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The potential impact of gut microbiota on your health: Current status and future challenges

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Abstract

Our health and probably also our behaviors and mood depend not only on what we eat or what we do (lifestyle behaviors), but also on what we host. It is well established for decades that all vertebrates including humans are colonized by a wide array of bacteria, fungi, eukaryotic parasites and viruses, and that, at steady state (homeostasis), this community of microbes establishes a friendly mutual relationship with the host. The term microbiota was originally meant to represent an ecological community of commensals and potentially pathogenic microbes that live within our bodies, but it is now used interchangeably with the term microbiome which was initially meant to represent a collective genome of the microbiota. Although the number of microbes that live in or on our body was previously estimated to outnumber that of their hosts by 10 to 1, the latest estimate put the ratio to be closer to 1:1. On the other hand, their collective genomes (microbiome) outnumber those of the host by 100-200 times. It is not surprising therefore that these microbes not only provide the host with a variety of metabolic impact, but can also modulate tissue integrity and immune defense, all of which lead to a healthy ecosystem (symbiosis) that is unfavorable for colonization and invasion of pathogens. Microbiota is well known for its role in development and education of immune system. However, its link with diseases is less known and it is only recently that there is a surge of interest in the potential impact of microbiota on human health and disease. The diversity and composition of microbiota (healthy microbiota profile) are dynamics, depending not only on the host physical status, genotype and immune phenotype, but also on the environmental factors like diet, antibiotic usage and lifestyle behaviors. These environmental factors may adversely alter gut ecosystem (dysbiosis) that is frequently associated with increased susceptibility to infections as well as to non-communicable diseases like obesity, metabolic syndromes (e.g., diabetes and cardiovascular diseases), allergy and other inflammatory diseases. Emerging evidence from more recent studies also demonstrate the existence of a bidirectional communication route linking gut and microbiota with brain, thus suggesting that these microbes may play a role in neurological disorders as well as in host perception, behavior and emotional response. However, whether the observed alteration of the microbiota profile in these diverse conditions is the cause or the consequence of the disease remains to be established. These observations imply that it may be possible to design new strategies for the management of diseases by manipulating gut microbiota. The common practice now available is the use of probiotics to rehabilitate gut ecosystem. The microbiota-based therapeutics like fecal transplantation for the treatment of recurrent antibiotic-resistant *Clostridium difficile* infection is now under clinical trial and reported to be highly successful. In the next decade, we will probably see even more exciting approaches, for example, using advanced microbiota engineering technologies to create "intelligent" or "smart" bacteria for use in diagnosis, prevention, prediction and treatment of inflammatory diseases and possibly of some gastrointestinal cancers. The microbiota-based therapeutics together with personalized medicine may be the most accurate and optimal strategy for the future treatment of some difficult-to-manage diseases. However, many challenges remain to be solved before the translational potential of this new knowledge can be implemented clinically. In this review, I highlight some important recent developments and advances that contribute to our understanding in the role of microbiota in human health and disease and on how to best manipulate the microbiome to promote greater human health.

Keywords: microbiota, microbiome, gut homeostasis, dysbiosis, microbiota-based therapeutics.

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It has been recognized for some time that a wide array of microbes colonizes all vertebrates including humans and that the mutual interactions between these organisms and their hosts are critical for our health. Recent advances in molecular biology and genetics including 16S ribosomal RNA sequencing and other molecular technologies have unraveled a diversity and complexity of the microbial communities present in or on our body. It has been estimated that human intestine is colonized by more than 1000 species of friendly bacteria. Although this symbiotic relationship is highly beneficial for both parties, in this review, I focused my discussion mainly on the impact of microbiota on our health, elaborating particularly on the potential mechanisms that these organisms establish and maintain homeostasis at steady state. When the balance is disturbed as in dysbiosis, it can have significant impact on susceptibility and occurrence of diseases, particularly obvious for those associated with low-grade chronic inflammatory condition.[1-5] However, more and more diseases have now been reported to be associated with altered gut flora (dysfunctional microbiome).

Abbreviations:

5-HT	= 5-hydroxytryptamine
ACTH	= adenocorticotropic hormone
AMP	= antimicrobial peptide
AhR	= aryl hydrocarbon receptor
APRIL	= a proliferation-inducing ligand
CNS	= central nervous system
BAFF	= B-cell activating factor
DC	= dendritic cell
ENS	= enteric nervous system
FMO	= Flavin mono-oxygenase
FMT	= fecal microbiota transplantation
GBA	= gut-brain axis
GF	= germ-free/germfree
GABA	= gamma-aminobutyric acid
GPCR	= G protein-coupled receptor
HMP	= Human Microbiome Project
HPA	= hypothalamic-pituitary-adrenal axis
IEC	= intestinal epithelial cell
IEL	= intraepithelial lymphocyte
Ig	= immunoglobulin
IL	= interleukin
ILC	= innate lymphoid cell
ILF	= isolated lymphoid follicle
LDL	= low-density lipoprotein
LP	= lamina propria
MAIT	= mucosal-associated invariant T cell
MGB	= microbiota-gut-brain
MLN	= mesenteric lymph node
MMP	= matrix metalloproteinase
NLRP	= NOD-like receptor protein
NOD	= neucleotide-binding oligodimerization domain
PPAR	= peroxisome proliferator-activated receptor
RA	= retinoic acid
RAG	= recombination activating gene
RAGE	= receptor for advanced glycation end product
REG	= regenerating islet-derived protein
SCFA	= short-chain fatty acid
TGF	= transforming growth factor
TLR	= Toll-like receptor
TMA	= trimethylamine
TMAO	= trimethylamine N-oxide
Treg	= regulatory T cell
TSLP	= thymic stromal lymphopoietin

The term microbiota (previously referred to as commensals or normal flora) was originally meant to represent an ecological community of commensals, symbionts and potentially pathogenic microbes (pathobionts) that live within our body. This collection of microbial community includes bacteria, archaea, fungi and viruses. The term microbiome was originally coined to represent all genomes in that ecosystem, but it is now routinely used interchangeably with microbiota. In humans, the number of bacterial cells has been estimated to outnumber that of their host by almost one magnitude and that their genes are altogether more than 100 times that of our own genes.[5-7] However, more recent estimate put the bacterial to host cell ratio to be closer to 1:1.[8] The major phyla of the more than 1000 species of the bacteria harboring our intestine are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and, to a lesser extent, Verrucomicrobia. It is now well established that human microbiota exhibit considerable intra- and inter-personal variations, however in any one adult individual the microbiota remains relatively stable over the years.[5-7] The diversity, composition and richness of microbiota have been reported to vary with habitats and, for example, those on the skin or in the vagina or respiratory tract are different from those in the gut which is the most dense and diverse.[6,7] The microbial communities from sites within the oral cavity appear to be least stable, whereas those in the vagina and gut are most stable. However, the latter varies considerably with locations (e.g., about 10^6 in small intestine vs 10^{14} in colon), age of the hosts, mode of delivery and early environmental exposures that include, for instance, diets and dietary habits, hygiene, antibiotic usage, and geography.

Current research interest has been focused on the potential causal impact of microbiota on some human diseases, aiming at a possible use of microbiota-based strategies to alleviate some of these disorders. This interest has reflected in a dramatic surge in a number of publications on microbiota during the last 5 years (**Figure 1**). The publication profiles in the figure show that the publications on microbiota and microbiome scarcely existed prior to the year 2000. The availability of the NIH-sponsored research funding for "Human Microbiome Project" (HMP) at the end of 2007[6,7,9] serves as significant seed money for much of the research that leads to a logarithmic increase of the publications on microbiota. The approximately 150 million dollars was allocated to support interdisciplinary research for 5 years, aiming at fully characterizing microbiome at major body sites (i.e., gut, mouth, vagina and skin) of normal human volunteers. The ultimate objective of these research projects was to demonstrate whether there are opportunities to improve human health through monitoring and manipulating microbiota. The early findings confirmed the previously established fact that healthy humans differ remarkably in the microbes that colonize different habitats, namely, oral cavity, gut, vagina, respiratory tract and skin. It can be concluded from these studies that although the microbiota profiles at each location vary considerably among individuals, it is nevertheless possible to identify the microbial taxa that make up a "core" microbiome at each site (**Figure 2**).[9] It has been said that the Human Microbiome Project (HMP) looks more like an extension of the previous NIH-funded "Human Genome

Project" whose goal was to sequence and identify human genomes. In fact, in the year 2007 when the HMP was about to be launched, the human microbiome was occasionally referred to as a "second human genome".

Observations from epidemiological and metagenomics studies, together with the data from different experimental animal models, demonstrate a shift in the microbiota profile in many diseases, particularly in the disorders associated with metabolic syndrome, and also more recently with mental health and behaviors.[5,10-14] However, it is not certain if the disturbed gut flora reported is really the cause or the consequence of these disorders because the data that favor one or the other are rather limited, particularly in humans. With the exception of obesity whereby a causal relationship has been more or less well established, the associative data for other diseases are not very convincing and the conclusion remains only speculative. Recently a link between microbiota and some neurological disorders has emerged, suggesting that microbiota can influence brain behaviors via gut-brain axis or via its metabolic by-products and bioactive mediators or hormone-like neurochemicals.[13,14] The ability of gut microbiota to influence brain functions and behaviors is a new frontier of research that has recently attracted considerable attention of many biomedical researchers. Although a radical conclusion from recent studies that microbes can influence the mind and behaviors has met with much skepticism, I personally feel that the time is changing, as emerging evidence are rather convincing and consistent with the conclusion that microbes can genuinely affect personality, behaviors, mood and cognitive functions. New research findings showing a link of microbiota to diet and immune response further suggest a potential implication in developing novel strategies for the treatment of metabolic diseases as well as others that are associated with low-grade chronic inflammation. However, a longitudinal study together with detailed characterization of the dynamics of microbiome in healthy persons is an essential first step needed to unravel the potential impact of microbiota to human health and disease.

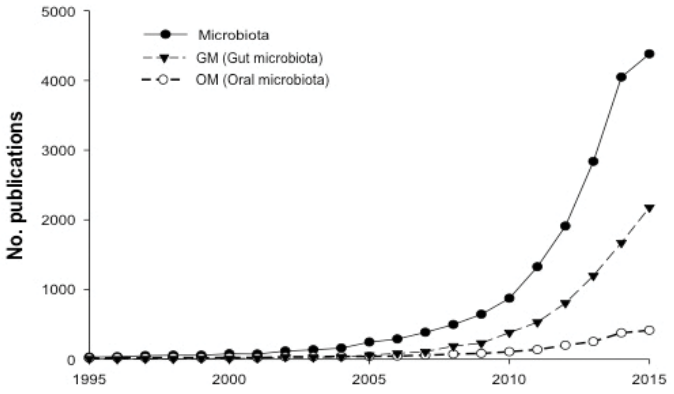

Figure 1 Number of publications on microbiota and microbiome during the last two decades (from 1995 to 2015). Data were obtained by searching PubMed database (January, 16, 2016) using the terms: **microbiota**, **gut microbiota** and **oral microbiota**.

Brief overview on diversity and composition of microbiota

All vertebrates including humans are colonized by a large number of microbes which predominantly consist of bacteria, but archaea, fungi, protozoa and viruses are also present, all of which are believed to have co-evolved with the multicellular hosts.[12] It was estimated that there are at least 1,000 bacterial species possessing a total of several million genes living inside the host or on its surface.[5-7] Among the various habitats, human gastrointestinal (GI) tract possesses the most abundant and diverse number of microbes, containing at least 10^{14} bacteria with a total weight of approximately one kilogram and with total genome (microbiome) in excess of 100 times as that of the host.[5-7,15] Of the approximately 200 common bacterial species in human gut, those belonging to the phyla Firmicutes and Bacteroidetes are most common, representing over 70-75 % of total. The next most common phyla are Actinobacteria, Proteobacteria and Verrucomicrobia. Most predominant genera are *Bacteroides*, *Clostridium*, *Faecalibacterium*, *Eubacterium*, *Ruminococcus*, *Peptidococcus*, *Peptidostreptococcus* and *Bifidobacterium*.[10,15,16] The most studied bacteria in the GI tract, *Lactobacillus* and *Escherichia*, are present to a much lesser extent. The gut of newborn humans is generally believed to be sterile but is immediately colonized after birth. At this time its diversity and number are much lower than those of adult gut which is dominated by bacteria in the phyla Actinobacteria and Proteobacteria[2,5,10,15,16] The microbiota in the newborn remains relatively stable to about one year of age and thereafter it quickly changes to resemble those of the adults, dominated by anaerobic Firmicutes and Bacteroidetes.[10,15,16] The composition and diversity of microbiota also vary with delivery mode, early childhood feeding (i.e., formula-feeding vs breast-feeding), host genetics and a number of environmental exposures, e.g., diet and dietary habits, early childhood infections, antibiotic usage, hygiene and geography (**Figure 2**). It has been suggested that breast feeding should help the right microbes to get established in the gut and help to set the baby growth and microbiota maturity on the right trajectory, as healthy mother is known to produce sialylated milk oligosaccharides that

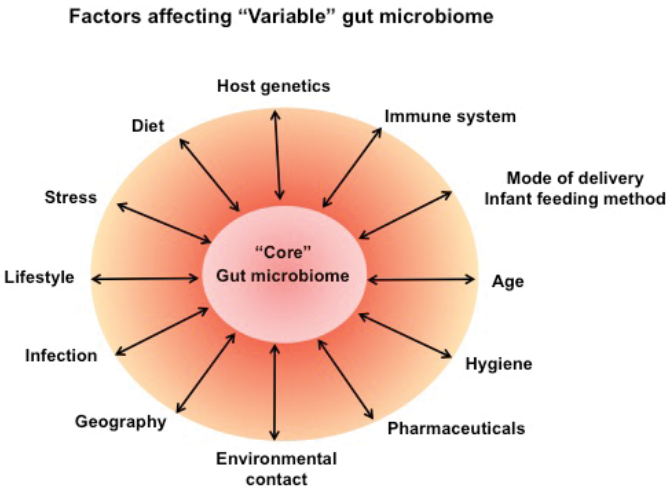

Figure 2. Factors affecting human microbiome profile. Modulations of "core" gut microbiome by host and environmental factors are shown.

facilitate gut microbes to colonize and thrive. Interference with the maturation of microbiome at early life, e.g., over use of antibiotics, would put the child at higher risk for asthma, allergies, inflammatory diseases and autoimmunity. It is possible that the antibiotics may deplete the gut microbes that normally keep immune activation under control, thus leaving it prone to overreact. Similar conclusion was reached in the study using GF animals. There is also evidence suggesting that aging is commonly associated with a shift in microbiota profile characterized by low levels of bacteria in the phylum Firmicutes but with an increase in Proteobacteria. Significant loss of microbial diversity has been observed to be associated with increased frailty in aging population.[16] Conclusions from the various studies in the Human Microbiome Project reveal a considerable variation in diversity of microbiota at different body sites, even within the same individuals or in different individuals in the same family. It is of interest to note from several animal studies that the composition of gut microbiota shows diurnal variation, i.e., it oscillates with the circadian clock.[17-19] The limited data in humans, as demonstrated in jet-lagged passengers and night-shift workers, also reveal circadian rhythmicity similar to those reported in animal experiments. Analyzing the microbiota data in fecal specimens from different individuals demonstrated that a small number of microbial species is shared by all individuals in a human population and these might be considered to represent a microbiota phylogenetic "core" (**Figure 2**).[6-8] However, there is a considerable variation in the composition and diversity of the remaining gut majority and this variability depends on host genotype, immune status, diet and a number of other environmental factors.[4,10-12,15,16] Host genotype is known to have considerable influence on the composition of its microbiome which can in turn shape an individual microbiota phenotype.[20,21] For example, people carrying known mutations (e.g., *NOD2* gene) associated with increased risk of some inflammatory bowel diseases have microbiomes that differ from those who do not have the mutation.[20] Another example is the alteration of the microbiota found in association with the mutation in the gene controlling fucosylation of mucosal surface carbohydrates (fucosyltransferase 2 enzyme) in Crohn's disease.[4,20] However, much of the remaining studies favor the conclusion that environmental factors are probably more important than host genotype in shaping the overall microbiota profile of the host.

Influence of diet on microbiota profile

It is most logical to expect that, among the environmental factors, diet should have the highest impact on the composition and diversity of gut microbiota. The observations that gut microbiota in malnutrition is different from that of the well-nourished control readily substantiate this claim.[22] Recent evidence suggested that an immature gut microbiome reported in earlier studies on malnutrition and kwashiorkor-like condition is probably not a consequence but a causal factor that contributes to the development of this problem.[23] Because the diet is also known to have significant influence on host immune response which in turn regulates the microbiota,[5,10,11] it is difficult therefore to identify the exact molecular mechanism on how the diet by itself regulates and maintains a healthy microbiota ecosystem. Emerging evidence from epidemiological and experimental studies reveal that changes in the diet, microbiota and immunity are linked to increased incidence in a number of diseases, particularly in obesity and related chronic inflammatory conditions (e.g., type 2 diabetes, atherosclerosis and cardiovascular diseases).

Although not yet a consensus, it is generally agreed that good health is associated with high degree of microbial diversity and richness.[24-28] Right combination of microbes can tip the balance between disease and healthy status. Unhealthy high-fat and high-sugar diet commonly associated with Western-style diet is known to have substantial adverse impact on the composition and diversity of gut microbiota leading to unbalanced ecosystem known as dysbiosis. Fecal specimens from a population in developed countries consuming this type of diet show microbiota profiles that are considerably different from those in developing countries consuming low-fat and low-sugar diet but rich in vegetables and dietary fiber content.[2-5,24-27] The gut microbiota profile of the latter is more commonly associated with marked increase of bacteria in the genus *Prevotella* and other SCFA-producing microbes.[27] Studies comparing the microbiota profiles of children from developing and developed countries reveal the stool from the latter to be dominated by the bacteria taxonomically belonging to the phyla Firmicutes and Proteobacteria compared with those in the phylum Bacteroidetes in the former group.[3,4,24,27] Data from several other studies comparing the gut microbiota of African children with other European children all agree that the microbiota of the former group is dominated by Bacteriodetes and a marked increase in diversity, whereas with the latter it is predominantly Firmicutes and reduced diversity.[3-5,26,27] Among the Bacteroidetes detected in the Africans, there was an abundance of bacteria in the genus *Prevotella* and *Xylanibacteria*.[12,26-29] It was hypothesized that gut microbiota might have coevolved with the polysaccharide-rich diet in more primitive individuals, allowing them to maximize energy production from fiber-rich diet, and at the same time protecting them from inflammation and inflammatory diseases.[12] The microbiota profile from vegetarians has been reported to be different from those of omnivores.[24] Altogether, these studies demonstrate the association of dietary type with gut microbiota.

Based on the microbiota profile, human population can be arbitrarily categorized into 3 enterotypes, namely, *Bacteroides*, *Prevotella* and *Ruminococcus*.[5,29] People consuming high protein and animal fats are colonized predominantly by *Bacteroides*, whereas those consuming high carbohydrate and simple sugars possess *Prevotella* in their gut, and those consuming polyunsaturated fats are dominated by *Ruminococcus*. The animals fed on high-fat diet showed reduced Bacteroidetes-Firmicutes ratio compared with those feeding on regular diet.[28,29] The microbiota of obese and overweight persons usually exhibited increased levels of Firmicutes and Proteobacteria and decreased levels of Bifidobacterium.[5,24,26,27] Diets high in certain fats and sugars deplete anti-inflammatory microbes, thin mucus later and foster systemic inflammation. Switching to a high fat, high protein diet favors the expansion of, for example, *Bilophila wadsworthia* and occurrence of

inflammatory diseases. On the other hand, adding fermentable fiber to diet high in fat keep "good" microbes happy, healthy mucus layer and intestinal barrier intact which prevent the occurrence of systemic inflammation. It should be mentioned that not only the energy intake and macronutrients, but micronutrients like vitamins and trace elements can also influence the characteristics of gut microbiota. For example, the gut microbiota profile in vitamin A deficiency has been reported to be different from that of normal control.[30] However, it is uncertain if the deficiency directly affects the gut microbes, or indirectly exerts its influence on the host immune machinery. It is known that vitamin A has a marked influence on development and differentiation of mucosal immune system[31] which can have a significant impact on gut microbiome and ecosystem.

Different lines of evidence are consistent with the conclusion that changes of the microbiota profile can occur rapidly following consuming unhealthy diet which, if left unattended and prolonged, can result in increased susceptibility and severity of several diseases.[32] However, it has been reported very recently in animals that it may take long time for the microbiota to return to "normal" after antibiotic treatment and if the treatment is prolonged, normal profile may not return and this is not uncommonly associated with weight gain and obesity.[5,32,33] An excellent example for this association is the common practice of adding antibiotics to animal feeds to accelerate weight gain in animal industry. Fully understanding the molecular pathway linking diet to microbiota may lead to a potential new intervention "pharmaco-nutrition", i.e., using gut microbiota to alleviate host metabolic alterations associated with dysbiosis. However, more studies are needed to unravel how specific changes in the gut microbial community might affect the development of obesity and related disorders. The beneficial outcome of probiotics in reconditioning the gut ecosystem has been used for decades. In the future, it may be possible to fabricate targeted probiotics containing specific bacterial strains to cure disease affecting the population in different parts of the world. A current approach using probiotics supplemented with gram-positive bacteria with high butyrate producing capacity to reverse dysbiosis is currently a promising strategy to specifically targeting certain metabolic diseases.[10,26,33] It has been shown for example that adding *Bifidobacterium* species (e.g., *B. breve* or *B. longum*) or *Lactobacillus* species (e.g., *L. rhamnosus*, *L. plantarum*, *L.paracasei* or *L. reuteri*) to probiotics can not only reducing body weight gain but also decreasing urinary cortisol output. Another possible approach to reconditioning gut homeostasis is the use of fecal microbiota transplantation. This strategy has been reported to be effective in clinical trials in the treatment of recurrent antibiotic-resistant *Clostridium difficile* infections.[34] It is quite possible also that in the near future we may have available a tailor-made "intelligent" bacteria that would be able to carry on a specific task using microbiota engineering technology.

Based on the information currently available on the interplay of diet on microbiota and host immunity, it is possible to arbitrarily classify food components into those with inflammatory (inflammogenic) or anti-inflammatory potential.[3-5,24] The inflammogenic foods include the well-known high-fat diet, milk-derived fat, free fatty acids, eggs and red meat.[24] Some of these are microbiota dependent while others are microbiota independent. For example, the popular inflammatory red meat and eggs containing phospholipid phosphatidylcholine and L-carnitine have to be metabolized by gut microbiota to trimethylamine (TMA) prior to being absorbed and then metabolized further by liver enzymes flavin mono-oxygenases (FMOs) to atherogenic trimethylamine N-oxide (TMAO).[35-37] The latter is taken up by macrophages in the process of plaque formation. Whether or not other food components with high fat content and free fatty acids require prior metabolizing by gut bacteria (e.g., Firmicutes and Bacteroidetes) is uncertain. However, some of them may need prior oxidization before acquiring the capacity to trigger dietary pattern recognition receptors, TLR or NALP3 inflammasome for proinflammatory cytokine production.[10,38] Advanced glycation end products from carbohydrate metabolism may induce inflammation via activation of receptor for advanced glycation end products (RAGE) present on mononuclear phagocytes.[38] Some free fatty acids, e.g., palmitic acid, impair intestinal permeability, resulting in enhanced intestinal absorption of lipopolysaccharide and other inflammatory-inducing components like peptidoglycans, ending up with metabolic endotoxemia and inflammation.[3,24] The anti-inflammatory food components are predominantly fruits and vegetables (particularly broccoli and cabbage), tryptophan and tryptophan-derived products, soluble dietary fibers, omega-3 fatty acid and some vitamins (e.g., vitamin A). Soluble dietary fibers are metabolized by gut bacteria to SCFAs (e.g., bioactive butyrate, propionate and acetate) and are used for energy by enterocytes or are absorbed systemically to promote anti-inflammatory microenvironment (e.g., suppression of inflammatory cytokines and chemokines, reduced production of adhesion molecules) in the mucosa. Many of the anti-inflammatory food components mentioned above function as microbiota-dependent ligands for a number of receptors (e.g., arylhydrocarbon receptor and G-protein-coupled receptors) that signal production of anti-inflammatory cytokines (IL-10 and IL-22).[39] In addition to inducing the production of cytokines, these food components can also promote differentiation and proliferation of Treg, ILC3 and intraepithelial lymphocytes (IELs), and enhance production of secretory IgA.[24,39] These effects, together with their ability to enhance production of antimicrobial peptides and proteins by enterocytes and mucin by goblet cells, promote and maintain homeostasis and tolerance in the gut. Integrating these data altogether, it is fair to state that the food components in Western diets are biased toward chronic inflammatory response associated with autoimmunity and inflammatory disease, whereas the Mediteranian diet (containing high polyphenols and polyunsaturated fatty acids) and the ones consumed by populations in developing countries are generally anti-inflammatory, favoring tolerance induction and maintaining gut homeostasis. Both inflammatory and anti-inflammatory dietary components may thus affect host physiology and metabolism directly or indirectly via microbiota-dependent mechanism.

A novel multifunctional metabolic sensor with a capacity to regulate cross-talks in the diet-microbiota-immunity

network mentioned above has only been identified and described recently.[39,40] This sensor, now known as Aryl hydrocarbon receptor (AhR), is a transcription factor ubiquitously expressed in immune cells and several non-immune cells like epithelial cells and cancer cells. It functions as if it is the host environmental sensor that conveys signals from external environment to cellular milieu inside the host. After binding to its ligands, the signals generated can exert significant impact on the development and functions of immune cells of both innate and adaptive arms. Its main role is to promote Treg cell differentiation and production of anti-inflammatory cytokine IL-22. Through ILC-dependent pathway, the AhR signaling is known to facilitate the formation of isolated lymphoid follicles and possibly of the Peyer's patches. It also modulates the AMP production by γδ T cells present in the intraepithelial compartment of the GI tract. The sources of the ligands for AhR are rather diverse, ranging from food and microbial pigments/metabolites to components generated endogenously by the host and cancer cells. Dietary components reactive with AhR are predominantly found in fruits and vegetables.[39,40] Generally speaking, the receptor and its ligands are found most abundantly in the tissues most exposed to environment. It should be noted that the AhR itself is uniquely positioned as the center of food, microbiota and immunity network, and functions not only as dietary pattern recognition receptor, but also as pathogen-associated molecular pattern receptor and danger/damage-associated molecular pattern recognition receptor. Strategically, this newly described receptor plays an important role as anti-inflammatory regulator that integrates dietary, microbial, and danger signals to regulate gut microbiota, elicit protective response and regulate gut tolerance and homeostasis. However, the final outcome from the interaction depends on the amount of AhR in a given cell and microenvironment or the abundance and potency of ligands in the tissue. Exciting data are emerging rapidly and we will most certainly have a chance to witness its potential and implication in the management of human diseases in the near future.

Bidirectional interactions between microbiota and the host

From the evolutionary point of view, microbes are believed to have co-evolved with and exerted control over the integrity of the immune system. In fact, it has been said that the microbiota functions as if it is another organ of our body, e.g., facilitating development and providing proper education for the developing immune system.[1] The final outcome of this education is to maintain homeostasis, i.e., the mature immune system must learn to tolerate the commensal microbes and at the same time must respond appropriately to protect the host against pathogens. The interactions between microbiota and the host are in essence bidirectional, but the outcome depends on differential impact from both genetic and environmental factors.[20,21,40] Because more than 90% of the microbes inhabiting the gastrointestinal tract and much of the information currently available on microbiota are from studying gut microbes (**Figure 1**), in this review, the remaining discussion will focus on gut microbiota if not specified otherwise. The information on modulation of immune development and function by microbiota relies to a considerable extent on animal studies using different approaches, e.g., germfree and mono- or di-colonized animals, antibiotic treatment and probiotic supplementation. Improper or "immature" microbiota can delay maturation of the immune system characterized by improperly developed tolerance with inappropriate response to resident microbes and pathogens and development of inflammatory and autoimmune diseasses. Although the studies carried out by different groups of investigators are well designed and carefully executed, the results must be interpreted with cautions because both the composition and diversity of gut microbiota and the development and function of the immune system are also subjected to modulation by several variable factors. Moreover, with the animal studies, even slight contamination of sterile animal feeds and drinking water with microbial components, e.g., lipopolysaccharide and peptidoglycan, is sufficient to trigger undesired immune reactivity in GF animals which may interfere with the final outcome of the complex interplays of the microbes-diet-immune network.

It has been shown in a number of studies that better health is associated with greater microbiota diversity.[5,10,24-26] Balanced intestinal microbiota, particularly regarding the degree of diversity, helps maintaining the host in healthy state and for whatever reason when the balance is disturbed, it may end up with dysbiosis and occurrence of diseases of not only the gastrointestinal tract but also of the organs at distant site.[2,27,28] A large volume of knowledge linking altered microbiota profile to a number of diseases have been reported over the last decade. However, the implication of this observation will not have significant impact clinically until it can be demonstrated that the relationship is a causal type and not a consequence of the diseases. Targeting gut microbiota could lead to a new strategy for preventing and correcting a variety of difficult-to-manage diseases, for example, from obesity and diabetes to cardiovascular diseases and cancers. It should be reminded that the study on the impact of microbiota on human health is not simple, and the results must be interpreted with caution as both the microbiota and the overall health of an individual are also subjected to regulation by other factors including host immune system which itself is also influenced by diet and other lifestyle factors.

Modulation of immune development and response by gut microbiota

Although the influence of commensals on development, maturation, education and regulation of both local and systemic arms of the immune system has been reported several decades earlier, the molecular mechanism responsible for this association is still not completely understood. The influence of gut microbiota is not limited just to the GI tract but it can extend far beyond to affect the response of systemic immune components in other organs as well as the central nervous system and its associated disorders. The interaction between commensal microbes and immune system is bidirectional, i.e., signals from one party can affect another party and vice versa (**Figure 3**). A large amount of information available reveal the microbiota to have extensive and long-lasting effects on the development and functions of both innate and adaptive immune cell populations in the gut.[40-45] They illustrate that the microbiota possess different mechanisms and use multiple

pathways to shape the differentiation and functions of intestinal regulatory and effector T cells and to facilitate IgA switching of B cells and IgA secretion. To generate appropriate signals and maintain mutual relationship at steady state, the contact of microbiota and its metabolites with the host (in this case, it is the epithelial cell surface) must be limited and carefully controlled and regulated, and this is achieved, for example, by various host components and mechanisms including mucus layer, antimicrobial peptides and proteins, secretory IgA and host own metabolites.

Much of the information available is from studies using germfree animals. In general, the immune components of these animals are immature and poorly developed.[5,40,41] The development and functional integrity of mucosal immune system, particularly of the GI tract of these animals, are adversely affected, e.g., lower frequencies and poorly developed isolated lymphoid follicles, smaller Peyer's patches and mesenteric lymph nodes and scattering presence of a relatively low number of resident myeloid cells like mononuclear phagocytes (particularly dendritic cells and macrophages). The $CD4^+$ and $CD8^+$T cells proliferate and differentiate poorly, whereas the IgA-producing cells and production of sIgA are much reduced. The Th17 cells are almost totally absent in these animals. Regulatory T cells and innate lymphoid cells are low compared to those of their conventional counterpart. The development and differentiation of GI epithelium is also affected, e.g., smaller villus thickness, enlarged cecum (due to accumulation of undegraded mucus), reduced intestinal motility and low expression of antimicrobial peptides. On the other hand, these GF animals exhibit exaggerated number of iNKT cells and substantially elevated IgE levels, thus consistent with the augmented inflammatory responses and airway hyperresponsiveness commonly observed in these animals.[41] Colonization of GF animals with commensal bacteria, e.g., *Bacteroides fragilis* and/or bacteria belonging to the *Clostridium* clusters XIV and IV can normalize these immune defects.[5,40,41] However, recolonization needs to occur during the critical "window of opportunity" (which is within one month of postnatal period in mice), and if it does not occur during this timeframe, the development may not be fully achieved in the adult. It has been demonstrated recently that purified polysaccharide A (PSA) of *B. fragilis* can restore the Th1 and Th2 balance of these GF animals. Similarly, segmented filamentous bacteria are known to promote the development of Th17 cells as well as playing a key role in the induction of sIgA. It is also known that sphingolipid of *B. fragilis* inhibits proliferation of iNKT cells and helps promoting healthy colon. Although the colonization with specific bacterial species may have the capacity to normalize immune development, the functional maturation of the immune system appears to be dependent on intestinal bacterial diversity.

1. Regulation of innate immune response by microbiota. At steady state, only a small fraction of the trillion microbes in the

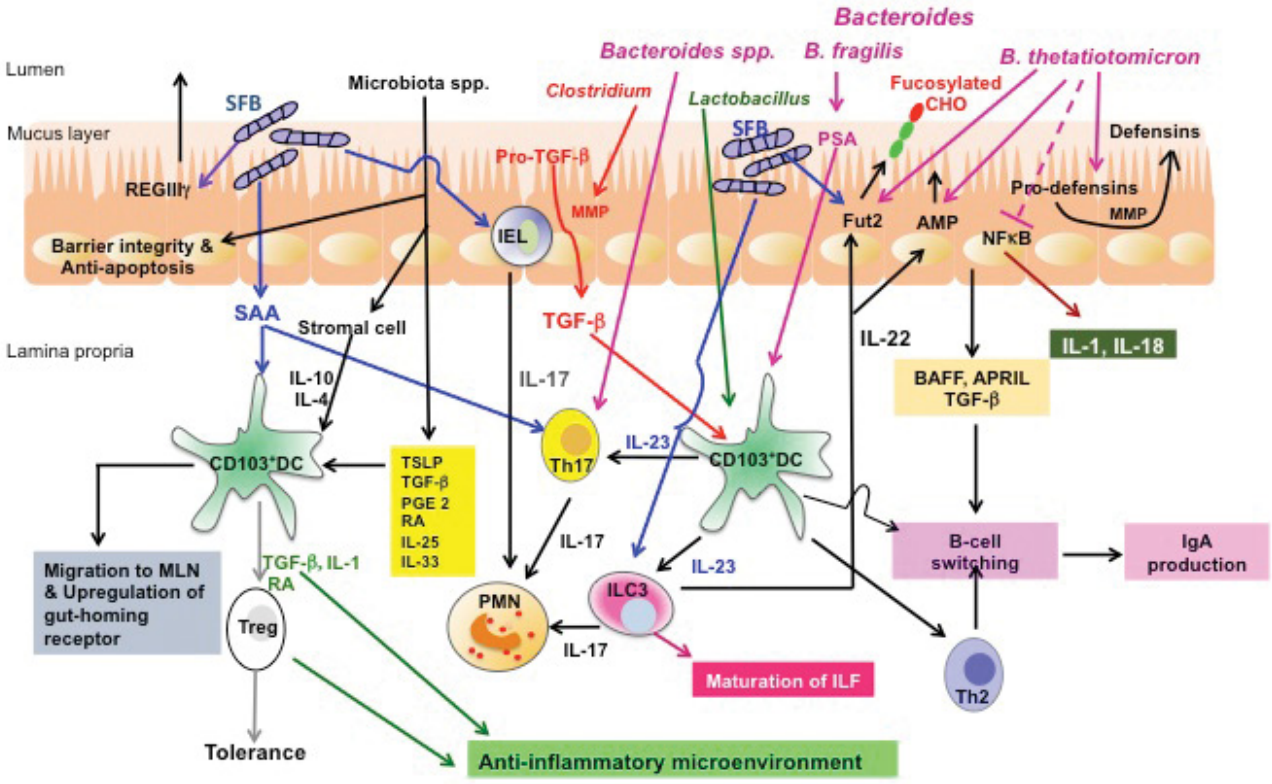

Figure 3. Examples of the impact of microbiota and some specific bacterial species on development and function of mucosal immune system. Although under steady state, only a small fraction of the microbes can come into direct contact with the surface of enterocytes, those in the lumen can nevertheless signal the epithelium via their soluble structural components and/or metabolites like SCFAs. SFB is the bacterium that can readily interact and contact, or even penetrate the mucosa, and exert influence on epithelium, innate and adaptive immune cells as well as innate-like lymphocytes. To facilitate the understanding of the complex host-microbial interactions, color-coded arrows are used to designate different signal pathways.

gut is in direct contact with the epithelial cell surface due to interference by mucus barrier. The predominant mucin MUC2 secreted by goblet cells forms a tight network that serves as a physical barrier that interferes with bacterial movement and attachment needed for invasion.[44] The commensal bacterium *Bacteroides thetatiotaomicron* stimulates IECs to produce AMP, C-type lectin (e.g., REGIIIγ) and to increase the expression of MMP needed for cleavage of inactive pro-defensins to active defensins (**Figure 3**).[40-43] Besides α- and β-defensins and REGIIIγ, other AMPs secreted by IECs include cathelicidins, lectins and a number of hydrolytic enzymes. The mucus layer formed by mucins secreted from goblet cells can trap these antimicrobial compounds, thus increasing their potential protective effects locally at or near mucosal surface. Some of these AMPs are not only induced and regulated by microbes and their metabolites, but they, in turn, play a fundamental role in shaping the composition and diversity of the microbiota.[40-44] For example, there is evidence suggesting that the MUC2 mucin can promote tolerogenic response to commensal antigens. *Bacteroides thetatiotaomicron* has the ability to down-regulate inflammatory response by interfering with the activation of NFκB by exporting the NFκB subunit out from the nucleus to cytoplasm in a PPARγ-dependent pathway.[40] This bacterium together with others like segmented filamentous bacteria (SFB) can activate and regulate production of enzyme fucosyltransferases that add fucose subunit to the carbohydrate moieties on the surface of IECs needed to maintain homeostasis.[4,45] The presence of several *Lactobacillus* species in the GI tract promote barrier integrity and sustain the Th1/Th2/Th3 cytokine balance.[40] The IECs can also respond to stimulation by *Clostridium* spp. by upregulating MMPs needed for the conversion of pro-TGF-β to its active form.[5,27,40,46] The latter provides anti-inflammatory microenvironment required for Treg differentiation and IgA production. In addition, some resident microbes possess the ability to regulate the repertoire and specificity IgA which can in turn modulate the composition of microbiota.

By responding to microbes and their metabolites, the intestinal epithelial cells can send signals to facilitate the development of immune system via several pathways (**Figure 3**). Direct contact of some commensal bacteria like SFB with pattern recognition receptors (e.g., TLRs, NOD1, NOD2) or inflammasome (NLRP3) can not only stimulate IECs to produce AMPs (e.g., defensins, cathericidin LL-37 and RegIIIγ),but can also activate mononuclear phagocytes (e.g., DCs and MPs) and innate lymphoid cells (particularly the ILC1 and ILC3 subpopulations) to upregulate the production of cytokines and chemokines.[40] Some of the microbiota-induced production of mediators by the IECs (e.g., TGF-β, TSLP and IL-25) are anti-inflammatory and can condition DCs (CD 103^+ DCs) and resident macrophages ($CX3CR1^+$) to retain an immature status that favors polarization of $CD4^+$ T cells toward a Th2 cell phenotype. These resident microbes also endow the DCs, and to a lesser extent the MPs, with a capacity to synthesize retinoic acid needed for mucosal homing of activated lymphocytes and for T cell differentiation toward regulatory T (Treg) cells and Th2 phenotypes.[5,31,47,48] These conditioned DCs and MPs also secrete anti-inflammatory mediators (e.g., IL-10 and TGF-β) that promote switching and differentiation of B cells to IgA-producing cells and sIgA secretion. In response to gut microbiota (e.g., SFB), the intestinal mononuclear cells also produce IL-23 that facilitates the development and response of mucosal Th17 and ILCs.

2. Regulation of adaptive immune response. It was mentioned earlier in this review that microbiota is required for proper development of mucosal T cell subpopulations, favoring Treg and Th2 phenotypes.[40,47,48] This can occur indirectly via IECs or directly via PRRs known to be present also on T cells which allow them to sense and respond directly to microbial structural components and their metabolites. Several species of bacteria present in the gut like SFB and several others that are in close contact with or invade IECs stimulate them to synthesize, for example, serum amyloid A (SAA) which can in turn influence the differentiation of Th17.[40,47,48] The SFB is also able to induce Th17 differentiation directly via a pathway that has not yet been completely characterized, but probably needs a recognition of specific SFB antigens.[27,40] Similarly, other members of gut microbiota provide microbial antigens and immunomodulatory signaling small molecules that modulate intestinal Treg cells which are unique and distinct from Treg cells in other organs.[48] Immunosuppressive polysaccharide A (PSA) produced by *Bacteroides fragilis* can also function as a TLR2 ligand, thus directly promoting Treg cell differentiation or indirectly through conditioning the DCs.[40] Some of the SCFAs produced as metabolites by many species of gut bacteria can recognize G protein-coupled receptors (GPCRs) present on T cells and IECs and promote Treg cell generation. Some species of *Clostridium* in the GI tract provide signals for the production of TGF-β and other anti-inflammatory cytokines needed to maintain anti-inflammatory microenvironment needed for Treg cell differentiation and tolerance induction. In addition to inducing differentiation of T cells, SFB and probably other bacterial species can facilitate B cell differentiation for IgA production by both T cell-dependent and T cell-independent pathways. For example, in the T-cell-independent pathway, the bacteria induce IELs and mononuclear phagocytes to secrete IgA-switching cytokines (e.g., BAFF, APRIL and TGF-β) that promote the synthesis and secretion of sIgA.[40] There is evidence suggesting that the BCR receptors of *Rag*-expressing immature B cells in gut lamina propria are susceptible to microbiota-dependent receptor editing, thus implying that specificity and repertoire of the IgA produced in response to microbiota stimulation play a role in regulating and maintaining of tolerance in the GI tract.[40,41]

There is also emerging evidence suggesting that development and functions of intraepithelial lymphocytes (IELs), predominantly dominated by T cells of the γδ phenotype, are also under the influence of microbiota.[40] In addition to producing IL-17, these γδT cells are able to produce AMPs in response to the bacteria that have penetrated the epithelial barrier. Although the mechanism behind this is still incomplete, it has nevertheless been proposed to involve the binding of microbial components to AhR known to be present in thisT cell subpopulation.[39,40]

3. Interplay between microbiota and innate-like immune cells. In addition to cells of the innate and adaptive compartments

mentioned above, there is another group of cells that has been recently described to be present in substantial number in the mucosa. These cells exhibit lymphoid morphology but lack specific antigen receptors.[31,49,50] This group of cells is functionally very diverse and has properties that do not fit into either innate or adaptive immune categories. These innate-like immune cells that have attracted much interest is known as innate lymphoid cells (ILCs).[49,50] They are predominantly located in close proximity to mucosal epithelium and can readily crosstalk and interact with local immune cells and epithelial cells. Because of their location in the lamina propria of mucosa, it should not be surprising if the gut microbiota would have considerable impact on their functions. Among the 3 ILC subgroups (ILC1, ILC2 and ILC3), the ILC2 andILC3 are best known for their role in regulating and containing commensals as well as protecting the host against enteric pathogens. The ILC2s secrete type 2 cytokines (i.e., IL-4, IL-5, IL-9 and IL-13), well known for their function in the mucosal tissues, whereas the ILC3s secrete predominantly IL-17 and IL-22. These cells are activated and attracted to the mucosal sites by the cytokines secreted from the IECs that have been stimulated by gut microbes. It should be reminded that some of these cytokines can activate the epithelial and goblet cells to produce and secrete AMPs and mucins that can in turn regulate and influence the composition and diversity of the microbiota. The SFB induces ILC3s to secrete IL-22 which can in turn activate IECs to upregulate expression of fucosyltransferase 2 enzyme that adds fucose subunit to the glycosylated surface of the epithelial cells.[31,45] Commensal bacteria possess fucosidase enzyme that can remove the fucose subunit from fucosylated carbohydrate on the surface of epithelium and use it for their own survival. Most pathogens do not have this enzyme and therefore cannot compete with the resident microbes for survival. In addition, dietary and microbial components and metabolites can bind and activate AhR known to be present also in these ILCs.[39] Currently very little is known about the interactions of microbiota with other innate-like immune cells, e.g., iNKT cells, MAIT cells, B1 and marginal zone B cells. The mucosal-associated invariant T (MAIT) cells are known to be present predominantly in mucosal lamina propria and rely on the presence of microbiota for their expansion.[51] They produce INF-γ TNF-α and IL-17 in response to vitamin B precursor.

Gut microbiota and metabolic disease

During the last few decades, an increase of non-communicable diseases worldwide has outnumbered that of communicable diseases. The prevalence of metabolic diseases, particularly obesity and obesity-related disorders has drastically increased as a result of changes in diet and dietary habits, lifestyle and environment factors like pollution.[2,3,5,28] An imbalance of gut microbiota, particularly regarding microbial diversity, has been suspected for a long time to have predisposed an individual to a variety of other diseases including malnutrition, asthma, allergies and inflammatory and metabolic diseases.[27] This is not at all surprising as gut microbiota is a rich source of inflammatory molecules (e.g., LPS and peptidoglycan) that can contribute to inflammation and metabolic diseases.[24,27] A large number of studies have consistently demonstrated a shift in the microbiota profile from a "lean" (anti-inflammatory) phenotype toward an "obese" (inflammatory/inflammogenic) phenotype in obese animals and humans.[3,5,52,53] Fecal transplantation of gut microbiota from lean healthy individuals into obese recipients also improved insulin sensitivity in the recipients and the apparent improvement of clinical manifestations and these changes correlated with changes in the levels of SCFAs and butyrate-producing bacteria.[54] Obese animals have a smaller proportion of Bacteroidetes than Firmicutes compared to normal animals, and the ratio of Bacteroidetes to Firmicutes gradually increases as fat mass decreases.[24,27,28] Similarly, study with human twins revealed a low Bacteroidetes to Firmicutes ratio in the obese members compared with the lean counterparts.[52] Results from several other studies altogether point toward a direction that among the various environmental factors, dietary components appear to play a major role in regulating and inducing a shift in gut microbiota, for example, high protein diet and high fat diet favors *Bacteroides*, high carbohydrate and simple sugar diet favors *Prevotella* and high fat diet favors *Ruminococcus*.[26-29] It has been shown very recently that the consumption of artificial sweeteners could induce changes in the gut microbiota profile toward an obese phenotype.[55] More recently, the number of mucin-degrading bacterium *Akkermansia muciniphila* was found to be markedly decreased in obese animals and colonizing the animals orally with this bacterium resulted in improvement of clinical manifestations related to obesity.[56] Evidence from the animal studies showed that the obese microbiota phenotype most likely mediated abnormal changes in epithelial integrity and barrier function which allow microbial components particularly the lipopolysaccharide to enter blood circulation, resulting in metabolic endotoxemia and inflammation. Chronic low-grade inflammation is known to be associated with a number of other diseases within the gut as well as with systemic diseases at distant sites. Moreover, it has been proposed several years earlier that some species of bacteria present in the obese microbiota phenotype can produce microbial toxins that can damage beta-cell function.[28,57] Regardless of the mechanism involved, it remains to be elucidated whether the change of microbial community is the cause or the consequence of diseases. However, the concurrence of microbiota change with increasing disease prevalence implies a causal association. Different lines of evidence from both animal and human studies showing a marked reduction in microbial diversity in obesity is consistent with the conclusion that an alteration of gut microbiota is the cause and not the consequence of disease. Data from another well-known metabolic disorder, namely atherosclerosis and cardiovascular disease, are consistent with a causal relationship.[35-37] The studies in humans and animals reveal the importance of gut microbiota in converting lecithin and carnitine present in red meat and egg yolk to TMA and after absorption the latter is oxidized to TMAO by FMO enzymes in the liver. The TMAO metabolite was previously believed to be just an inert nitrogenous waste product of protein metabolism, but it has been shown in the studies just mentioned to have important atherosclerotic potential. Mechanistically, the TMAO appears to have the ability to upregulate scavenger receptors on macrophages, leading to foam cell formation and inflammatory cytokine synthesis needed for activation of endothelial cells in the process leading to atherosclerotic plaque formation.[37]

However, the genuine "receptors" for TMAO, if exist at all, have not yet been identified or characterized. In the mouse model in this study, the TMAO was shown to suppress reverse cholesterol transport. Its level in human volunteers correlated with an increased risk of CVD. In consistent with these observations, it was observed that vegetarians and vegans had much reduced capacity to degrade L-carnitine to TMA and TMAO than omnivores. Gut metagenome data from patients with symptomatic atherosclerosis was different from healthy controls.[58] In this study, the bacteria in the genus *Collinsella* was found to be enriched in the patients, whereas those in the genus *Roseburia* and *Eubacterium* were enriched in healthy controls. Functionally,the patient metagenomes were enriched in the genes encoding peptidoglycan synthesis pathway, whereas in the healthy group the genes involved were associated with synthesis of anti-inflammatory molecules, e.g., the SCFA butyrate and anti-oxidant species.

In addition to the metabolic disorders, inflammatory bowel diseases and allergy mentioned earlier, a shift in the microbiota profile has now been reported in patients with, for example, gout, autoimmune uveitis and Behcet's syndrome.[59] There is also recent evidence suggesting that altered community of gut microbiota may be associated with increased risk for colorectal carcinoma in human population.[5] However, the causal effect of these associations remains to be elucidated. It should be reminded that the profile of microbiota is also known to be under the influence of intestinal motility, and that the variations in transit time can alter the environment in which the microbes live and therefore may favor the survival of some species over that of others. Thus, unless the transit time is taken into consideration, what one observes on gut microbiota may or may not be a genuine correlation with the disease process.

Microbiota-gut-brain network

The ability of gut microbiota to communicate and influence brain functions and behavior, and vice versa is another new frontier of biomedical science that has attracted much attention of a great number of investigators in recent years.[13,14,60,61] Results from the HMP have brought the link between the gut and the brain into a clearer focus. It has been observed for sometimes that disturbance of intestinal integrity and activity can influence brain functions. One factor in the gastrointestinal tract with obvious potential to influence its activity is the resident

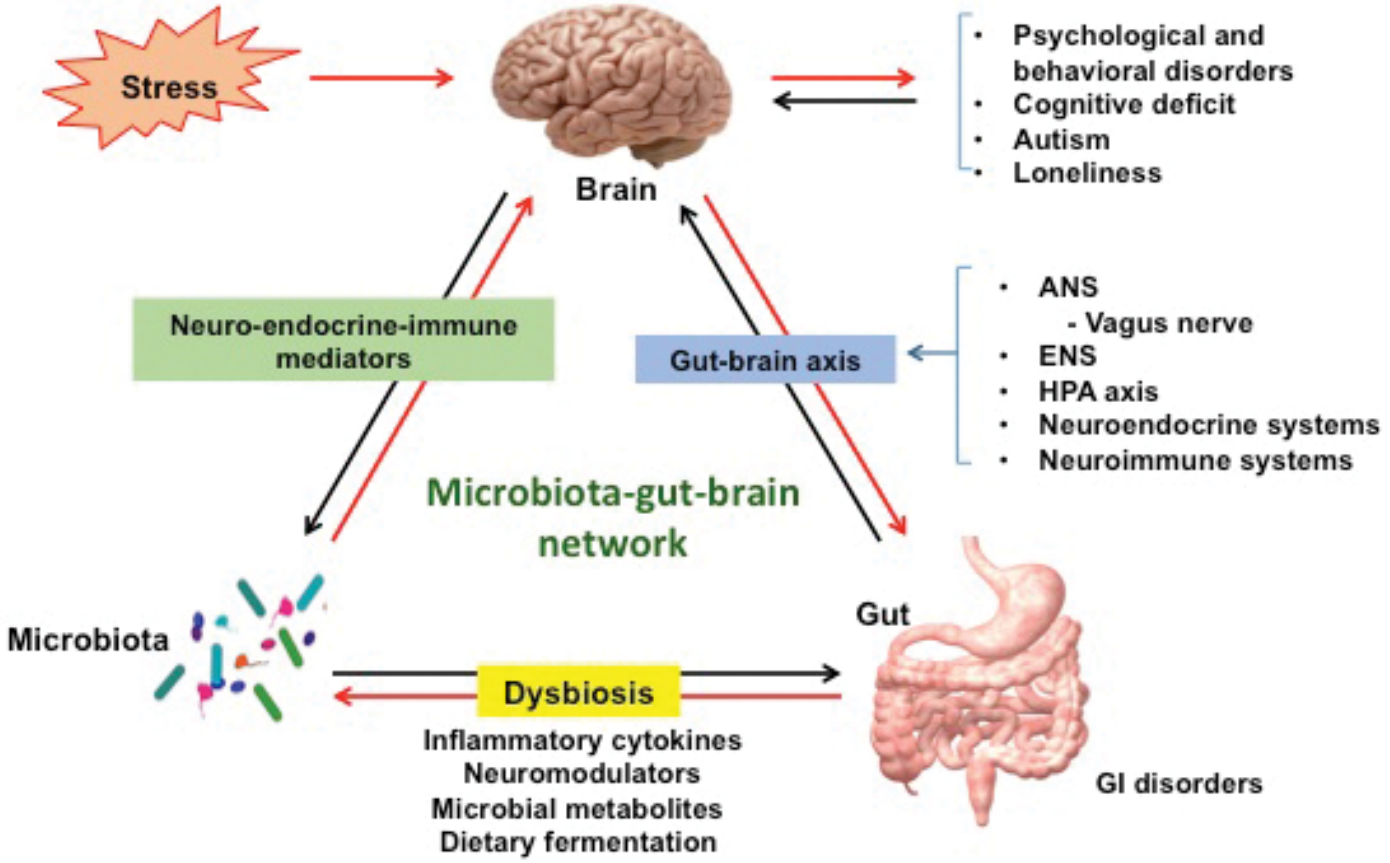

Figure 4. Microbiota-Gut-Brain (MGB) network. Proposed role of the MGB network in modulating stress response. Microbiota communicates with the gut-brain axis (GBA) via mediators from epithelial cells, immune cells and neurons to influence behavioral response. Stress, on the other hand, can affect GI disorders directly via GBA or indirectly via gut-microbiota interaction. Abnormal or unhealthy microbiota (dysbiosis) alters microbial metabolism and synthesis of mediators (e.g., via immune and enteroendocrine cells) which can readily gain access to the brain. Abbreviations: ANS, autonomic nervous system; ENS, enteric nervous system; GBA, gut-brain axis; HPA, hypothalamo-pituitary-adrenal axis; GI, gastrointestinal. Color-coded arrows are used to show the existence of bidirectional communications within the MGB network. (Based on information taken from references 13, 14, 60 and 61).

microbiota. A bidirectional communication and interaction between gut and brain via a so-called gut-brain axis (**Figure 4**) have been suspected for more than one decade.[14,60] In addition to autonomic nervous system and vagus nerve that contribute to this communication network, the interaction is also influenced by signals from hypothalamic pituitary adrenal (HPA) axis. These neural and hormonal signals, together with soluble factors from other sources like the immune and endocrine systems, can modulate the activities of intestinal effector cells (e.g., epithelial cells, enterochromaffin cells, smooth muscle cells, immune cells and enteric neurons). Under the influence of gut microbiota, these same cells in the GI tract can send signals back to regulate the activity of central nervous system. There is evidence showing that a number of bacterial species in the gut possess receptors that can respond to neurotransmitters and neuromodulators, e.g., noradrenalin, that the neurons themselves use to communicate and regulate mood and stress-related behaviors.[13,14,60,61] Likewise, these microbes have the ability to produce biologically active neurochemical-like substances, e.g., serotonin (5-HT), acetylcholine, melatonin and histamine, that can modulate the activity and function of enteric nervous system (ENS) and vagus nerve and therefore these neurochemical-like mediators can be perceived by CNS. Moreover, the bioactive metabolites from gut microbes, such as SCFAs, can also target ENS and stimulate autonomic nervous system. With these new observations, a novel concept of a microbiota-gut-brain network has emerged.[13,14,60] The concept is well accepted as it has important implication, i.e., explaining a possible link between gut microbiota and neurological and psychiatric disorders, e.g., depressive-like behaviors, anxiety and mood, or even social and developmental disorder like autism.[5,60,62,63] Although different lines of evidence from a series of investigations are consistent with this concept, its validity needs to be proven in the future using well-controlled animal models. Nevertheless, it appears that more and more neurological disorders and diseases have now been reported to be associated with altered gut microbiota, but again whether or not the alteration observed is the cause or consequence of the disease remains to be investigated. Different approaches have been employed to investigate this interrelationship including the use of GF animal models, prebiotic/probiotic supplementations, exposure to antibiotics, infection studies using known commensals and pathogens and fecal microbiota transplantation.[5,64]

A number of different pathways for the communication among the various components in the microbiota-gut-brain circuitry (**Figure 4**) have been proposed. For example, gut microbiota stimulates production, expression and turnover of neurotransmitters (e.g., GABA and serotonin) which can convey signals from the enteric sensory system to the brain and influence its behavior. These mediators can also influence the integrity of intestinal barrier and tight junction which, in turn, regulate gut permeability and mucosal immune response. Bacterial metabolites like SCFAs are known to have significant impact on CNS and peripheral immune activity.[60,61] In an opposite direction, stress and emotion signals from the brain can modulate production of mucus, regulate biofilm formation and production of antimicrobial peptides, as well as regulating intestinal permeability and motility. Noradrenalin produced and released in the GI tract can affect bacterial gene expression which may have some impacts on bacteria-bacteria communication.[1,13,33,60,61] Such a mutual interaction can in turn alter the composition, diversity and richness of gut microbiota.

The findings that have attracted my attention into this exciting new field of biomedical science is the evidence suggesting that alteration of gut microbiota might be a causative factor in autistic patients and autistic-like mice and that by restricting the diets and/or antibacterial treatment, some improvement was noted.[63,65] Subsequent studies have convinced me further that the link between neurological diseases like autism and stress-related behaviors with gut microbiota is not just a speculation, but it is a real phenomenon waiting to be reconfirmed by carefully designed and well-controlled animal and human study.[14,66,67] The interconnection of microbiota-gut-brain network with the immune system and nutrition has now turned out to be another very interesting but neglected research area. This complex network of interactions and its potential implications in the management of neurological and psychological diseases has attracted considerable interest from not only investigators in the scientific circle, but also from a general public as demonstrated in an article entitled "Can the bacteria in your gut explain your mood?" that appeared in The *New York Times Magazine* last year.[68]

Gut microbiota and neurological disorders

It has been observed several years earlier that neurological problems including psychiatric disorders and stress-related behavior often coexist with some gastrointestinal conditions which are now known to be associated with alterations of gut microbiota. Epidemiological, clinical and experimental animal studies reveal the existence of communication pathways between gut and brain that depends on signals from neural, hormonal and immune systems and from the microbiota itself (**Figure 4**). Gut microbiota is known to possess properties that can either suppress or enhance the activity of HPA axis.[14,60,61,69] These microbes can directly signal the afferent limb of vagus nerve or can produce neurotransmitters and neurochemicals, e.g., GABA, serotonin, noradrenalin, dopamine and acetylcholine that can gain access to the brain and affect its behavior. Bacterial metabolites like SCFAs are also known to have impact on host response.[13,14,60,61] In an opposite direction, exposure to physical and psychosocial stress can have substantial impact on gut microbiota but the molecular mechanisms operated in this microbiota-gut-brain network need to be further dissected (**Figure 4**). Much of the information in this research area have been obtained from animal studies, particularly from GF animals and therefore need to be validated in humans. The gap of information between animal studies and clinical studies in humans must be narrowed down before we can apply this knowledge in a practical sense. Targeting the microbiome could prove to be a powerful tool and more cost-effective for the future treatment of some neurological disorders and mental health problems.

1. Impact of microbiome on mood, anxiety and depression. Although there are emerging exciting data showing a link

between gut pathology and/or altered microbiota with various neurological conditions (e.g., anxiety, loneliness and depression, autism and other neurodegenerative disorders), this is just an association; solid data are rather limited, particularly in humans. Disruptions of the microbiome and gut ecosystem as seen in a GF mouse model induce changes in their behavior that mimics human anxiety, depression or even autism, and these behavioral changes can be readily reversed by colonizing them with appropriate strains of microbes. Much of what has been said is just a speculation and now we are only at an exploratory stage. A number of people are still skeptical about the conclusion on a link between microbes and behaviors and whether or not it would have any significant impact on human health remains to be proven. It was shown several years earlier that stress could induce an exaggerated HPA response in GF mice, judging from enhanced production of corticosterone and ACTH compared to the controls.[14,60,61,68,69] The brain chemistry of these animals was also different from that of controls. These alterations, together with the aberrant stress response, could be reversed following monocolonization of these GF animals with *Bifidobacterium infantis*.[14,60,61] Probiotics containing appropriate species of bacteria can lower cortisol output in urine from healthy human volunteers, hinting at a value of probiotics in the management of these neurological disorders.[13,32,33] It has been shown also that the stress phenotype could be induced in conventional wild type animals with microbiota transplanted from the animal with a similar phenotype, thus confirming the previous studies which demonstrated that gut microbiota is essential for the development of normal neuronal circuitry.[31-33,66-68] Taken these information altogether, it is fair to conclude that the composition and diversity of gut microbiota can have significant impact on stress and emotion in animals and humans, and vice versa. However, one should not forget that the fluctuations in microbial composition and metabolic activity are also subjected to environmental factors that are difficult to control, e.g., dietary changes, antibiotic usage and lifestyle of the host.

2. Influence of microbiota on cognitive function. There is scattering data to support the viewpoint that microbiota and its metabolites can also influence cognitive activity of the host.[1,13,14,60,62] GF animals displayed cognitive defects compared with conventional controls, suggesting a possible impairment of hippocampal development.[5,14,33,66-68] Elderly human volunteers with impaired cognitive function, as in those with Alzheimer's disease, have altered microbiota profile, showing low diversity compared with those without cognitive dysfunction.[14,70] However, it remains to be determined whether or not there is a causal association between degree of microbial diversity and cognitive decline. Current evidence however only allow one to speculate that abnormal microbiota profile can modulate the inflammatory response in the CNS, a process known to be associated with some psychological disorders and age-associated neurological diseases.[70,71]

3. Potential influence of microbiota on social and stress-related behaviors. Data from studies on maternal separation of newborn rodents show that this neonatal stress can lead to long-term changes in the diversity and composition of gut microbiota. It was reported that maternal separation was associated with increased *Clostridium* and decreased *Bacteroides* in the fecal specimens of these animals.[1,5,14,33,60] Giving probiotics (e.g., *Bifidobacterium* and *Lactobacillus* species) to these animals during the early stage of life can reverse these changes, judging from normalization of the exaggerated corticosterone levels after treatment.[33,60,68] Reconstitution of microbiota in GF animals for example with *Bifidobacterium infantis* can also normalize the stress response and behaviors of the animals.[14,60,68] These studies, together with those reported elsewhere, permit one to conclude that gut microbiota have the capacity to regulate the functions of HPA axis from the very early stage of life as well as to control stress reactivity over the whole lifespan of the animals.[69] Induction of gut inflammation by microbes, parasites or chemicals (e.g., dextran sodium sulfate) is known to be associated with increased anxiety-like behavior that can be reversed by probiotic treatment. However, with the exception of those reported for autism, there is only limited information on the impact of microbiota on social behaviors, even though the role of microbiota in the development of sociability in animals has been proposed several years earlier.[33,62,66,68] Well-controlled studies on the relation between microbiota and autistic-like behaviors in an animal model have been reported only recently.[63,66-68] In one such study, an alteration in the composition of microbiota noted could be readily reversed by *B. fragilis* treatment, thus suggesting that it may be possible to manipulate a social behavioral response by manipulating gut microbiota, at least in the rodent model. In agreement with the reports in animals, alterations of the microbiota profile have also been noted in autistic patients.[1,5,33,65] For example, there was a reduction of bacteria in the phylum Bacteroidetes (e.g., *Bifidobacterium* spp.) in the gut the patients. It is logical to speculate that such an association might have been related in part to bacterial metabolites like SCFA. These observations suggest that autism, once believed to be strictly a neurological disorder, appears to have additionally some dysregulatory functions of the gut-brain-microbiome axis.[60,66] Interest on the interplay between microbiota and brain has been extended to include skeptical but interesting observations that microbes may also have considerable impact on the host eating habits and craving for foods and dietary choices to favor consumption of particular nutrients they grow best rather than passively living on what is provided, or to induce dysphoria until the host consume foods that enhance their fitness.[14,33]

Conclusions

It is now well established that our physical and mental health depends not only on our lifestyle or what we eat, but also on what we host, and therefore microbial communities, particularly those in the gut, should play a key role as regulators of our health. The science of microbiota is still in its infancy state and the greatest challenge in microbiota research remains the establishment of a causal correlation between microbiota profile and human disease. Microbiome-modulating strategies should prove to be highly beneficial in the near future, either as preventive therapy or as adjunctive treatment for some difficult-to-manage diseases. The Human Microbiome Project

initiated at the beginning of 2008 has served as a cornerstone for our current understanding and progress on human microbiota/microbiome. The research output from this Project reveals not only unprecedented diverse microbial communities that inhabit different sites of our bodies, but also shows marked variability within one individual to the next. Its composition can also change rapidly over time in a single individual, depending on variable factors like early environmental exposure, diet as well as antibiotic usage. All these studies have disclosed many exiting aspects regarding microbial communities and established the association between microbiota profile and some complex human diseases, particularly obesity and other chronic low-grade inflammatory diseases like cardiovascular disease or a disease in an oral cavity known as periodontitis.[2-5,10-14,27,72] Furthermore, the data provide us with interesting new thoughts regarding the pathogenesis of several diseases, that is, instead of trying to identify "pathogenic" microbes or "keystone" pathobionts responsible for certain disease, would it be possible to identify or to detect instead good "peacekeeping" microbes that prevent the occurrence of disease?[72-75] There are studies demonstrating a depletion of the clostridial microbe *Faecalibacterium prausnitzii* in some inflammatory bowel diseases.[12,70] This mucin-promoting bacterium represents an anti-inflammatory member of resident microbiota, revealed by its capacity to make anti-inflammatory protein as well as ability to metabolize high-fiber diet to SCFAs that regulate anti-inflammatory signals.[73-75] A shift in the microbial community (e.g., a loss of key anti-inflammatory microbes and weakening of their peace-keeping function) can contribute to hyperactive immune response via influence on Treg cells as well as on Th17 effector cells, thus leading to a possible increased occurrence of autoimmunity. Thus, the balance of commensal bacteria in the gut microbial ecosystem is essential for the maintenance of epithelial homeostasis. If disturbed, this may result in dysbiosis, for example, as seen following routine use of antibiotics. Drug-induced alterations of the microbiome can also influence the effectiveness of certain drugs as well as side-effects that they cause. It remains hopeful that one day in the near future, we will have "targeted" antibiotics that can selectively kill only the pathogens without simultaneously taking away good peace-keeping symbionts in our gut.

The increased prevalence of metabolic syndrome and other inflammation-related diseases as well as some neurological

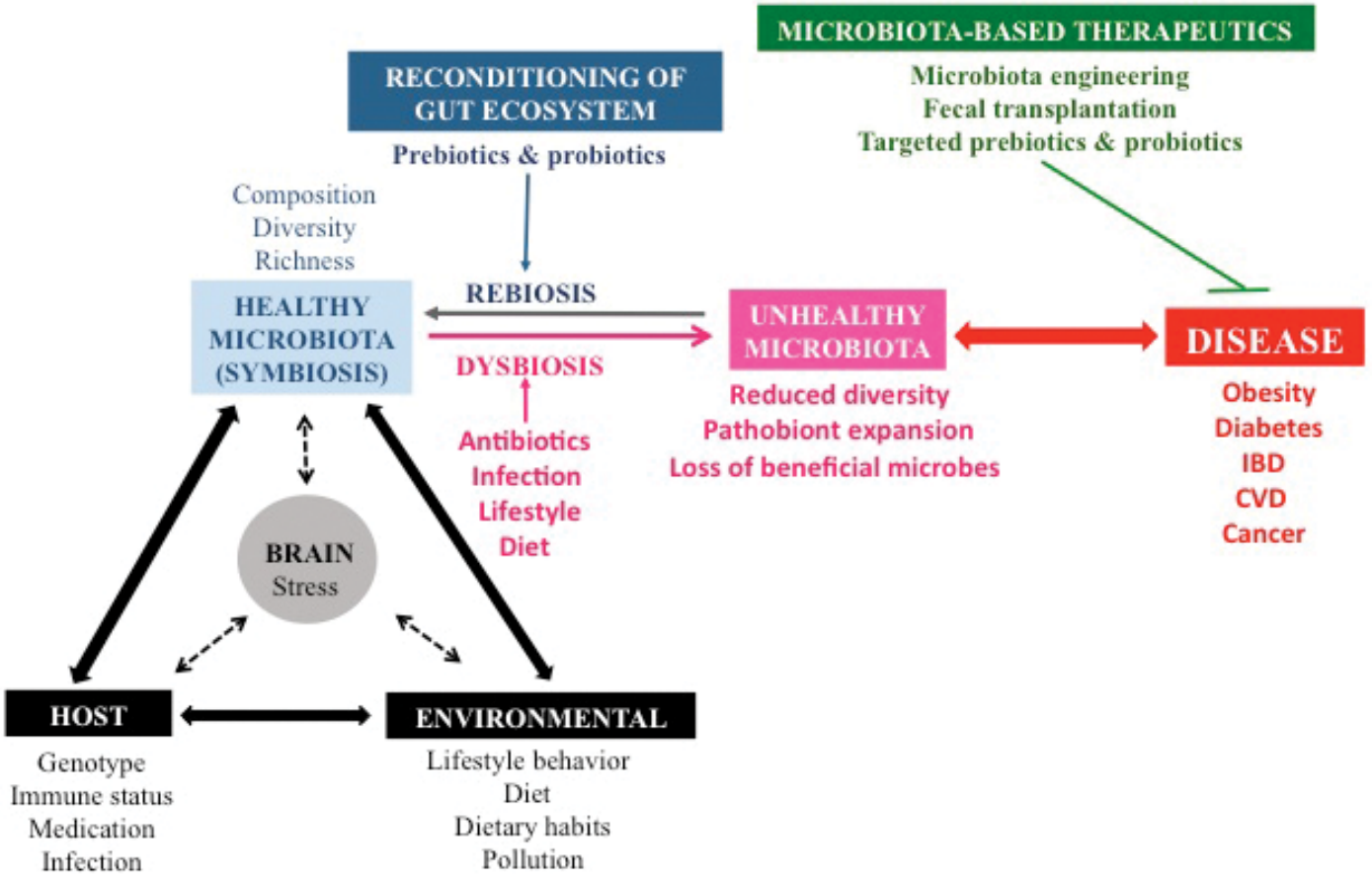

Figure 5. Proposed microbiota-based therapeutics to reestablish (rebiosis) gut homeostasis. At steady state, host, environment and brain interact to achieve and maintain a healthy balance microbiota status (symbiosis). This network also crosstalks with central nervous system. When the balance is disturbed, such as, via antibiotic usage and unhealthy diet, there is a shift to unhealthy status (dysbiosis) which may end up with associated diseases. It is possible to rehabilitate the gut ecosystem back to a healthy status simply by using targeted prebiotic and probiotic supplementations. Several microbiota-based approaches like microbiota engineering and fecal transplantation are possible to treat some of these illnesses.

disorders is becoming a major health problem worldwide and this has triggered a surge of research interest in this area over the past decade. Although host genotype is known to play a role in the pathogenesis of these diseases, environmental factors, for example, diet and dietary habits appear to be crucial in the development and progression of the diseases, particularly in those associated with metabolic syndrome. Different lines of evidence from human and animal studies reveal a profound impact of microbiota on human health and a shift in the diversity of gut microbiota has been documented in obesity and CVD. Difference in the gut microbiota composition between obese and lean hosts has been reported in both humans and animals.[52-54] In obesity, the weight of evidence suggests that the association is one of a causal relationship and the change is not a consequence of the disease. However, with the exception of *Akkermansia muciniphila*,[56] it is not possible at this time to identify with high degree of precision specific gut microbes that are either positively or negatively correlated with each disease phenotype. One complication, particularly in the analysis and interpretation of the large and complicated metagenomics data generated from these studies, is a well-known impact of diet directly on the composition of gut microbiota. Both the diet and microbiota are known to have considerable influence on the development and function of immune system which can in turn regulate the final outcome of diseases (**Figure 5**).[40-42] Therefore, a thorough understanding on the basic crosstalk in microbiota-diet-immune network is a prerequisite knowledge needed before it is ready for clinical implications. A possible involvement of microbiota in neurological diseases and psychological or stress-related behavior and disorders including autism has emerged over the past decade. In fact, the reported improvement of clinical conditions in autistic children taking antibiotics is consistent with the possible causal involvement of microbiota in this illness.[33,60,65] A communication between gut microbiota and brain via a so-called microbiota-gut-brain axis has emerged as a new exciting frontier of biomedical science.

Microbiota-based therapeutics for the management of diseases in humans remains an attractive possibility, but we need to have the currently available animal data transformed and validated in humans. Reconditioning gut microbiota profile is probably more simple and more cost-effective approach than, for example, developing new drugs or gene therapy. The current approach using conventional prebiotics and probiotics as food supplement to rehabilitate gut homeostasis is now a common practice for a number of human diseases (**Figure 5**). Altering gut microbiota via dietary intervention for brain dysfunction or mood disorders is not beyond the realm of possibility (**Figure 4**). The recent report on the value of *B. fragilis* in alleviating autism-like symptoms in animals is highly encouraging.[67,68] Future improvement of this approach is to identify and select specific microbes (e.g., those known as "clostridial cocktail" experimentally used to rehabilitate the gut of GF animals) or to create genetically modified microbes to be included in the next-generation probiotic supplements to maximize the treatment. Fabrication of microbes with desirable properties is not impossible, as the synthesis of new organism containing a few hundred genes is now achievable. It should be possible for instant to improve the next-generation prebiotics by adding new fermentable fibers and dietary nutrients to promote growth of stunted infants.[22] It has been reported, for instance, that adding sialylated milk oligosaccharides to diet can readily promote growth of undernourished infants by enhancing rehabilitation of healthy microbiome, e.g., *F. prausnitzii* and *B. thetaiotaomicron*.[22,75] Another approach of microbiota-based therapeutics currently under clinical trial and reported to be highly successful is fecal transplantation for the treatment of recurrent antibiotic-resistant *Clostridium difficile* infection.[34]

Lastly, it is not too far-fetching that in the near future, we will be able to colonize our gut with genetically modified "intelligent" bacteria that can detect and eliminate disease of the GI tract at the earliest possible moment. These "smart" bacteria, created through microbiome engineering, can theoretically sense local environmental changes, make decisions based on the information perceived and then trigger appropriate responses to correct pathological conditions. In other words, these "smart" bacteria will be able to perform desired tasks like facilitating diagnosis, prevention, prediction and treatment of complicated gastrointestinal diseases, and possibly as well as of those occurring at distant sites like respiratory tract, heart and brain. One can imagine further that in the near future there will be a genetically modified bacterium that can synthesize and secrete, say, anti-inflammatory cytokine-like molecules when inflammation is detected and would automatically shut off the production when the inflammation is eliminated. Furthermore, one could additionally insert a "kill" switch into the bacterial gene that we can turn it on in case this bacterium goes out of control. We can even go one step further to create bacteria that will help the host combating incoming pathogens, diagnosing early stages of cancers, correcting diarrhea and other GI tract problems or even regulating our mood and behavior. I understand that there are some ongoing experiments being carried out to create "smart" bacteria using resident microbe like *Bacteroides thetaiotaomicron* as a model.[75,76] The microbiota-based therapeutics together with personalized medicine may one day be the most effective strategy to maintain good health of our population. Advanced molecular and genetic engineering technologies together with a rapid progress in human metagenomics and bioinformatics will make it possible to systematically analyze and integrate complicated data generated from this complex microbiome-gut-diet-brain-immune circuit and to advancing our microbiota engineering strategy . Not being a molecular biologist and rather naïve in molecular technologies, I would opt to choose microbiota engineering or rehabilitating gut microbiota with the next-generation prebiotics and probiotics or fecal transplantation rather than the more sophisticated human gene therapy approach to improve or promote human health (**Figure 5**). However, I will not be surprising that in the near future technologies for the latter approach will become more attractive and more simple to perform, for instance, with the availability of the newly described CRISP-Cas9 gene-editing system. Currently, the research in microbiota to promote our health remains a relatively unexplored area of investigation. In fact, I feel that research on the contributions of gut microbiota and its crosstalk with diet and host immune system to improve human health is now one of the most exciting frontiers in

biomedical science in the coming decades.

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