Impact of the Gut Microbiota, Prebiotics, and Probiotics on Human Health and Disease

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Recent studies have revealed that the gut microbiota regulates many physiological functions, ranging from energy regulation and cognitive processes to toxin neutralization and immunity against pathogens. Accordingly, alterations in the composition of the gut microbiota have been shown to contribute to the development of various chronic diseases. The main objectives of this review are to present recent breakthroughs in the study of the gut microbiota and show that intestinal bacteria play a critical role in the development of different disease conditions, including obesity, fatty liver disease, and lung infection. We also highlight the potential application of prebiotics and probiotics in maintaining optimal health and treating chronic inflammatory and immunity-related diseases. *(Biomed J 2014;37:259-268)*

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More than 100 trillion (10^{14}) microbes inhabit the human gastrointestinal (GI) tract, and the total number of genes derived from these microbes exceeds that of the human genome by at least 100-fold. In the stomach of healthy human adults, a relatively low number of bacteria can be found, approximately 10^4 colony forming units (CFU) per milliliter of gastric content, which mainly correspond to bacilli, catenabacteria, enterococci, and lactobacilli [Figure 1].[1] *Helicobacter pylori* is also present in the stomach of more than half of the human population.[2] The first part of the small intestine, the duodenum, is acidic (pH 4-5) and a relatively large number of bacteria (10^2-10^4 CFU/ml) are found in this section.[3] Lactobacilli, streptococci, veillonellae, staphylococci, actinobacilli, and yeasts are the most prominent organisms in the duodenum and jejunum.[4] The GI microbiota changes markedly from the duodenum to the ileum due to an increase in pH and reduction of oxidation-reduction potentials, leading to an increase of the bacterial load which can reach up to 10^6-10^8

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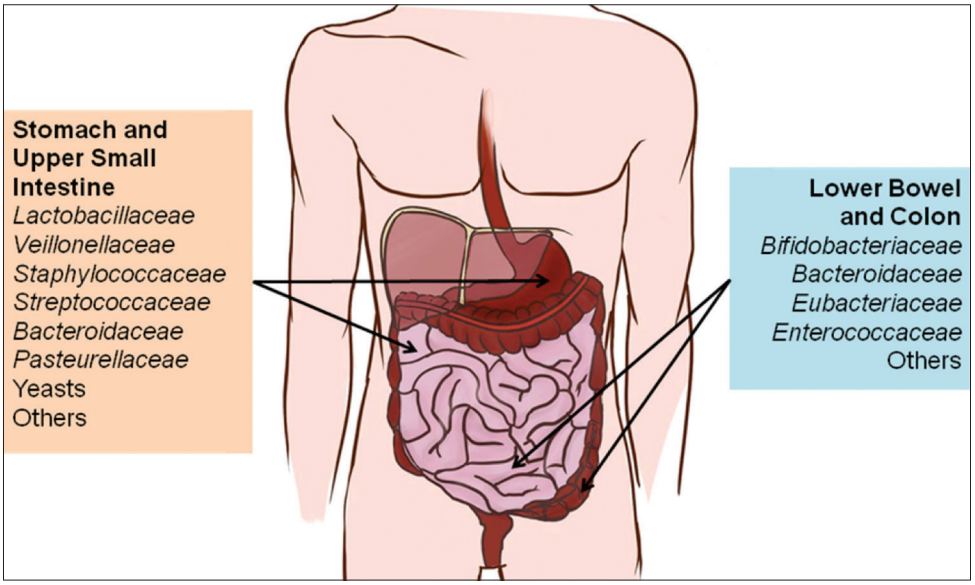

Figure 1: Overview of the human gut microbiota. The stomach and upper small intestine contain bacteria and yeasts which together can reach up to 10^4 CFU/ml of gastric juice. In comparison, the lower bowel and colon contain a wider variety of bacteria which can reach up to 10^6 CFU/ml in the lower bowel and as high as 10^{12} CFU/ml in the colon.

CFU/ml. In the large intestine, the pH is neutral and bowel transit time increases, which ensures that bacteria reach high numbers (10^7-10^{12} CFU/ml) and are extremely diverse. At the same time, as the environment of the colon is strictly anaerobic, obligate anaerobes, which derive their energy from fermentation, prevail in this section. More than 1200 bacterial species have been identified in the colon of humans, with each healthy individual harboring at least 160 shared species.[5] However, a large fraction (>80%) of the GI microbiota cannot be cultured *in vitro*, necessitating the use of molecular techniques.[6]

Human gut microbiota and host homeostasis

The number and diversity of bacterial species found within the GI tract are affected by various factors, including pH, peristalsis, transit time, nutrient availability, host age and health status, and mucin secretion, among others.[1] Under healthy conditions, the gut microbiota exists in a state of "normobiosis" in which microorganisms with beneficial effects on health predominate over harmful species. This situation is crucial for normal gut homeostasis and optimal development of the host.

The gut microbiota exhibits many important physiological functions that include regulation of energy levels and metabolism, neutralization of drugs and carcinogens, modulation of intestinal motility, regulation of immunity, barrier effects, and protection against pathogens.[7] Host behavior and cognitive functions such as learning, memory, and decision-making are also believed to be affected by the gut microbiota.[8] In a broad sense, the gut microbiota appears to be critical to maintain host homeostasis and health [Figure 2].

Inability to regulate intestinal mucosal immunity can result in local and systemic inflammation.[9] Gut microorganisms may stimulate production of pro-inflammatory cytokines and infiltration of immune cells. A persistent low level of inflammation in different organs also contributes to diabetes, heart disease, and obesity.[10]

Nutrient metabolism by the intestinal microbiota

Genetic and environmental factors influence the abundance and type of beneficial and pathogenic bacteria in the gut, with each type of bacteria possibly having preferred substrates for growth and producing unique fermentation products. Diet composition influences the composition of the gut microbiota and the subsequent fermentation products that in turn affect the host. While some fermentation products and metabolites promote gut functions and health, others impair these processes, leading to impaired digestion and barrier functions. Such fermentation end products may also influence food intake, energy levels, and insulin activity, thereby influencing adiposity and related metabolic pathways.

Unlike the small intestine, the large intestine is involved in the fermentation of food nutrients, such as carbohydrates and some polysaccharides that cannot be digested by the host. These are converted into short-chain fatty acids (SCFAs) that can be assimilated by the host.[11] Some endogenous carbohydrates derived from mucins and chondroitin sulfate can also be fermented in the large intestine.[1] The main bacterial species that play a role in this process are of the genus *Ruminococcus*, *Lactobacillus*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Eubacterium*.[1]

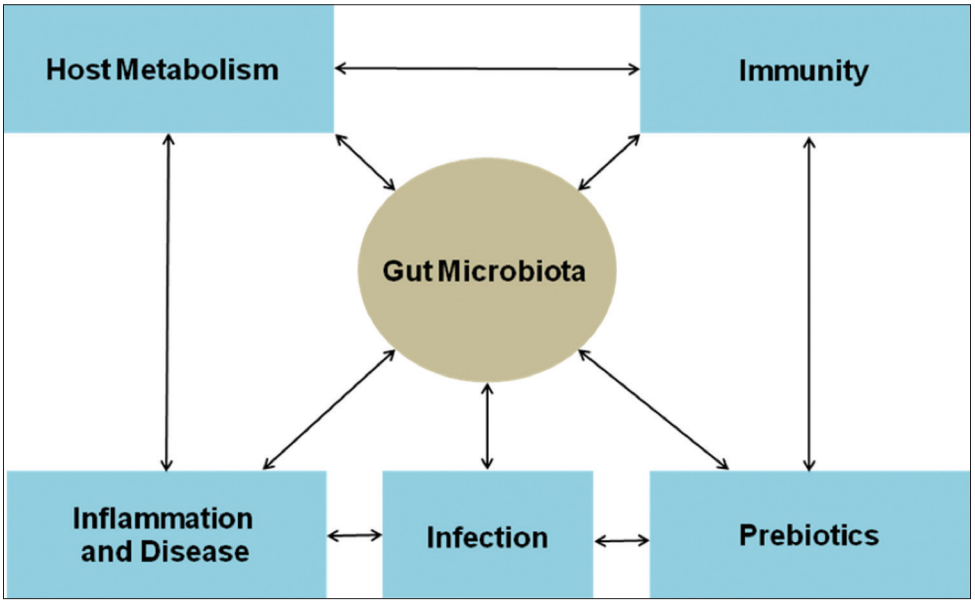

Figure 2: Interactions between the gut microbiota and the host. The gut microbiota interacts with the host to regulate metabolism and immunity. Under a state of dysbiosis, chronic inflammation occurs and may be involved in disease progression and infection. The structure and activities of the gut microbiota can be modulated by prebiotics, which induce the growth of probiotic bacteria and produce beneficial effects on the host.

To cope with the need to ferment food substrates, the gut microbiota has developed a large reservoir of enzymes that facilitates food degradation and nutrient utilization. For instance, genomic analysis of Bacteroides thetaiotaomicron identified a series of enzymes that can process the whole range of host glycans.[12] This bacterium also possesses numerous sulfatase enzymes that allow degradation of highly sulfated glycans such as mucins.[13] A new bifunctional α-galactosidase/sucrose kinase enzyme found in the intestinal bacterium *Ruminococcus gnavus* can hydrolyze melibiose and raffinose into galactose and glucose/sucrose, respectively.[14] A novel β-glucuronidase enzymatic activity was also identified in Firmicutes using a functional metagenomic approach.[15] Similarly, xenobiotic-responsive genes involved in pathways related to antibiotic resistance, drug metabolism, and stress response were recently identified.[16] Intestinal microbes can also generate catecholamines that have an effect on gut physiology.[17] This enzymatic machinery suggests an evolutionary adaptation of commensals to life in the human GI tract. In addition, the SCFAs produced in the colon, such as formic acid, acetic acid, propionic acid, and butyric acid, may have an impact on the intestinal mucosa[18] as well as other peripheral tissues that regulate host metabolism.[19]

The protective role of omega-3 fatty acids on intestinal inflammation is well established. But recent studies show that dietary lipids also affect specific populations of intestinal microbes. Conversely, the gut microbiota influences liver metabolism through modulation of the bile acid profile.[20] Fatty acids are distributed to every organ by transporting proteins, and can act as ligands of G protein-coupled receptors (GPRs), such as GPR41 and GPR43, and peroxisome proliferator-activated receptor-alpha (PPAR-α), which play crucial roles in the regulation of energy expenditure.

Proteins and peptides reaching the colon are fermented by intestinal bacteria to yield a great diversity of end products, including branched-chain fatty acids, such as isobutyrate and isovalerate, along with ammonia, amines, N-nitroso compounds, phenols, in doles, thiols, CO_2, H_2, and sulfur-containing compounds such as H_2S, many of which have toxic properties[21] that have been associated with colon cancer[22] and inflammatory bowel disease.[23] An increase of the dietary protein load in healthy individuals results in enhanced generation of these toxins, many of which are cleared by the kidneys.[24]

The gut microbiota also plays an important role in the pathogenesis of metabolic syndrome, diabetes, non-alcoholic fatty liver disease (NAFLD), and cognition, which extend well beyond the traditional view that the function of intestinal bacteria is limited to promoting nutrient digestion and absorption.[25]

Impact of microbial fermentation products on the host

The gut microbiota is involved in the production of metabolites [trimethylamine (TMA) and trimethylamine N-oxide (TMAO)] that increase the risk of cardiovascular disease.[26,27] Production of TMAO by the gut microbiota appears to originate from two major sources, phosphatidylcholine/choline and L-carnitine. It has been postulated that consumption of these dietary nutrients (which are found in high amounts in red meat), and their conversion into TMAO,

may have a detrimental effect on the cardiovascular system and promote atherosclerosis. In contrast, a number of studies have demonstrated that L-carnitine has beneficial effects against disease conditions that include insulin resistance and ischemic heart disease. In addition, fish represent a significant source of TMAO, but consumption of fish and fish oils is associated with beneficial effects on cardiovascular health,[28] underlying the need for further studies to clarify this discrepancy.

Besides the risk of cardiovascular events, the digestion of red meat by the gut microbiota is also associated with increased risk of colorectal cancer. In addition to the compounds found in meat (e.g. proteins, heme) and the compounds generated by the cooking process (e.g. N-nitroso compounds, heterocyclic amines), increased bacterial fermentation (putrefaction) of undigested proteins and production of bacterial metabolites derived from amino acids may affect the functions and renewal of epithelial cells lining the colon. Consistent with this possibility, colon cancers are mainly detected in the distal colon and rectum where protein fermentation actively occurs.[29]

Carbohydrate fermentation products in the colon are generally recognized as beneficial for maintaining host homeostasis. SCFAs are fermentation end products of the intestinal microbiota that exert an extensive influence on host physiology through nutritional, regulatory, and immune-modulatory properties. Moreover, SCFAs act as signals for the regulation of virulence genes in enteric pathogens.[30,31] As a whole, SCFAs acidify the luminal pH, which suppresses the growth of pathogens; SCFAs also influence intestinal motility.[32] Among SCFAs, acetate is mainly seen as a lipogenic compound while propionate acts as a glucogenic substrate and an inhibitor of lipogenesis.[33,34] Butyrate serves as a major energy substrate as well as a regulator of cell growth and differentiation.[35,36] Of note, butyrate may reduce the risk of colon cancer by stimulating apoptosis of colonocytes. We summarize in Figure 3 several diet-independent and diet-dependent microbial effects on host metabolism.

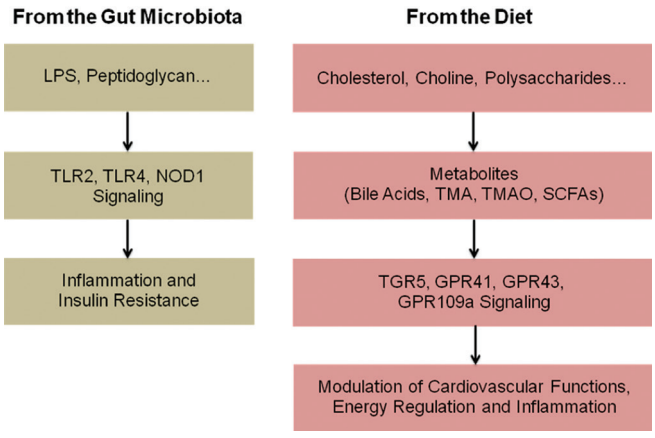

Figure 3: Effects of the gut microbiota and diet on health and disease. The gut microbiota releases LPS and peptidogly can that may in turn activate TLRs (TLR2 and TLR4) and the NOD1 receptor on host cells, eventually leading to inflammation and insulin resistance. Dietary nutrients like cholesterol, choline, and polysaccharides may be modified by the gut microbiota to produce various metabolites. These compounds may affect host signaling pathways that modulate cardiovascular functions, energy regulation, and inflammation. TGR5 is also known as G protein-coupled bile acid receptor Gpbar1.

Intestinal immune cells monitor the gut microbiota

Mononuclear phagocytes, such as macrophages and dendritic cells (DCs), are located in the intestinal lamina propria where they prevent immunological reactions against commensal bacteria, a process which is important for maintaining gut homeostasis.[37,38] Gut-resident phagocytes do not produce significant levels of pro-inflammatory cytokines upon stimulation and are hypo-responsive to bacterial components and commensal bacteria.[38,39] Stimulation of intraepithelial cells by damage-associated molecular patterns (DAMPs) and commensal bacteria-derived microbe-associated molecular patterns is sensed by a wide repertoire of pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and NOD-like receptors (NLRs).[40] Activation of intraepithelial cells by intestinal bacteria is required for epithelium development, maintenance of intestinal barrier integrity, and defense against pathogens.[41] Ligation of DAMP receptors induces the assembly of inflammasomes, which also contribute to maintaining the integrity of the intestinal epithelium.[42] The NLR family of PRRs is thought to modulate the gut microbiota and innate immunity in order to prevent intestinal inflammation and cancer. For instance, NLRP6 and NLRP12 protect the host against the development of colitis and cancer.[43] Pathogenic bacteria that possess a type III secretion system and flagellin can activate the NLRC4 inflammasome in intestinal phagocytes, resulting in the production of interleukin-1β (IL-1β) which promotes clearance of pathogens by inducing the expression of endothelial adhesion molecules that facilitate neutrophil recruitment.[37] Therefore, the production of IL-1β by intestinal phagocytes contributes to distinguishing pathogenic versus commensal bacteria in the gut.

Adaptive immunity is also involved in microbiota homeostasis. For instance, Th17 cell differentiation, which can be induced by colonization with segmented filamentous bacteria, may protect against *Citrobacter rodentium* infection.[44] In addition, induction of Treg cells by the microbiota attenuates intestinal damage produced by overt immune response against pathogens. For instance, *Bacillus fragilis* activates Treg cells which in turn protect against *Helicobacter hepaticus* infection.[45,46] Similarly, *Bifidobacterium infantis* may enhance proliferation of Treg cells which attenuate intestinal damage caused by infection with

Salmonella enterica Typhimurium.[47] Commensal bacteria may also activate immune reactions such as the production of IgA that are directed against the bacteria's own antigens.[48,49] Several studies indicate that epithelial sensing of intestinal bacteria greatly influences the numbers and types of microbes found in the GI tract through the production of various metabolites.[10,41]

Dysbiosis and disease

A complex relationship exists between diet, microbes, and the gut epithelium. During "dysbiosis," in which a few potentially harmful bacterial genera or species have been shown to propagate, a disease-prone situation is created. Diet-induced dysbiosis was identified as a contributing factor for the development of diseases such as allergy, autoimmune disease, Crohn's disease, obesity, type-2 diabetes, and ulcerative colitis. In addition, new diseases were recently added to the list, including cardiovascular disorders, colorectal cancer, irritable bowel syndrome, and NAFLD.[8,50] Based on this new knowledge, great interest has developed in using antibiotics, probiotics, and prebiotics to reduce the risk of dysbiosis in the colon and modulate the gut microbiota to prevent or even treat human diseases.[51]

Microbiota and obesity

Studies performed in germ-free mice suggest that the gut microbiota plays a major role in harvesting energy from food. Transfer of the gut microbiota of normal mice into germ-free mice increases the body fat and insulin resistance of the latter by 60% within 2 weeks, despite being associated with reduced chow consumption and increased physical activity.[52] Similarly, transfer of the microbiota of genetically obese mice (ob/ob) into germ-free mice is sufficient to transfer the obese phenotype into the recipients.[53] This study showed that obese mice harvested more energy from fecal matter than their lean counterparts, suggesting that the intestinal microbiota may contribute to obesity. Indeed, sequencing of the gut microbiota of lean mice and ob/ob mice showed differences in the two major bacterial phyla, Bacteroidetes and Firmicutes.[54] Compared with their lean littermates, obese mice showed a 50% reduction of Bacteroidetes and a proportional increase of Firmicutes.[54] Obesity is associated with a number of other metabolic disorders characterized by chronic, systemic, low-grade inflammation. While endotoxins such as lipopolysaccharide (LPS) derived from the cell wall of Gram-negative bacteria may circulate at low concentrations in the blood of healthy individuals, the presence of genetic and diet-induced obesity has been associated with a substantial increase in blood LPS concentration, a condition termed "metabolic endotoxemia." Consumption of a high-fat diet increases blood endotoxin concentrations and alters the composition of the gut microbiota in both animals and humans.[55] An increase in blood endotoxin levels may be due to increased intestinal permeability caused by changes in the gut microbiota.[56] Endotoxemia is thought to contribute to low-grade inflammation and insulin resistance. Antibiotic treatment in high-fat-fed mice and ob/ob mice reduces the LPS levels in blood and feces.[57] Taken together, these studies suggest that intestinal microbes may contribute to obesity by inducing chronic inflammation.

Gut microbiota and liver disease

The hepatic portal vein conducts large amounts of venous blood from the GI tract and the spleen to the liver, therefore constantly exposing the liver to diet- and microbe-derived compounds.[58] The liver is equipped with various immune cells that recognize microbial products, toxins, and food antigens. Recent studies have shown that the innate immune system interacts with the intestinal microbiota during the development of obesity and autoimmunity and promotes the progression of chronic liver disease.[59-61] For instance, the impact of the gut microbiota on NAFLD pathogenesis has been established recently. NAFLD is a complex metabolic disease associated with perturbations of multiple triggering factors, including the gut microbiota and diet.[62] Studies have also uncovered roles for the gut microbiota, bile acid receptors, and vitamin D in regulating the progression from NAFLD to hepatocellular carcinoma (HCC). Senescence and autophagy play a role in the progression of hepatic stellate cells to HCC. Similarly, a link between dysregulated progenitor cell regulation and HCC has been identified.[63]

Importantly, high fat intake and changes in the gut microbiota may increase intestinal permeability and lead to absorption of compounds that would not normally penetrate the gut barrier. These substances may enter the liver and cause inflammation, oxidative stress, and lipid accumulation, leading to fatty liver, a condition found in pre-diabetic states. Visceral adipose tissues represent another source of pro-inflammatory substances and oxidative stress signals, which may ultimately activate Kupffer cells. Release of these substances, in particular triglycerides and pro-inflammatory cytokines, into the blood may lead to ectopic fat deposition and blood vessel damage. For these reasons, the liver appears to be involved in the development of the pre-diabetic syndrome. Treatments that prevent leakage from the gut are currently being developed to treat insulin resistance and liver steatosis.[64,65]

Over-consumption of alcohol leads to alcoholic liver disease (ALD), a term used to describe various conditions that include steatosis, progressive fibrosis, cirrhosis, and HCC. New treatments for this condition are urgently needed since alcohol abstinence, corticosteroids, and nutritional changes produce relatively poor outcomes. Recent studies

have identified numerous potential targets for new treatments, including chemokines, endocannabinoids, IL-22/signal transducer and activator of transcription 3 (STAT3), the tumor necrosis factor (TNF) receptor super family, and osteopontin, as well as the gut microbiota, LPS, and inflammasomes.[66]

Intestinal microbiota and lung immunity

Given that the lungs continually encounter a large array of foreign antigens, maintenance of lung immunity is crucial to prevent allergic reactions and microbial infections in the respiratory tract. Surprisingly, perturbation in the gut microbiota due to dietary interventions and antibiotic treatment is important for lung immunity and pulmonary diseases. Similarly, alteration of metabolites derived from the gut microbiota and circulating systemically in the body may influence lung immunity. Recently, a high-fiber diet has been shown to protect mice against allergic inflammation by decreasing the Firmicutes to Bacteroidetes ratio while increasing the SCFA levels.[67] The protective effects mediated by SCFAs were characterized by increased infiltration of macrophage and DC precursors, along with an attenuated ability to promote the activity of T helper type 2 (Th2) cells. Conversely, mice fed with a diet low in fiber exhibited more severe allergic pulmonary inflammation than chow-fed mice. This study showed that consumption of fermentable fiber may be used to shape lung immunity and reduce the severity of allergic inflammation.

Antibiotics have been used to kill specific bacterial pathogens and combat infections. However, broad-spectrum antibiotics may target various intestinal bacteria and leave a long-lasting effect on the gut microbiota.[68-70] It was recently shown that gut dysbiosis induced by antibiotics exacerbates allergic lung inflammation by promoting allergy-prone M2 macrophage polarization.[71] Aberrant macrophage polarization and allergic inflammation was attributed to increased *Candida* species in the cecum, which induces the production of prostaglandin E_2 from arachidonic acid. Increased *Candida* species might be due to decreased levels of *Lactobacillus*[71] since these probiotic bacteria show antifungal properties by inducing the aryl hydrocarbon receptor-IL-22 signaling pathway.[72] The results of these studies suggest that probiotics and prebiotics may be used to treat allergic reactions in the airway by boosting *Lactobacillus* species within the gut microbiota.

Disruption of the gut microbiota by antibiotics leads to a loss of "colonization resistance" due to the depletion of commensal bacteria. Microbiota–host mutualism depends on the cooperative flexibility established by the innate and adaptive immune system.[73] Antibiotic-induced dysbiosis has been linked to inflammatory diseases, such as obesity, GI tract inflammatory diseases, and autoimmunity.[74,75] The absence of PAMPs, including metabolites and components derived from gut microbiota, may result in hypo-responsiveness of host immunity and, subsequently, uncontrolled dissemination of potential pathogenic infection.[68,76,77] Mice orally fed with broad-spectrum antibiotics (e.g. metronidazole, ampicillin, neomycin, vancomycin) exhibit hypo-responsiveness to pulmonary influenza A virus infection.[78] Intact microbiota provides signals for optimal activation of inflammasomes and expression of pro-IL-1β and pro-IL-18 at steady state and facilitates DC-mediated induction of adaptive immunity against influenza A virus infection in lungs. Furthermore, commensal-derived signals determine the activation threshold of macrophages in response to both lung and systemic viral infections.[79] Antibiotic-treated mice are highly susceptible to pulmonary viral infection due to impaired innate and adaptive immunity, which eventually leads to a substantially delayed clearance of virus and lethal viral dissemination.[78,79] Notably, antibiotic-induced hypo-immunity in lungs is restricted to specific microbial pathogens,[78] suggesting that unique PRRs and signaling pathways are selectively modulated under antibiotic-induced gut dysbiosis. Recently, we observed that the gut microbiota is required for the establishment of anti-mycobacterial and anti-fungal pulmonary immunity (Lai *et al*., manuscript in preparation). In our experiments, mice treated with broad-spectrum antibiotics exhibited attenuated innate responses against mycobacterial and fungal pathogens introduced in the lungs and were susceptible to lethal mycobacterial and fungal pulmonary infection. Taken together, these results suggest that an intact gut microbiota is essential to maintain lung immunity in combating microbial pathogens of the respiratory tract.

Probiotics

Numerous organisms meet the criteria established by the World Health Organization to define probiotics: "A live organism which provides a benefit to the host when provided in adequate quantities."[80] The Gramnegative *Escherichia coli* strain Nissle 1917, various lactic acid producing *Lactobacillus* strains, and a number of bifidobacteria represent the primary microorganisms classified as probiotic agents. Probably the most effective strategy to select probiotic species is based on production of beneficial clinical outcomes in humans.[81] The beneficial effects of probiotics may be related to their capacity to produce vitamins, antioxidants, and defensins against pathogenic competitors.[82] Probiotics are also characterized by their production of SCFAs and absence of toxins.[1] Probiotic bacteria may also inhibit the growth of pathogens through various mechanisms.

Many beneficial probiotics such as bifidobacteria and lactobacilli are Gram-positive bacteria, which are devoid of LPS. Such bacteria may reduce the risk of infection by competing with pathogens for dietary nutrients or receptors on the gut wall.[83] Other bacterial genera that include

bacteroides, enterococci, eubacteria, and streptococci are potentially beneficial or harmful to the host, depending on the particular bacterial species under study. Moreover, the butyrate producer *Roseburia*[84] and the mucin-degrading bacterium *Akkermansia muciniphila* have also been reported as potential probiotics.[85] The use of *Bifidobacterium longum* and *Bifidobacterium breve* for prevention and treatment of acute diarrhea in newborns and infants has gained interest.[86]

Prebiotics

Nutrients that restore a healthy gut microbiota by modulating its composition are being developed as new therapeutic approaches to treat inflammatory diseases. Since the gut microbiota plays a major role in maintaining physiological reactions in the host, new dietary treatments based on the use of dietary supplements (organic selenium and *Lithothamnium muelleri* algae) and probiotics (*Saccharomyces boulardii* UFMG 905 and *Bifidobacterium*) have been developed to modulate the gut immune response and restore intestinal homeostasis.[87-89] In addition, changes in the diet of the host could be used to modulate the gut microbiota and restore homeostasis. Accordingly, the fecal microbiota of children from Europe or rural Africa showed major differences that might be attributed at least in part to different dietary habits.[90] Currently, protein and animal fat consumption appears to be more closely linked with disease than the intake of carbohydrates.

Prebiotics stimulate the growth or activities of specific microbial genera and species in the gut microbiota in order to confer health benefits to the host. In general, prebiotics favor the growth of bifidobacteria and lactobacilli over potentially harmful proteolytic and putrefactive bacteria. Prebiotics have been classified mainly into two groups, the inulin-type fructans (ITF) and the galacto-oligosaccharides (GOS), based on their chemical structures.[91]

High consumption of dietary fiber has long been recognized to provide health benefits,[92] and foods rich in dietary fiber have been shown to enrich Bacteroidetes, especially Prevotella and Xylanibacter, and to reduce Firmicutes and Enterobacteriaceae, and high-level dietary fiber supplements increase the level of several bacteria including *Bifidobacterium*, the clostridial cluster XIVa, and *Faecalibacterium* prausnitzii, bacteria usually associated with a healthy status.[93]

In addition to traditional foods, pure polyphenols and polyphenol-rich foods (such as cocoa, tea, wine, soy products, and fruits) may significantly affect the composition of the gut microbiota.[94] Based on these results, the enhancement of the number of bifidobacteria and lactobacilli is currently regarded as a marker of intestinal health and as a possible screening marker for the identification of prebiotics. A clear classification of beneficial versus harmful bacteria remains to be made.

We recently conducted experiments in order to identify novel prebiotics based on therapies used in traditional Chinese medicine. We observed that treatment of high-fat diet mice with a water mycelium extract of *Ganoderma lucidum*, a fungus used for centuries as a health tonic in Asia, reduced body weight and inhibited obesity-induced complications such as inflammation, insulin resistance, and LPS-induced endotoxemia (manuscript in preparation). Notably, the effects of *G. lucidum* could be reproduced by transferring the feces of mycelium-treated mice to obese mice, indicating that the mechanism of action of the mycelium extract involved the gut microbiota. Similar results were obtained with other fungal remedies used in traditional Chinese medicine, including *Hirsutella sinensis* (the anamorph of *Cordyceps sinensis*) and *Antrodia cinnamomea* (a fungus found predominantly in Taiwan) (unpublished results). Our results suggest that these fungal products may be used in the future as prebiotic agents.

Fecal transplantation

Fecal transplantation represents a potential therapy that is effective against many diseases, including anorexia nervosa, autoimmunity, infections, inflammatory bowel disease, obesity, and multiple sclerosis.[95] In a recent randomized clinical trial, researchers found that recurrent diarrhea caused by *Clostridium difficile* could be treated by duodenal transfer of feces from healthy individuals.[96] Notably, the researchers showed that feces transfer restored normal bacterial diversity in the recipients. Cultured strain mix has been proposed as a potential alternative for treatment of *C. difficile* infections.[97] Fecal microbiota transplantation from lean donors to patients with metabolic syndrome has also been reported to induce changes in intestinal microbiota composition and improve insulin resistance.[8,98]

Conclusions

While the gut microbiota has been studied for many decades, recent studies have considerably expanded the role of intestinal microbes in human health and disease. Advances in the fields of bioinformatics, metagenomics, metatranscriptomics, and metabolomics have allowed researchers to gain important insights into the function of intestinal bacteria.[31] The gut microbiota has been shown to modulate the activity of a broad range of tissues and organs, with effects ranging from immunity to stimulation of brain centers responsible for appetite and food intake control. Furthermore, recent studies suggest that the gut microbiota could be manipulated using diet, prebiotics, and probiotics in order to maintain health. Diets containing nutrients that are fermentable by intestinal bacteria may be used to stimulate

the growth of beneficial bacteria. In fact, the gut microbiota is now considered as a separate organ of the body which shows both physiological and pathological effects.[99]

Metagenomics provides an excellent tool to assess the whole composition of the gut microbiota, including the microbes that currently cannot be cultivated *in vitro*. The potential of metagenomic analysis is particularly interesting for the identification of novel treatment options related to the gut microbiome, including the discovery of novel genes and development of the so-called "bio-engineered probiotics."[100]

In summary, the human intestinal microbiota is similar to a true organ, which plays critical roles in human health and disease. Given the complexity of the bacterial flora found in each individual, defining a normal microbiota represents an important challenge. Dysbiosis of the gut microbiota is associated with many diseases, suggesting that intestinal bacteria could be used as a signature for disease conditions. Modifying the gut microbiota using prebiotics and probiotics represents an important therapeutic strategy for prevention and treatment of human diseases.

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