor function. Of note, SCFAs derived from dietary fiber have been reported to potently prevent the development of colonic mucosal 5HT receptor hypersensitivity.[47−49]

Recently, it was reported that stress is one of the key risk factors and that the microbiota plays an important role in the pathogenesis of IBS.[50,51] In addition, it was previously reported that space-flight, a condition inducing serious stress, decreased the numbers of three different kinds of fecal Lactobacilli, although dietary factors and other conditions might also have contributed to the change in microbiota in these subjects.[52] Dysbiosis caused by infection, dietary changes, or overuse of antibiotics is reported to produce low-grade inflammation, as observed in a subset of IBS patients.[53] On the other hand, changes in the gastrointestinal transit time may also contribute to the abnormalities observed in enteric microbiota metabolic activity, including fermentation processes.[54] The microbiota of the gastrointestinal tract form a complex ecosystem, and the use of probiotics or prebiotics has been reported to change the diversity and quantity of microbiota in IBS patients.[55]

Although the mechanisms remain unclear, it has been reported that probiotics change the microbiota in IBS patients and improve the mucosal immune system[56] and motor function of the bowel.[57] These effects subsequently result in positive changes in fermentation and visceral sensitivity. Furthermore, prebiotics have also been reported to alleviate IBS symptoms, mainly through the modulation of the microbiota and the increase of SCFA production.[58] Indeed, prebiotics as indigestible food constituents provide specific substrates ready to be metabolized by the beneficial gut microbiota, thereby stimulating their growth and/or activity.[59,60] Prebiotics are utilized as one source of SCFAs that provide energy to the epithelium and exhibit anti-inflammatory properties.[61] In a recent systematic review, the effects of probiotics and prebiotics on IBS were described.[62] Some probiotics have considerable potential in the management of IBS, although the benefits are strain-specific.[63] Therefore, the selection of the probiotic strain is one of the most important factors that needs to be considered for its clinical use.[64] With regard to the effects of prebiotics on IBS, the evidence is limited, because there have not been enough trials performed to evaluate their effects.[57,62,65]

MECHANISM(S) UNDERLYING MICROBIAL MODULATION OF THE MUCOSAL IMMUNE SYSTEM INDUCED BY PROBIOTICS OR PREBIOTICS

Prebiotics and their microbial metabolites, including SCFAs, are reported to have several beneficial effects on the mucosa, including metabolic (anti-inflammatory effects), trophic (epithelial energy), and protective (absorbing bile acids) functions in IBD.[61] Probiotics have also been reported to have a potent ability to attenuate the symptoms of IBD by manipulating the microbiota and modulating the mucosal immune function.[66] In particular, butyrate has a unique ability to down-regulate the activation of NF-κB or phosphorylation of the signal transducer and activator of transcription 3 (STAT-3).[67] Interestingly, modulation of the microbiota by prebiotics was also shown to change the characteristics of naïve T cells (CD4+CD45RB^{high}), and this characteristic is transferable to another host.[68]

Briefly, this naïve T cell transfer model is helpful for understanding the initiation, as well as regulation, of chronic colitis.[69] Because SCID or RAG knockout mice are immunodeficient recipients, which lack a regulatory T cell system, chronic colitis develops as a result of enteric antigen-driven activation and polarization of naïve T cells to disease-producing Th1 cells in the colon, where enteric bacterial antigens gain access to the secondary lymphoid tissue draining to the GALT and naïve T cells interact with antigen-presenting cells to become activated Th1 cells with a disease-producing phenotype.[69,70]

In our previous study, prebiotic enzyme-treated rice fiber (ERF) showed preventive effects on colitis in this T cell transferred model. After they encountered and responded to their cognate antigens, T cells acquired immune function; furthermore, this unique characteristic was inherited by severe combined immune-deficient mice following splenocyte transplantation. In that study, ERF significantly changed the SCFA production pattern and microbial profile. Although the two groups of mice (control group and ERF group) were fed the

same diet (AIN93G), these phenotypes were distinct between the two groups, and the ERF-derived CD4+ T cells had significantly attenuated inflammatory parameters.[61]

Butyrate has antineoplastic effects at physiological concentrations due to its effects on cell cycle regulation, leading to an increase of apoptosis in colonic tumor cell lines[71] as well as anti-inflammatory effects.[72] Butyrate has also been shown to suppress TLR4 gene expression.[73] Lipopolysaccharide is a ligand of TLR4, which induces the degradation of IκBα, resulting in the induction of pro-inflammatory cytokines via the transmigration of NF-κB. One of the anti-inflammatory actions mediated by butyrate due to prebiotic fermentation may be explained by this down-regulation of TLR4 mRNA expression. In a clinical study, TLR4 was reported to be up-regulated in ulcerative colitis patients, and TLR4 was reported to detect Gram-negative microbiota and to activate the innate immune system.[74] Furthermore, a recent study revealed that TLR4 had the ability to regulate cyclooxygenase-2 (COX2) expression in a chronic colon cancer model via NF-κB expression.[74] In our previous study, prebiotics, including germinated barley foodstuff (GBF), decreased TLR4 and COX2 expression, compared with that observed in the control group.[29]

Probiotics can modulate or change the microbiota directly, whereas prebiotics cannot. However, prebiotics have a unique characteristic: the fecal bulking effect (in other words, a high water-holding capacity). McBurney focused on the relationship between the bulking effects and changes in microbiota using the SCFA production as a marker. That study revealed that high bulking fiber increased SCFA production. Therefore, changes in the physical condition (bulking effects) in the lower intestinal tract may also contribute to the composition of microbiota.[75]

MECHANISM(S) UNDERLYING MICROBIAL MODULATION OF BOWEL FUNCTION INDUCED BY PROBIOTICS OR PREBIOTICS

To date, there have been three important mechanisms suggested to underlie how the microbiota influences gut motor function. First, bacterial substances or the end products of bacterial fermentation may modulate the gut motor function. Second, changes in intrinsic intestinal neuroendocrine factors may affect the central nervous system. Finally, mediators released by the gut immune response may lead to the change in function.[57,76]

With regard to the first point, the SCFA described in this review and bacterial deconjugated bile salts[77] were shown to modulate the bowel motor function. The gas production resulting from the fermentation of unabsorbed carbohydrates is also an important factor. Indeed, the overproduction of methane has been shown to directly inhibit motor activity.[78] With regard to the second point, 5HT has already been described to modulate the gut motor function in this review. Furthermore, it has been reported that dysbiosis due to antibiotic administration can increase the substance P immunoreactivity and induce hypersensitivity in the colon.[79] Certain commensal strains have the capacity to modulate intestinal pain through the induction of opioid receptors.[80]

A postinfectious IBS model was used to study the relationship between the immune response and motor function. Akiho et al. reviewed the role of many kinds of cytokines and chemokines, as well as the imbalance of Th1/Th2 or Th17/Treg, in the malfunction of IBS based on the low-grade inflammation theory and showed the increase of proinflammatory cytokine production and abnormality of contractility.[81]

It was reported that a high concentration of SCFAs in the colon following prebiotic ERF treatment prevented the enhancement of colonic motility under restraint stress due to suppression of 5HT secretion, compared with the control and polycarbophil Ca groups.[58] Interestingly, ERF prevents abnormal fermentation under restraint stress and also maintains good fermentation under stress-free control conditions. Another study showed that the 5HT released from enterochromaffin (EC) cells is stimulated by mucosal stroking, microbiota, and SCFAs and that the presence of mechanical stimuli is the most important factor affecting 5HT release.[82] Because of their high water-holding capacity and lattice-like physical structure, prebiotic ERF might attenuate the colonic mucosal 5HT release during restraint stress compared with the control condition. Finally, acute gastroenteritis is reported to be one of the strongest risk factors for the development of IBS. Therefore, transient infection seems to play an important role in persistent gut dysfunction by inducing an increase in intestinal permeability and activating the mucosal immune system[57,83]

FUTURE PERSPECTIVE OF PREBIOTIC AND PROBIOTIC TREATMENT FOR IBD AND IBS

Although the mechanisms underlying IBD and IBS are quite different, both diseases have a common pathogenesis, an overwhelming immune response, in some subtypes.[84,85] In addition, IBS and IBD show heterogeneous clinical symptoms, which include abnormal bowel movements and abdominal pain.[86] As shown in this review, microbiota and the regulation of the diversity and number of microbiota play an important role in the pathogenesis of both IBS and IBD by pleiotropic mechanisms, including modulation of the immune system, bowel motor function, central nervous system, and bulking effects (water-holding capacity and absorption of bile acids). Therapeutic approaches for IBS and IBD that modulate the microbiota are associated with a low incidence of adverse effects (if any). Therefore, for patients with chronic gastrointestinal dysfunction or inflammation, treatment using these approaches is considered to be highly safe and beneficial (Figure 1).

In addition, it has been reported that modulating the microbiota using prebiotics or probiotics can improve human health in several ways.[87] Sommer and Backhed reviewed the unique effects of gut microbiota on the morphogenesis of the

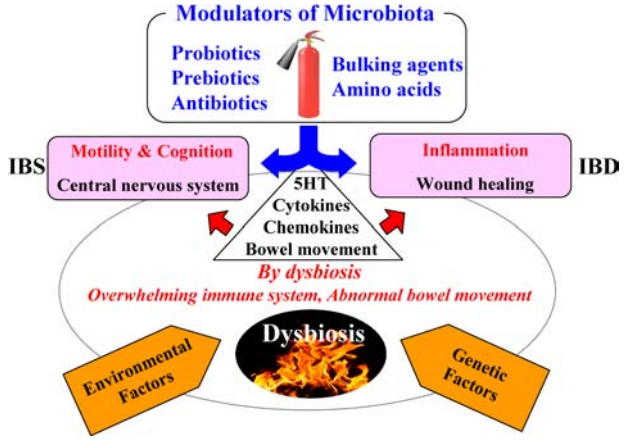

Figure 1. Concept of modulating the microbiota and potential remedies for gastrointestinal disease.

intestinal tract and remodeling of the vascular pattern, adiposity, and organ homeostasis.[87,88] Although further detailed studies are required to provide deeper insight into the mechanisms of action, nutraceuticals (probiotics, prebiotics, and other agents) may also be helpful for modulating the colonic environment in healthy humans, in addition to patients.

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The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are deeply appreciative to Motoyasu Ishihara (Kirin Holdings, Director) for his encouragement and permission to submit this manuscript and to Noriko Kuriyama (Kirin Holdings) for grammatical corrections.

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