Perspective

Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications

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Indigenous microbiota are an essential component in the modern concept of human health, but the composition and functional characteristics of a healthy microbiome remain to be precisely defined. Patterns of microbial colonization associated with disease states have been documented, but the health-associated microbial patterns and their functional characteristics are less clear. A healthy microbiome, considered in the context of body habitat or body site, could be described in terms of ecologic stability (i.e., ability to resist community structure change under stress or to rapidly return to baseline following a stress-related change), by an idealized (presumably health-associated) composition or by a desirable functional profile (including metabolic and trophic provisions to the host). Elucidation of the properties of healthy microbiota would provide a target for dietary interventions and/or microbial modifications aimed at sustaining health in generally healthy populations and improving the health of individuals exhibiting disrupted microbiota and associated diseases.

Introduction

The nature of microbial colonization of humans is being increasingly understood through regional microbiome projects that are linked as a single global network, such as the International Human Microbiome Consortium, the European Commission's Metagenomics of the Human Intestinal Tract project, the US National Institutes of Health's Human Microbiome Project, and the Canadian Microbiome Initiative, among others. These projects are focused on the identity, genetic potential, and metabolic activities of microbes (bacteria, viruses, archaea, and eukaryotes) associated with numerous body sites. Although there is agreement that microbes are important to human health, with the exception of defined pathogens, the roles that these microbes play in health and disease remain to be fully elucidated. Different patterns of microbial colonization associated with disease states compared to healthy controls have been documented (Table 1), although a causal relationship has not been established. However, the patterns of microbial colonization associated with health are more difficult to define. Definition of healthy microbiota would provide a target for interventions aimed at sustaining health in the generally healthy populations and improving the health status of people exhibiting disrupted microbiota and diseases associated with these disruptions. However, the definition of a healthy human microbiome is not yet defined.

This Perspective focuses on key concepts related to defining a healthy gut microbiome. Briefly, it addresses how a healthy microbiome is defined and the current evidence that relates the microbiome to human health. Many factors must be addressed before considering dietary or therapeutic interventions that target the microbiome. The microbial composition, metabolic activities (Figure 1A), host responses to microbes (Figure 1B) including genetic susceptibility to disease, and environmental factors (such as diet) that may influence host response may all impact the success of any intervention. A better understanding of factors that shape microbiome structure and function can result in defining specific microbial community properties that can be targeted through interventions with diet, functional foods, chemicals (drugs), or live organisms. Pathways to achieve this goal are discussed, including how the "health" of a gut microbiome might be assessed. Although the technical aspects of sampling and analysis are important considerations in defining a healthy microbiome, those topics are beyond the scope of this paper. The reader is referred to several excellent references regarding human sampling (Grice et al., 2009; Jalanka-Tuovinen et al., 2011; Mai et al., 2011; Saulnier et al., 2011) and analysis (Kuczynski et al., 2012).

Definition of a Healthy Microbiome

How is a healthy microbiome defined? From the ecologic standpoint, the stability of a community (bacterial or otherwise) can be thought of as a functional property descriptive of the health of that community. Stability refers to the ability of a community to

Table 1. Dysbiosis Associated with Intestinal and Systemic Diseases

Dysbiosis-Associated Diseases or Conditions
Obesity
Metabolic syndrome
Nonalcoholic steatohepatitis
Inflammatory bowel diseases (Crohn's disease, ulcerative colitis, pouchitis)
Irritable bowel syndrome, functional bowel disorders
Atherosclerosis
Type 1 diabetes
Autism
Allergy
Asthma
Celiac disease

See reviews by Bäckhed et al. (2005), Honda and Littman (2012), Ringel and Carroll (2009), and Sartor (2008, 2010).

resist change in the setting of an ecologic stress (resistance) or to return to an equilibrium state following a stress-related perturbation (resilience). These concepts of resistance and resilience as key features of healthy microbiome are consistent with established views of microbial ecology. However, the concept that a healthy microbiome can be defined by some idealized community composed of defined populations of specific microbes is too simplistic in light of the consistent interindividual differences observed in many studies. An alternative conceptualization is an idealized collection of genes and pathways rather than specific populations; although, a "healthy" set of metabolic functions remains to be defined (Nicholson et al., 2012). A recent survey of 4788 samples collected from 242 healthy adults noted marked interindividual variations in fecal bacterial communities, but metabolic pathways were conserved (Human Microbiome Project Consortium, 2012a).

Certain microbial distributions may make a person more susceptible to infection or disease. For example, alteration of the indigenous gut microbiota by antibiotics can put an individual at risk for developing infections from an opportunistic pathogen, such as *Clostridium difficile*. From animal studies, it is known that different microbial populations can dramatically affect susceptibility to chronic inflammation (Ferreira et al., 2011; Sekirov et al., 2010; Willing et al., 2011b; Wlodarska et al., 2011). The presence of microbes that convert luminal compounds into potential carcinogens also puts one at increased risk for cancer and can lead to adverse responses to chemotherapeutic agents (Wallace et al., 2010). The lack of sufficient diversity or evenness in a bacterial community structure appears to diminish its ability to withstand perturbation (Virgin and Todd, 2011). Hosts with such bacterial communities may not exert overt disease in most environments. However, their bacterial communities may be considered less than optimal for preventing disease, and these individuals may be more susceptible to developing different diseases. An emerging paradigm is that diseases such as obesity and inflammatory bowel diseases (IBD) (Turnbaugh et al., 2006; Manichanh et al., 2006; Sartor, 2010) are associated with reduced diversity in the intestinal microbiome, which may represent evidence of a suboptimal microbiome.

Much of the initial research focus on the microbiome has been on understanding disease (Chang et al., 2008; Giongo et al., 2011; Sepehri et al., 2007; Young et al., 2011). As detailed below, the typical design of such a study is to compare the composition of the microbial community obtained from a person with a given disease and that of a control microbial community. This control specimen could be obtained from a person without such a disease. In some cases, an additional specimen can be obtained from the individual with the disease, but from a site either temporally or spatially distinct from the "disease" specimen. The concept of dysbiosis arose from such studies, and altered microbial communities were defined as dysbiotic in the setting of the disease. However, current evidence is insufficient to distinguish between dysbiosis as a cause or consequence of the disease. Thus, prospective studies are needed.

A recently proposed definition of human health suggested that health can be considered "a dynamic state of wellbeing characterized by a physical, mental, and social potential, which satisfies the demands of a life commensurate with age, culture, and personal responsibility" (Bircher, 2005). There are several important aspects of this definition that can be applied when considering microbiome health. The first is the idea that health is a dynamic state. Many recent studies of the role of the microbiome in human health follow the dynamics of microbial communities (Dethlefsen and Relman, 2011; Frank et al., 2010; Hartman et al., 2009; Human Microbiome Project Consortium, 2012a, 2012b; Morowitz et al., 2011). Following temporal changes in the microbiome and (presumably) the concomitant changes in microbial gene expression, especially as they relate to the overall dynamics of an individual's health status, can lead to testable hypotheses regarding the role of a given microbial community in bodily function and disease pathogenesis. In addition, characterization of the state of health as satisfying the demands of life places a primacy on the functional aspects of the microbiome and the genes they encode. Multiple microbial community populations are likely to be able to satisfy the demands of life, reflected in nearly equivalent function. This point is supported by recent data showing that differences in microbial composition at different body sites are contrasted by the relative conservation of metabolic or functional modules in the human microbiome (Human Microbiome Project Consortium, 2012b).

Core Microbiome and Enterotypes

When considering the gut environment, it is essential to recognize that it is not one homogenous environment; it reflects substantive differences in microbial community structure along both the axial (mucosal to lumen) and longitudinal (proximal to distal) gradients of the gastrointestinal tract. Our insights to date extend from studies done primarily on extensive research of fecal samples as a surrogate for the gut. The concept that all humans are populated by a core microbiome has recently been addressed and suggests that we all share some of the same microbes (Human Microbiome Project Consortium, 2012b). Qin et al. (2010) demonstrated that 18 gut bacterial species were shared among 124 individuals from Denmark and Spain. Furthermore, 57 bacterial species were found to be present in >90% and 75 in >50% of the individuals. In contrast, a core microbiome was not found in a recent study, although 46% of subjects had detectable *Bacteroides thetaiotaomicron*
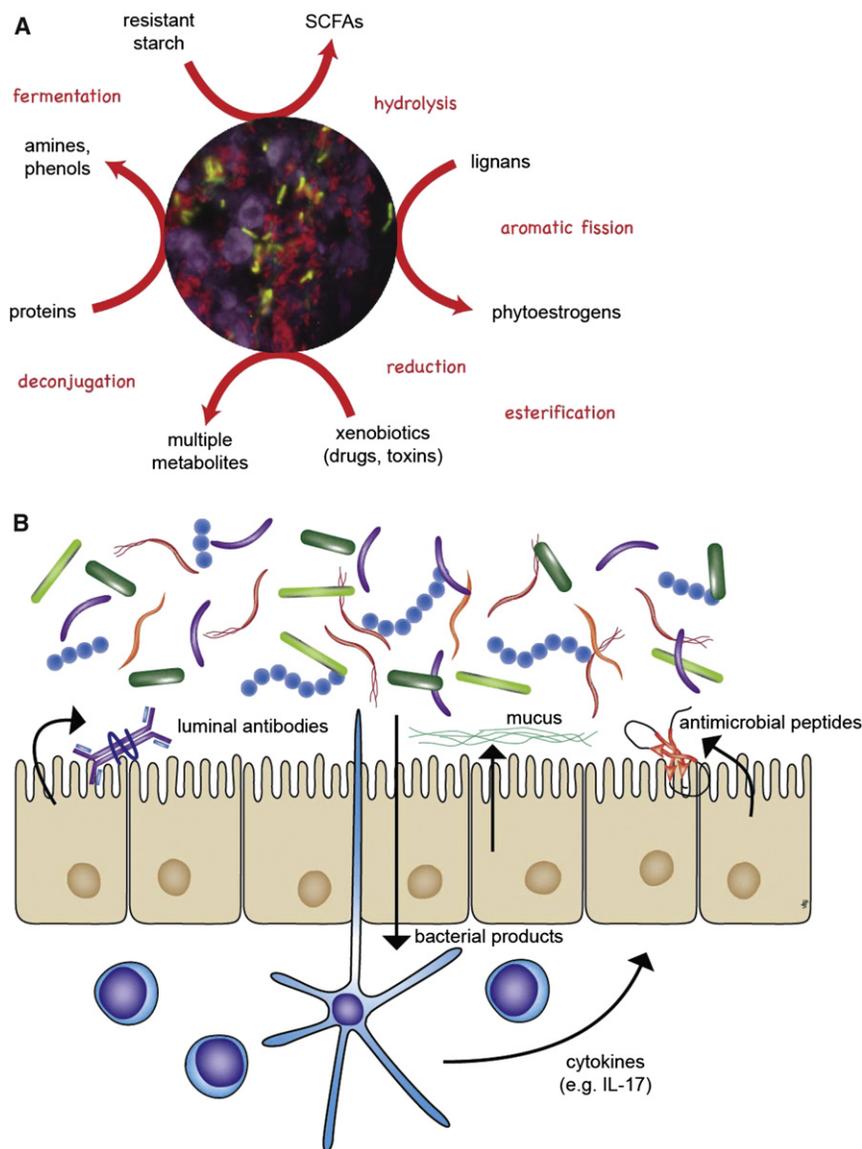
Figure 1. Metabolic and Immunologic Functions of the Indigenous Gut Microbiota

(A) The gut microbiota can carry out a suite of biochemical activities that can convert luminal compounds to secondary metabolites. These conversion reactions (examples listed in red) can alternately detoxify ingested toxins but in other cases can result in the production of compounds that can be deleterious. The specific composition of the gut microbiota can thus determine the balance between beneficial and harmful chemical conversion reactions in the gut lumen.

(B) In an analogous manner, the gut microbiota can signal to the host immune system and gut epithelium to set the tone of mucosal immunity. In response to the gut microbiota, the host will secrete a variety of cytokines and host defense effector molecules that can in turn shape the indigenous microbiota community and prime host responses to environmental stimuli.

but can shift with changes in age, diet, geographical location, intake of food supplements and drugs, and likely other causes as well (Yatsunenko et al., 2012). Accordingly, distinct developmental states may have unique core microbiomes (Segata and Huttenhower, 2011), but the role of these different colonization patterns in maintaining health is not yet known.

Recently, the concept that all humans can be divided into one of three discrete gut enterotypes based on the composition of the microbiota was proposed (Arumugam et al., 2011). Enterotypes appear to be independent of gender and nationality; however, a recent study suggested that long-term food preferences may contribute to formation of different enterotypes (Wu et al., 2011). The function and implications of different enterotypes are still unknown, and enterotype boundaries may be less clear than initially suggested. For example, analysis of the gut microbiota in >310 Old Order Amish subjects reveals three major bacterial networks resembling the three known enterotypes (Arumugam et al., 2011) but also exhibited overlap in community structure (Zupancic et al., 2012) (Figure 3). These data suggest a more straightforward view of gut bacterial community structure driven by greater abundance and high variability in populations of *Prevotella* and *Bacteroides* against a diverse background assemblage of Firmicutes. *Prevotella* and *Bacteroides* appear to coexist at lower levels if the community is predominant in Firmicutes, but these two Gram-negative genera are nearly mutually exclusive when either is abundant in communities that are *Bacteroidetes* predominant. Further research will be required to determine if discrete enterotypes or more fluid "enterogradients," describing a continuum of microbial community structures, better describe the relative composition of human microbiomes. Further research will also reveal if different enterotypes have specific core microbiomes
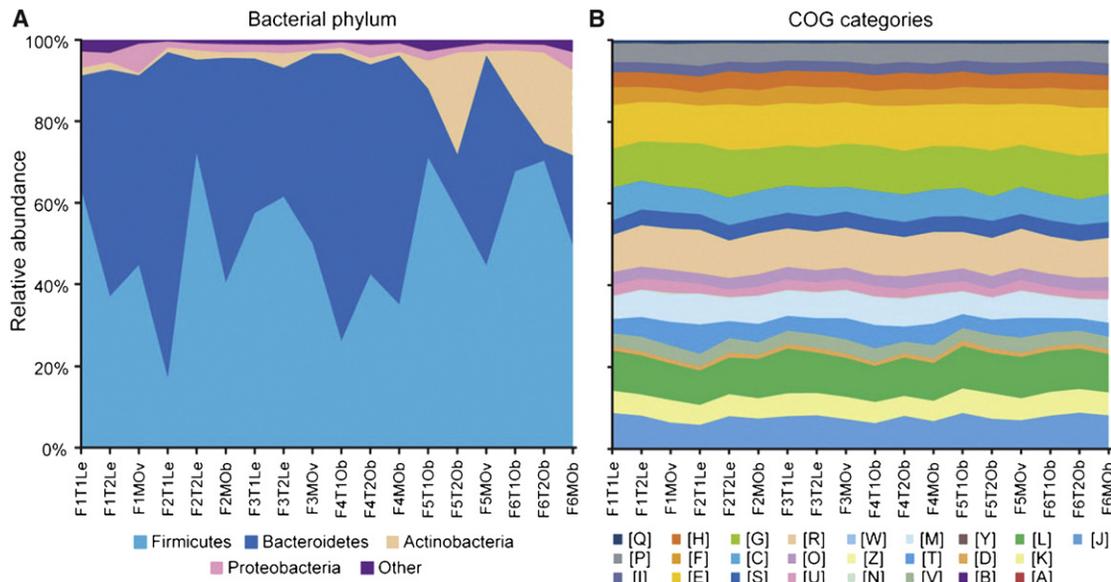
(Human Microbiome Project Consortium, 2012b). The concept of a core microbiome depends on the definition and inclusion criteria. For example, current conceptualization does not consider abundance, although it is evident that the relative quantities of specific bacterial species vary greatly among individuals (>2000-fold) (Qin et al., 2010; Turnbaugh et al., 2009). The core microbiome concept targets the most prevalent bacterial species, while several less-abundant bacterial species in the feces may reach high local densities in the gut (e.g., in the mucus niche) and may provide the host with fundamental functions at a particular site. By contrast, genes encoding specific metabolic functions, or clusters of orthologous groups, are similar among individuals and thus provide evidence for a functional core microbiome or core microbiome-encoded gene set (Turnbaugh et al., 2009) (Figure 2). The core microbiome (based on clusters of orthologous groups) may differ across continents, ethnicities, diets, or other factors (Human Microbiome Project Consortium, 2012b). The colonizing microbiota are established early in life

Figure 2. Disparity Between Microbial Community Composition and Function among Individuals
As seen through relative abundance of major phyla across 18 fecal microbiomes from monozygotic twins and their mothers compared to relative abundance of categories of genes (reproduced with permission from Turnbaugh et al., [2009]).

exhibiting altered resilience to perturbations and disease predisposition.

Structure and Function in Characterizing the Microbiome

Microbial community structure relates to the numbers and types of microbes present, whereas microbial community function relates to the metabolic activities and end products that result from microbial activity (Nicholson et al., 2012). Although both structure and function of microbial communities are important and are likely to be strongly correlated, function may be the more important measure of microbiome health. Evidence from bacterial ecology suggests that similar ecosystems have similar function but may have quite diverse compositions. Although bacterial composition of the gut varies widely among different subjects, the distribution of different functional genes of the microbiome is fairly constant, suggesting that it is more important that key functions in the gut are carried out rather than specific microbes be present to carry them out (Human Microbiome Project Consortium, 2012b). Because the microbiome is also transcriptionally regulated, it will be essential to apply metatranscriptomics and metaproteomics to fully understand the functional capacity of the gut microbiota.

It can be demonstrated that specific members of a bacterial community can play an important functional role in the realm of resistance to infection. The ability of specific resident microbes to occupy host niches and mount an effective resistance to pathogens may depend on a unique functional activity. For example, transfer of fecal microbes, and specifically one species of *Prevotella*, conferred resistance to *Citrobacter rodentium* infection in a mouse model of infectious colitis (Willing et al., 2011b). In addition, different members of the mouse microbiota were associated with successful *Salmonella typhimurium* colonization and colitis, and in particular *Porphyromo-*

nadacea was associated with protection against *Salmonella* (Ferreira et al., 2011).

Microbiome Stability

Canadian ecologist C.S. Holling first introduced the concept of resilience in ecological systems (Holling, 1973). This concept has been defined in two ways in the ecological literature: First, as the time required for an ecosystem to return to an equilibrium or steady-state following a perturbation; and second, as "the capacity of a system to absorb disturbance and reorganize while undergoing change so as to retain essentially the same function, structure, identity, and feedbacks" (Holling, 1973). In this article, we have included the concept of resilience along with resistance under the term stability. As in other ecosystems, the ability of the human gut microbiota to remain stable in the face of continuous and potentially disruptive perturbations is likely important for health. Longitudinal studies of the human gut microbiota based on 16S rRNA analysis have suggested that bacterial community structure is relatively stable over time in the absence of perturbations (Costello et al., 2009; Franks et al., 1998; Human Microbiome Project Consortium, 2012b; Zoetendal et al., 1998). However, extrinsic factors, diet, and exposure to antibiotics in particular cause significant changes in the gut microbiota. The long-term effects of such external influences on the health of the gut microbiota are still to be determined. Table 2 lists factors that perturb the microbiota, whereas factors that promote stability of healthy microbiota are discussed by Spor et al. (2011).

Both molecular- and cultivation-based approaches have revealed ecological disturbances in the microbiota after antibiotic administration (Dethlefsen and Relman, 2011; Jernberg et al., 2007). Specific members of the bacterial community that are particularly susceptible or resistant to the antibiotic in question contribute significantly to the antibiotic-associated gut microbome perturbations. In addition, antibiotic-resistant strains can
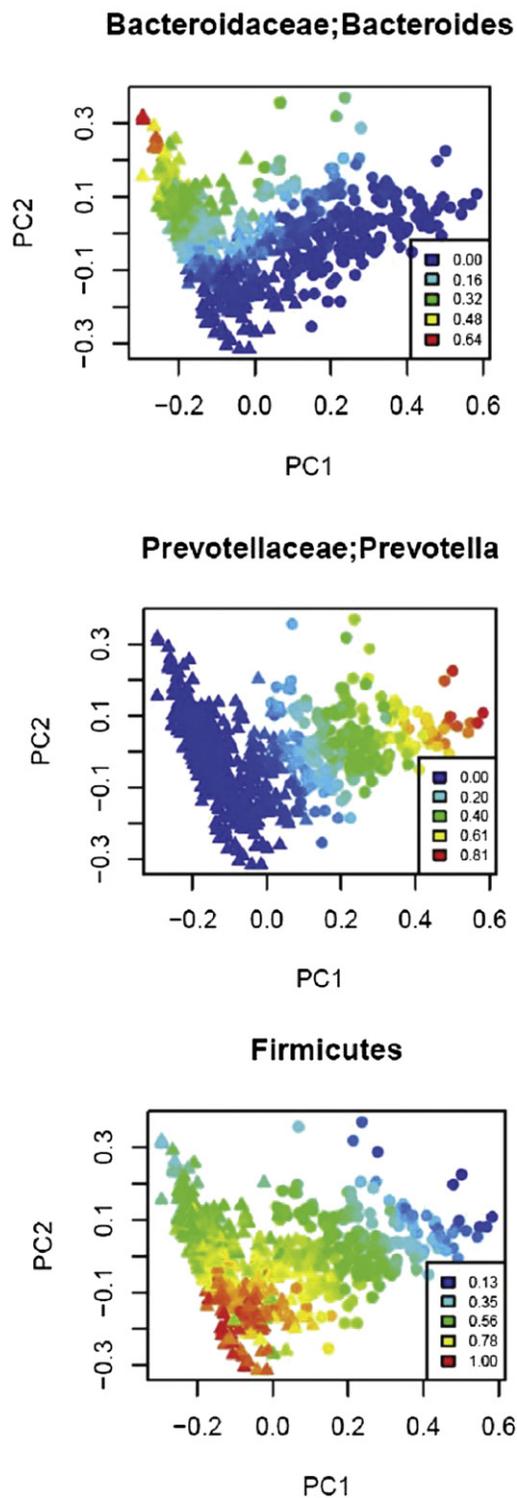
Bacteroidaceae;Bacteroides

Prevotellaceae;Prevotella

Firmicutes

Figure 3. Principal Component Analysis of 16S rRNA Pyrosequencing Data from 310 Old Order Amish Subjects
Each circle represents a single individual, and the data in each panel are identical but have been color-coded based on the relative abundance of *Bacteroides* (phylum *Bacteroidetes*) (top panel), *Prevotella* (phylum *Bacteroidetes*) (middle panel), or *Firmicutes* (bottom panel). From this analysis, it is apparent that the subjects do not cluster into three distinct enterotypes but instead represent a continuum with regard to the relative abundance of dominant taxa (reproduced with permission from Zupancic et al. [2012]).

Table 2. Factors That Can Influence the Gut Microbiota

Factor	Evidence from	Selected References
Mode of fetus delivery	Humans	Dominguez-Bello et al. (2010), Palmer et al. (2007)
Geographic origin	Humans	De Filippo et al. (2010)
Host genotype	Humans	Spor et al. (2011) and references therein; Li et al. (2012)
	Mice	Kovacs et al. (2011), Ley et al. (2005), Benson et al. (2010)
Diet	Humans	Walker et al. (2011), Wu et al. (2011)
	Animals	Turnbaugh et al. (2008), Hildebrandt et al. (2009), Turnbaugh et al. (2009)
Antibiotics	Humans	Willing et al. (2011a), Jernberg et al. (2007), Dethlefsen and Relman (2011)
	Mice	Yap et al. (2008), Cani et al. (2008)
Probiotics	Humans	Rauch and Lynch (2012) and references therein
	Animals	Garcia-Mazcorro et al. (2011)
Age	Humans	Tiihonen et al. (2010), Biagi et al. (2010)
Stress	Humans	Konturek et al. (2011) and references therein

persist in the human host environment even in the absence of selective pressure (Karami et al., 2007; Sommer et al., 2009). Although typically short-term, the impact of some antibiotics on the human microbiome is documented to persist for extended periods of time (Blaser and Falkow, 2009). These long-term imbalances to the microbiome may increase the individual's susceptibility to infections and disease. Excessive use of antibiotics could be fuelling the dramatic increase in conditions such as obesity, type 1 diabetes, IBD, allergies, and asthma, which have increased dramatically in many populations over the past few decades (Blaser and Falkow, 2009). Another disturbing consequence of antibiotic exposure, occurring either through directed antibiotic treatment regimens or exposure to antibiotic residues in the environment, has been the long-term persistence of antibiotic resistance genes in the human gut. Taken together, these data warrant prudence in the administration of antibiotics that could be altering the structure and function of healthy gut microbiota.

That microbial community stability is important in maintaining health is indirectly supported by the nonbeneficial consequences of altering the normal intestinal ecosystem. Consistent with this, alterations of the indigenous microbiota have been associated with many diseases (Table 1). For example, disrupting microbial colonization by antibiotics is frequently associated with diarrhea, altered gastrointestinal physiology, and abnormal carbohydrate metabolism. Antibiotic-mediated disruption of the microbiome may also result in the proliferation of *C. difficile* and can lead to aggressive bacterial toxin-induced colitis (Reeves et al., 2011). Antibiotic use in low-birth-weight neonates can contribute to the onset of necrotizing enterocolitis (NEC), a disease in which microbial dysbiosis is considered to directly contribute to disease onset. Likewise, epidemiologic studies link multiple courses of antibiotics in early childhood with increased risk of

Crohn's disease (Hviid et al., 2011). Although the tools for measuring perturbations in gut microbiota exist, the extent to which maintenance of microbial community stability is a causal, relevant measure of the health of the microbiome remains to be confirmed through studies tracking both physiological and clinical measures in addition to gut microbiota profiles.

Current Evidence on Interventions to Reverse Dysbiosis

The intricate symbiotic relationship between commensal gut microbiota and their host causes physiologic functions to be disrupted when microbial composition is altered. The concept that maintaining gut microbial structure and function is beneficial is supported by the following: (1) normal gut bacteria as a group and as selected individual species and their metabolites have essential physiologic activities; (2) dysbiosis is associated with a number of infectious, inflammatory, functional, and/or nutritional conditions; and (3) manipulating the microbiota can improve or prevent some pathologic conditions.

Several interventions targeting the intestinal microbiota have been used to maintain and improve host health. These include antibiotics, probiotics, prebiotics, fecal transplantation, immune modulators, and phage therapy.

Antibiotics

Although antibiotics can disrupt the microbiota, specific antibiotics can also be used to target dysbiosis, resulting in a beneficial clinical outcome. Antibiotics are an established and effective treatment for numerous infectious gastrointestinal conditions (e.g., infectious diarrhea and *C. difficile*-associated diarrhea). However, clinical data have shown that they can also be beneficial in certain disease conditions that do not have established pathogen-associated etiology such as IBD and irritable bowel syndrome (IBS), although the precise mechanism of effect is not completely understood (Khan et al., 2011; Pimentel et al., 2011). Such an application of antibiotics may reflect their potential for reversing or attenuating the effect of dysbiosis to yield a beneficial effect, similar to what has been found in mouse model studies (Willing et al., 2011a). However, confirmation that any disease-ameliorating effect is directly due to a correction of dysbiosis, rather than direct killing of an infectious agent or a direct effect on human physiology, remains to be established. Any use of antibiotics as microbiota-shifting agents should also address concerns regarding expanded reservoirs of antibiotic-resistant pathogens.

Probiotics

Probiotics are defined as live microorganisms that are not constituents of the host microbiome but confer a health benefit to the host when administered in adequate amounts (M. Araya et al., 2001, conference). Probiotics have been studied in recent decades as a means to modulate microbial populations and functions in order to promote health and prevent or manage intestinal disorders (Guarner et al., 2008; Ringel et al., 2012).

Accumulated data from clinical trials indicate that certain intestinal disease conditions associated with intestinal dysbiosis—such as antibiotic-associated diarrhea, NEC, pouchitis, ulcerative colitis, and IBS—have yielded clinical benefits with some probiotic interventions (Ringel et al., 2012). Because probiotics largely act directly or indirectly on the intestinal micro-

biota (O'Toole and Cooney, 2008), a possible hypothesis is that some probiotics correct or reduce the effect of dysbiosis. However, the mechanisms underlying the beneficial effects of probiotics remain incompletely understood, especially as they relate to modifying the gut microbiota and associated microbial functions. A recent study of consumption of a probiotic yogurt revealed no impact on populations of fecal microbes, but identified changes in expression of microbiome-encoded enzymes, mostly related to carbohydrate metabolism (McNulty et al., 2011). Possible extensions of the probiotic concept might include use of a genetically engineered probiotic for targeted, enhanced benefits. Another possibility might be to develop multispecies probiotics representing minimal microbiomes that emulate the three enterotypes (Arumugam et al., 2011) (or a reasonable representative of each group) for use as an orally or rectally administered intervention. Such a population approach to probiotics may be more effective at enhancing bacterial community function than administration of a single probiotic strain. Alternatively, using a small number of antagonistic organisms may achieve desired effects.

Prebiotics

Prebiotics are nondigestible food components that are selectively fermented by beneficial members of the gut microbial community (Gibson, 2010). This concept addresses the notion that it is feasible to enrich specific microbial subpopulations by feeding prebiotics, typically carbohydrates. Prebiotics have the potential to change the microbiota to favor a more beneficial microbial community. Ample evidence demonstrates prebiotic-induced changes in gut microbial populations (Gibson et al., 2004), but it is unclear if prebiotic intervention promotes stability or results in population shifts that are beneficial but not reflective of the subjects' original colonization patterns. There is great potential in future research focused on the role of diet, including prebiotics, in modifying the microbiota toward a more beneficial microbial community (Brownawell et al., 2012). Toward this end, Sonnenburg et al. (2010) demonstrated selective proliferation of *Bacteroides* in response to dietary fructans in a gnotobiotic mouse model. Matching a probiotic with a prebiotic that it can selectively use as a growth substrate (forming a synbiotic) is another strategy to make dietary-induced changes in the gut microbiota. A focus on diet provides a template for broader considerations of health-promoting interventions that go well beyond a narrower focus on prebiotics. The challenge remains, however, to define the components and activities that should be targeted to promote a beneficial microbial population and overall health.

Fecal Transplantation

Whereas the use of probiotics is meant to modulate the microbiota, in some cases indirectly, an alternative approach is to directly restore a dysbiotic community through the administration of a complete, complex microbiota in the form of feces. Fecal transplantation has been promoted recently in the setting of recurrent, refractory *C. difficile* infection (Gough et al., 2011), although it should be noted that the use of fecal transplantation in the setting of antibiotic-associated colitis was proposed more than 50 years ago (Eiseman et al., 1958). In addition to this application, for which it is remarkably effective, fecal transplantation
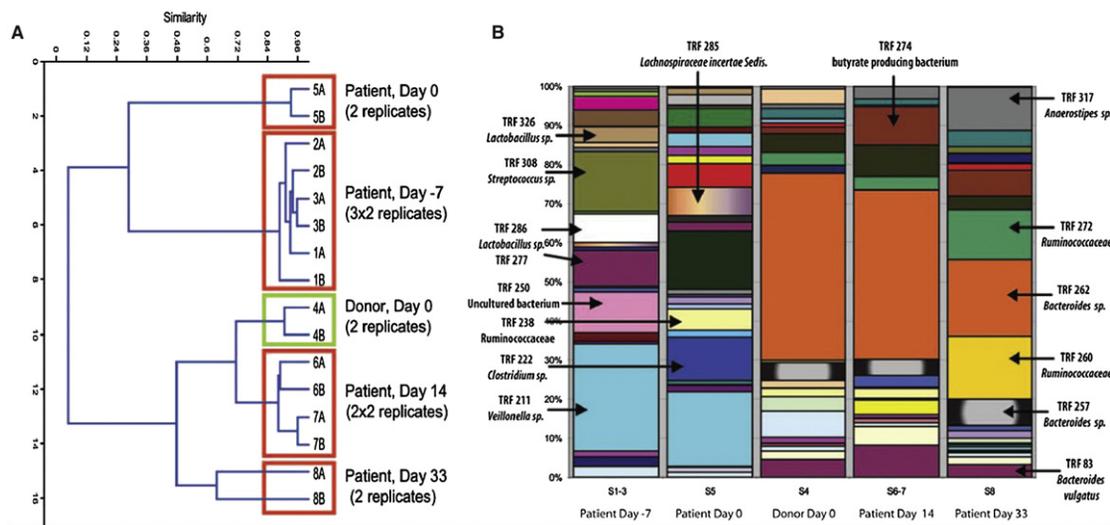
Figure 4. Changes in the Composition of the Human Fecal Microbiome after Bacteriotherapy for Recurrent *Clostridium difficile*-Associated Diarrhea

(A) Dendrogram of the 16S-based terminal-restriction fragment length polymorphism (T-RFLP) obtained from fecal material from the patient and the donor before and after fecal transplantation.

(B) Distribution of bacterial species in feces of the donor and the patient before and after fecal transplantation. Purgative wash-out occurred on day 0, shortly before fecal transplantation. The similarity of the intestinal microbiota of the recipient and donor following bacteriotherapy suggests that the donor's bacteria occupied their intestinal niches resulting in restoration of the structure of the microbial communities. The change in bacterial composition was accompanied by resolution of the patient's symptoms (reproduced with permission from Khoruts et al. [2010]).

has also been proposed as a therapeutic intervention in the setting of refractory IBD (Borody et al., 2003) and IBS (Andrews, 1992).

The most extensively reported application of fecal transplantation is in patients with recurrent *C. difficile* infection. In this setting, it is thought that prolonged decrease in the diversity of the indigenous microbiota prevents effective control of, or a less antagonistic environment for, toxigenic *C. difficile*. The transplanted fecal bacteria are meant to restore overall microbial diversity and stability. It has been demonstrated that administration of feces has a major impact on the recipient's gut microbiome, associated with the successful treatment of refractory *C. difficile* infection (Khoruts et al., 2010) (Figure 4). It should be noted that despite the clinical efficacy, this therapy has not been approved by the U.S. Food and Drug Administration. It is hoped that additional research will define the minimal set of microbes that can be administered (in pure, cultured form) to replace unfractionated feces for this application. Carefully defined microbial communities or minimal microbiomes may be just as effective, more sanitary, and safer for patients than "whole" fecal transplantation.

Immune Modulators

It is known that altered immune status can affect the microbiota (e.g., IBD, enteric infectious diseases). Mice lacking immune modulators such as interleukin-10 or nuclear factor κ B have different microbiota than wild-type animals (Lupp et al., 2007). An attractive (but unproven) concept is that it may be possible to use immune modulators to reshape the composition of the microbiota for benefit. To date, there are scant data in this area, other than evidence showing that immunosuppressive agents such as steroids can treat inflammatory diseases

(although it is not clear if these effects are due to changing the microbiota or dampening inflammation) (Siegmund, 2009). Most of the known immune effects of the microbiota are noted early in life, including T cell (Treg) development and Th1/Th2 ratios, which may limit the use of immune modulators to infancy. Emerging data support the role of the intestinal microbiota in the maturation of immunity including the differentiation of regulatory Treg populations (Honda and Littman, 2012). As yet, there are no convincing data in humans to indicate the value of using an immune modulator later in life, sustainability of effects, or if effects are due to changing the structure or function of microbial populations.

Phage Therapy

Another strategy to create a beneficial shift in the microbiome is to develop specific bacteriophages to target a particular microbe. There are several difficulties inherent to this yet-unproven approach, including the likelihood that phage resistance would develop rapidly.

In summary, although several interventions targeting the microbiota have been successfully used to improve clinical outcome, with the exception of inactivation of specific pathogens by antibiotics, it has not been established that improved clinical outcome is due to changes in the structure or function of bacterial communities. More targeted clinical studies are needed to establish such causality.

Clinical Investigation of the Impact of the Intestinal Microbiome on Human Health

Human trials that investigate the effects of interventions targeting the intestinal microbiome can address the following: (1) the effect of the intervention on the clinical outcome of interest;

(2) the effect of the intervention on the composition and function of the intestinal microbiota; and (3) the association between the effect of the intervention on the microbiome and the clinical response. Response variables used in these studies can be clinical or microbial, their choice driven by variables that relate most closely to the primary study question.

Clinical response variables must reliably detect a change from one discrete clinical state to another (including in diseases that vary over time), must be capable of being measured without bias in the same way in all participants, and must be ascertained as completely as possible. Selecting clinically relevant response variables in interventional studies of specific disease conditions is often relatively straightforward given that the clinical endpoints are usually well defined (e.g., symptom severity or inflammatory indices). However, the selection of clinically relevant response variables for interventional studies related to maintaining or promoting health in healthy individuals can be difficult and challenging. Examples of clinically relevant endpoints appropriate for studies in healthy subjects might include validated scales of overall wellbeing and quality of life, normalization of bowel habits, assessment of incidence of common infectious diseases (such as symptoms of colds and flu), and absenteeism from school or work. In addition, performance measures (e.g., mental, physical, and social) for healthy populations might be relevant (presuming a reasonable mechanistic hypothesis).

Microbial response variables may be incorporated into future clinical studies in digestive diseases and gastroenterology. Global microbial parameters such as microbial diversity or richness may serve as useful surrogate indicators for the relative stability or "fitness" of the gut microbiome. Several studies have shown that reduced microbial diversity, calculated with well-accepted indices, is correlated with different intestinal disease states. This has been shown, for example, in IBS (Carroll et al., 2011, 2012) and IBD (Sartor, 2010). Other aggregate biomarkers may include classes of microbes or taxonomic groups of bacteria that may be relatively abundant in disease states. Individual microbial taxa (e.g., species) may also serve as biomarkers of a healthy gut microbiome. An example is the usefulness of the commensal *Faecalibacterium prausnitzii* in predicting postoperative recurrence of Crohn's disease (Sokol et al., 2008) and subgroups of IBS patients (Carroll et al., 2012; Rajilić-Stojanović et al., 2011). Microbial biomarkers may include molecules derived from microbes by de novo biosynthesis or bioconversion, and different analytes may include DNA, RNA, proteins, or metabolites. Some of these compounds may also be human derived so that quantitative changes in specific specimen types should be carefully considered. For example, molecules or macromolecular components such as lipopolysaccharide (LPS) or ribosomal RNA sequences may be specific to microbes, whereas compounds such as lactate or histamine may be derived from human or microbial cells. Conversely, human biomarkers such as fecal calprotectin may indicate the presence of active inflammation in the intestine, and these human indicators may be combined with microbial biomarkers to enhance stratification of patients into disease subcategories or to improve prognostic utility.

Observational studies demonstrating the association between intestinal dysbiosis and diseases, or suboptimal health states, suggest the intestinal microbiota as a potential biomarker for health and certain disease conditions. However, despite the important information arising from these studies regarding differences in the intestinal microbiota between patients with specific diseases and healthy controls, the current data have two major limitations. First, they address only composition (and oftentimes at only a crude level), and not function, of the microbiota. Second, they provide only a single "snap-shot" of the associations, thereby missing important dynamic effects of the host-microbial interaction on the individual's clinical outcome over time. Future prospective studies with long-term follow-up on the dynamics of microbial composition and function associated with host health status will hopefully provide the needed validation for using intestinal microbiota as a biomarker for health or disease states.

Although randomized controlled trials are considered the "gold standard" of clinical research, they are not always practical (e.g., in investigating the effects of the intestinal microbiota on aging, cancer development, and longevity). In these situations, surrogate endpoints (biomarkers) are often used to substitute for clinical endpoints. Examples of possible surrogate endpoints in microbiome research include the effect(s) of interventions targeting the intestinal microbiome on intestinal motor, sensory, and absorptive functions; innate and adaptive immune responses; mucosal barrier function; and mucosal cell differentiation and growth and death cycles. The use of biomarkers can help reduce the cost of investigating the effects of interventions targeting the intestinal microbiota in clinical studies. Furthermore, the use of surrogate response variables/biomarkers can often be helpful in demonstrating proof of concept and providing the rationale for planning future clinical trials focused on clinical endpoints. However, investigators should recognize that changes observed in studies using surrogate response variables do not always reflect the expected clinical outcome. Thus, the beneficial effect of clinical intervention targeting the intestinal microbiota should ideally be confirmed by demonstrating improved clinical outcome.

Pathways to Advance the Field

Efforts to date have greatly increased knowledge about how the indigenous gut microbiota may influence human health and disease. In order to move the field forward, there are a number of key questions and directions to be considered.

Association of the Microbiome with Human Health and Disease

As noted above, much of the information regarding the role of the indigenous gut microbiota in human health and disease comes from studies in which specific community structures are altered in individuals with disease compared to healthy individuals. As such, many of these studies are cross-sectional studies, and such associations cannot be equated with proof of causation. Despite this shortcoming of associative studies, such studies are still important to the exploration of the complex relationship between the gut microbiome and human health.

To increase the power of such studies, future associative/observational studies would benefit from a longitudinal design, rather, that encompasses individuals with multiple clinical characteristics, including varied ages, races, and health status with particular microbiota characteristics or fluctuations. The

beneficial outcomes of such well-designed studies are many. Such work would help define a "core functional microbiome," which consists of a minimally redundant functional set of organisms. This is useful in helping design either therapeutic replacements for dysbiotic microbiota or a therapeutic target for nonreplacement interventions. In addition, such work could also lead to microbiome biomarkers of health and disease states. If validated, such biomarkers (e.g., specific community structures associated with health or disease) could be clinically useful even if they do not provide mechanistic insight into health and disease mediated by the microbiota.

To justify the time and expense of these studies, and to allow robust analysis to be performed, it is important to design these studies such that appropriate data and material are collected. Extensive clinical metadata need to be collected to permit adequate phenotyping of the subjects enrolled in the study. This includes cataloging and characterizing genetic and environmental (e.g., diet, medications) variables along with clinical phenotypes (including metabolic and other functional readouts).

The question of where to sample in the gastrointestinal tract is also very important. Feces is currently the most commonly used analyte, but it is likely that additional information would be gained by finer mapping of microbial community structure and function along the length of the gastrointestinal tract and possibly across axial gradients as well (i.e., from lumen to mucosa).

Linking Microbial Community Structure With Function

Identifying the structure (community profile) and function (metagenomics, metatranscriptomics, and metabolomics) of the microbiota that are critical to health are tasks that may require methods of assessment still to be developed. Recent advances in DNA sequencing and proteomics technologies have enabled insight into the structure and function of the gut microbiota without the necessity for cultivation. However, very few efforts to date have used a multi-"omics" approach to study the complex ecosystem in the human gut. Integrating information about the identities of microbial community members (obtained from 16S rRNA gene-based measurements), metabolic potential (obtained from metagenome sequence data), expressed proteins (obtained from metaproteome data), as well as metabolites produced (metabolomics) would enable explorations of the gut microbiota at multiple molecular levels simultaneously.

Technological and Resource Needs to Move Forward

Although many of the current advances in the area of microbiome research have been driven by advances in nucleic acid sequencing, technologic advances including computational infrastructure and bioinformatics are required to ensure progress. Technology that allows investigation of structural and functional aspects of the microbiome, as well as the analytic tools needed to process and draw conclusions from these data, will be crucial for future advances in this field. For example, it is currently difficult to make taxonomic assignments for a majority of the data from metagenomic and metaproteomic data sets. This limitation reflects the fact that genome sequence data for many of the bacterial species in the human gut microbiota are not available. The aim of the Human Microbiome Reference Genomes initiative is to generate a total of 3000 reference genome sequences from various human body sites, an effort that will greatly facilitate subsequent taxonomic analysis. It is likely that additional genome sequences, including those derived from multiple isolates of a given phylotype, will be required to gain a greater appreciation of the information present in these large data sets. Beyond taxonomic analysis, whole genome shotgun sequencing and functional metagenomics place major demands on computational infrastructure and software tools development. Ongoing refinements in computational server architecture and automated data analysis pipelines will facilitate metabolic pathway reconstruction and de-noising processes necessary for advances in metagenomics.

To obtain these genomic sequences, and to also permit hypothesis testing such as recolonization experiments and therapeutic trials with isolated groups of organisms, cultivation retains an important role even in the age of molecular microbial ecology. Researchers still struggle to cultivate the bacteria that are present in the human environment, although much progress is being made in this area. Growing bacteria in pure culture may not be possible, and coculture may be required, given that the gut microbiota are a complex, interdependent community.

Future preclinical studies of the microbiome would likely benefit from the refinement and expansion of current animal models. Because of the difficulty in conducting studies that address causality in humans, animal models will remain important. Although much information has been gained from the use of murine model systems, other mammalian systems including alternate rodent, porcine, and primate systems may have significant advantages. In addition, alternate nonmammalian systems including *Drosophila*, zebrafish, and nematodes have shown promise, allowing an increase in throughput, replication, and the ability to look more intensively at host genetics in the host/microbe interaction.

Ascribing the Gut Microbiome to Health and Disease

Two key questions regarding the microbiome in human health are (1) whether the microbiome should be manipulated therapeutically and (2) the appropriate means to do so. As noted above, there are multiple proposed approaches for specific microbiota manipulation, including probiotics, prebiotics, diet-based therapies, antibiotics, immune modulation, and fecal transplantation. Assessment of these potential therapies will require preclinical studies, likely to involve various animal model systems in addition to well-designed human trials.

Studies designed to assess a causative role for the microbiota in health or disease states are critically needed. Animal systems, in particular murine model systems, are useful in that interindividual variation in the baseline microbiota can be minimized by controlling for host genotype and housing conditions, including caging and diet. As such, these systems allow for experimental replication and to test hypotheses regarding causation. It has been argued that differences in microbiota prevent generalizing results observed in animal models to the human state. One approach proposed to circumvent this shortcoming is to colonize germ-free mice with human-derived microbiota. This can be in the form of human intestinal contents or by introducing mixtures of cultivated organisms that were originally isolated from a human source. It remains to be seen how broadly applicable findings in such "humanized mice" will be to human clinical conditions.

In summary, human colonizing microbiota are essential to health, but the composition and functional characteristics of a healthy microbiome remain to be defined. Although some disease states have been correlated with dysbiosis, it is not clear if the dysbiosis is a cause or consequence. Elucidation of the properties of healthy microbiota would provide a target for dietary interventions and microbial modifications aimed at sustaining health in generally healthy populations and improving the health status of people exhibiting disrupted microbiota and associated diseases. Future research as delineated above will enable application of the rapidly expanding knowledge of the human microbiome to improve dietary recommendations.

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