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STATE OF THE ART REVIEW

The role of the microbiome in human health and disease: an introduction for clinicians

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ABSTRACT

Research into the microbiome—the indigenous microbial communities (microbiota) and the host environment that they inhabit—has changed clinicians' ideas about microbes in human health and disease. Perhaps the most radical change is the realization that most of the microbes that inhabit our body supply crucial ecosystem services that benefit the entire host-microbe system. These services include the production of important resources, bioconversion of nutrients, and protection against pathogenic microbes. Thus disease can result from a loss of beneficial functions or the introduction of maladaptive functions by invading microbes.
This review will show how an understanding of the dynamics and function of the indigenous microbiota has altered our view of microbes in maintaining homeostasis and causing disease. It will discuss how disruption of the beneficial functions of the microbiota can lead to disease. Methods for studying the microbiota will be introduced as part of a conceptual framework for using these methods to delineate novel roles for microbes in health. Key associations between specific changes in the microbiome and disease will be discussed. This will lead to an explanation of how the intentional manipulation of the microbiota, either by restoring missing functions or eliminating harmful functions, may lead to novel methods to prevent or treat a variety of diseases. With the explosion of studies relating the microbiome to health and disease, this review aims to provide a foundation for clinicians to follow this developing area of biomedical research.

Introduction

The recent announcement of the United States' National Microbiome Initiative reflects the rise of microbiome science in the past decade.[1] An understanding of how complex microbial communities can influence the pathogenesis of multiple diseases has implications for prevention, diagnosis, and treatment. But because this area of microbiology has not traditionally been part of the premedical or medical curriculum,[2] practicing physicians and those in training often find it hard to understand the increasing attention paid to the microbiome in clinical practice.

This review aims to help doctors understand the basics of how the microbiome is being studied, and to provide an overview of how knowledge of the structure and function of microbial communities may eventually affect the practice of medicine. Because interest in the microbiome spans several biomedical disciplines, detailed discussion of many topics is not possible. However, high quality reviews are cited for readers to discover more about specific areas of interest.

Sources and selection criteria

The references used in this review were identified by PubMed and Medline searches of articles published between 1965 and 2016 and through my personal library. Given the exponential increase in the use of the term microbiome (fig 1), I focused on the past 10 years. Search terms included "microbiome", "microbiota", "probiotic", "prebiotic", and "metabolome" in conjunction with specific diseases and health states including "obesity", "infectious diseases", "inflammatory bowel disease", "diabetes", "cardiovascular disease", and "chronic obstructive pulmonary disease". Few high quality systematic reviews and randomized controlled trials (RCTs) have assessed the role of the microbiome in disease so it is difficult to assess the literature using formal quality criteria. Because of the broad nature of the subject, I prioritized recent high quality reviews and RCTs in which multiple references would be relevant. Research literature (much of it observational or using animal models) was included if it was published in high impact journals by investigators who are generally respected by others in the

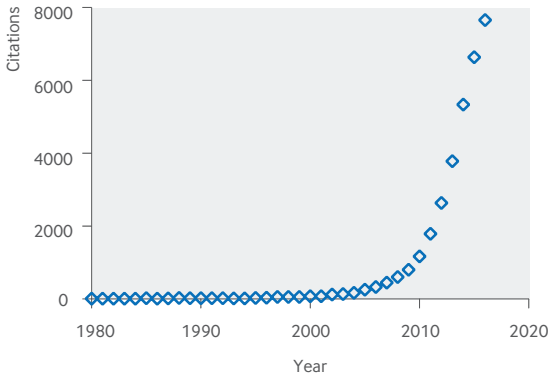

Fig 1 | Increase in publications on the microbiome. When the search term "microbiome" was used to query PubMed from 1980 to 2016 an exponential increase in publications was seen in the past decade

field. Important primary literature was cited to provide an example of a specific disease association or novel method or approach.

Evolving concepts on the role of microbes in health and disease

Some of the difficulty in understanding how microbial communities affect human health comes from the fact that the history of this field is different from that of the standard microbiology and infectious diseases that is taught during medical training. Our initial understanding of the role of microbes in human health relied on the germ theory of disease proposed by Louis Pasteur and refined by Robert Koch and others.[3] This early work focused on microbes as agents of disease (pathogens)—Koch's postulates sought individual microbes as disease causing agents. This led to a focus on the attributes of a micro-organism that enabled it to disrupt homeostasis of the host. This focus on single organisms (initially bacteria but then moving on to fungi, viruses, and prions) and pathogenesis has led to tremendous advances in medicine.[4] The development of methods to control infectious diseases secondary to this understanding of microbiology resulted in public health and sanitation practices as well as the development of antibiotics.

Parallel to the development of medical microbiology and infectious diseases, other scientists began to study the role of microbes in the natural environment, such as those found in soil and seawater.[5] Investigators observed that microbes in these environments were rarely found in isolation but were most commonly found as members of complex consortia. These microbiologists developed closer intellectual ties to researchers in the areas of ecology and evolutionary biology rather than medicine.

When doctors and medical researchers considered complex microbial communities it was usually in the setting of the alimentary tract, where microbes were considered to be commensals (box 1)—organisms that obtain benefit by living in close association with their hosts but have no positive or detrimental effects on the host. However, it has recently been realized that this relationship may not be one sided.[6]

Medical researchers have realized that the concepts developed by environmental microbiologists to study and understand microbial communities are applicable to humans. Large scale projects such as the National Institutes of Health (NIH) Human Microbiome Project and the MetaHIT consortium were initiated to foster this line of research.[7 8] As the study of human associated microbial communities started to flourish, investigators had to adopt new ways of thinking and discussing concepts of microbiology. Part of the difficulty that clinicians and medical researchers face when they try to understand the literature is the unfamiliar terminology. For this reason this review will start by discussing some common topics, terms, and working definitions (see box).

What is the microbiome?

The microbiome is defined in box 1. This term is now commonly used when referring to the complex community of microbes that inhabit a specific site on the body[9]; for example, in a discussion of the gut microbiome and its relation to various health and disease states. In this review, I will use the term microbiota when referring purely to the micro-organisms that are present in a specific site. The term microbiome refers not only to the microbes but also to the environment that they inhabit.[10] Using the example above, the gut microbiome refers not only to the microbes but also to elements of the host such as the host epithelium, immune components, and products of both the microbes and host including metabolites. Furthermore, although much of the work studying the indigenous microbiota has focused on bacteria, viruses and fungi also inhabit most body sites that are occupied by microbes. This focus on bacteria partly results from the fact that the sequencing methods used to examine microbial communities were developed to study bacteria (see below). However, more recent work is beginning to focus on the role of viral and fungal members of the indigenous microbiota.

What are the functions of the indigenous microbiota?

It might seem that the distinction between microbiome and microbiota is simply an exercise in semantics. However, it is important to understand whether we are discussing just the microbes in a given site or the sum total of the organisms and their environment when considering the multiple functions that a given microbial community might carry out (fig 2).

The indigenous microbiota can carry out functions related to the effects of their metabolism on the abiotic elements of the microbiome or through interactions with their host.[11] Although microbial genomes are much smaller than that of the host, the microbiota has a potentially greater metabolic capability in total.[12] Additionally, some metabolic activities are carried out jointly, with contributions from both microbes and the host.[13] Similarly, signaling between the host and indigenous microbiota can alter the structure and function of both partners in this symbiosis.[14]

The microbiota can carry out multiple metabolic activities ranging from catabolism and bioconversion of complex molecules to synthesis of a wide range of compounds that can have effects on both the microbiota and the host. In some cases the microbiota can augment pathways that are present in the host, but in others the microbiota

DEFINITIONS

Functional metagenomics
A method that looks for specific biochemical functions within a metagenome. As first described, the metagenome is cloned into a vector such as a plasmid or bacterial artificial chromosome This library is then moved into a test microbe (such as *Escherichia coli*) and screened for functions of interest such as antibiotic resistance or the ability to metabolize a given substrate

Metabolome
The total of small metabolites (peptides, oligosaccharides or sugars, lipids and so on) present in a given environment. In terms of a host associated microbiome, the metabolome generally reflects the combined metabolic activity of the host and the microbiota

Metagenome
The collective genomes of a given community of micro-organisms. It is a measure of the functional potential of a given microbiota. Metagenomics is the study of metagenomes

Microbiome
A characteristic microbial community that occupies a reasonably well defined habitat and has distinct physicochemical properties. The term not only refers to the micro-organisms involved but also encompasses their theatre of activity. Some people use the term microbiome to refer just to the organisms themselves (however, see "microbiota" below). In addition, some also use the term microbiome to refer to the collective genome of a microbial consortium or community (however, see "metagenome"above)

Microbiota
A community of micro-organisms that occupy a particular site or habitat

Operational taxonomic unit (OTU)
A classification unit based on the DNA sequence similarity of a taxonomic marker gene (such as the 16S rRNA encoding gene). One commonly applied threshold for binning organisms on the basis of 16S gene sequencing is to use a similarity of 97% or greater as the definition of an OTU

Phylotype
A classification unit that is based on comparing a query sequence to a given database and assigning membership to a given bin based on closest similarity to the database. Computationally, this method is generally faster than OTU based methods but is very dependent on the comprehensiveness of the database and the accuracy of the underlying taxonomy

Prebiotic
Nutrients that favor the growth and predominance of beneficial microbes and their inherent functions. Most of these have been carbohydrates that cannot be broken down by the human digestive machinery but are metabolized by specific members of the microbiota

Probiotic
Commonly defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host." In the past, these were often organisms that were first recognized in fermented food products. Currently, there is interest in identifying potential probiotics that are members of the microbiota of healthy people

Proteome
The comprehensive collection of proteins within a given environment

Symbiosis
Literally means "living together." When referring to a host-microbe interaction, this is classically subdivided into "mutualism" where both parties benefit; "parasitism" when the microbe benefits at the cost of damage to the host; and "commensalism" when the microbe benefits but there is neither harm nor benefit to the host. As noted in the text, true commensalism is probably rare but is a form a mutualism where the benefit to the host is not readily apparent

Transcriptome
The collection of transcriptionally active genes of a microbiota under a given condition. Analysis of the transcriptome typically involves harvesting and sequencing the collective RNA of the microbiota

encodes for pathways that are unique to the microbial component of the microbiome. One important area is the ability of the microbiota of the intestinal tract to ferment resistant starch (polysaccharides that cannot be digested by the host) to produce a variety of compounds, most notably short chain fatty acids,[15] which can have a variety of effects on the host. For example, the short chain fatty acid butyrate is the preferred energy source for colonic enterocytes and has a variety of effects on host physiology, ranging from anti-inflammatory effects to antitumor activity.[16]

Another interesting example of how the metabolic activity of the indigenous microbiota can influence host health is related to the metabolism of small molecules such as drugs.[17] Microbial metabolism can affect the bioavailability of certain oral drugs, as has been shown for the cardiac glycoside digoxin.[18] Because of the narrow therapeutic range of this drug, alteration of its bioavailability can greatly influence the development of toxicity. It was recently shown that certain strains of the bacterium *Eggerthella lenta* can reduce digoxin owing to the presence of the cardiac glycoside reductase operon.[18]

One example of host-microbe co-metabolism is the conversion of bile salts and bile acids in the gut.[19] These compounds, synthesized in the host's liver and secreted as conjugated bile salts, can undergo microbially mediated conversions within the intestine to release unconjugated bile acids and generate secondary bile acids.[19] Although these compounds have distinct activities from the parent ones, the host has evolved the ability to recognize and respond to these microbially generated compounds in a manner similar to the response to bacterially generated short chain fatty acids. Farnesoid X receptors (FXRs) are nuclear hormone receptors that respond to bile acids.[20] Signaling through FXRs and other bile acid receptors can have a variety of effects on the host. Because bile acids are the end products of cholesterol catabolism, changes to bile acid metabolism can have effects on cholesterol and lipid metabolism.[20] Changes in the gut microbiota are associated with altered lipid metabolism and various FXR agonists are being developed as potential treatments for various metabolic disorders, ranging from obesity and insulin resistance to liver fibrosis and non-alcoholic steatohepatitis.[21 22]

The indigenous microbiota can modify epithelial responses and systemic responses, such as the development and activity of the immune system.[23] Germ-free animals have underdeveloped peripheral lymphoid organisms and immune responses,[24] and colonization with a complex microbiota or specific members of the normal microbiota can reverse this immature state.[25 26] Similarly, mucosal epithelia modify their expression of mucus and nutrient receptors and differentiate in response to the presence of the microbiota.[27-29] In turn the host epithelium and immune system can alter the structure and function of the microbiota.[30] In addition, two reports have shown that the microbiota can alter the antitumor responses to immunotherapies that affect checkpoint blockades through targeting cytotoxic T lymphocyte associated protein 4 (CTLA-4) or programmed cell death 1 (PD-1).[31 32] These altered responses to immunotherapy were associated with specific members of the microbiota, although the precise mechanism has yet to be defined.

A final global function that has been attributed to the indigenous microbiota is that of colonization resistance, where the presence of the microbiota protects the host from colonization by and disease from potentially pathogenic microbes.[33] The mechanisms by which this phenomenon is mediated by the microbiota are still being

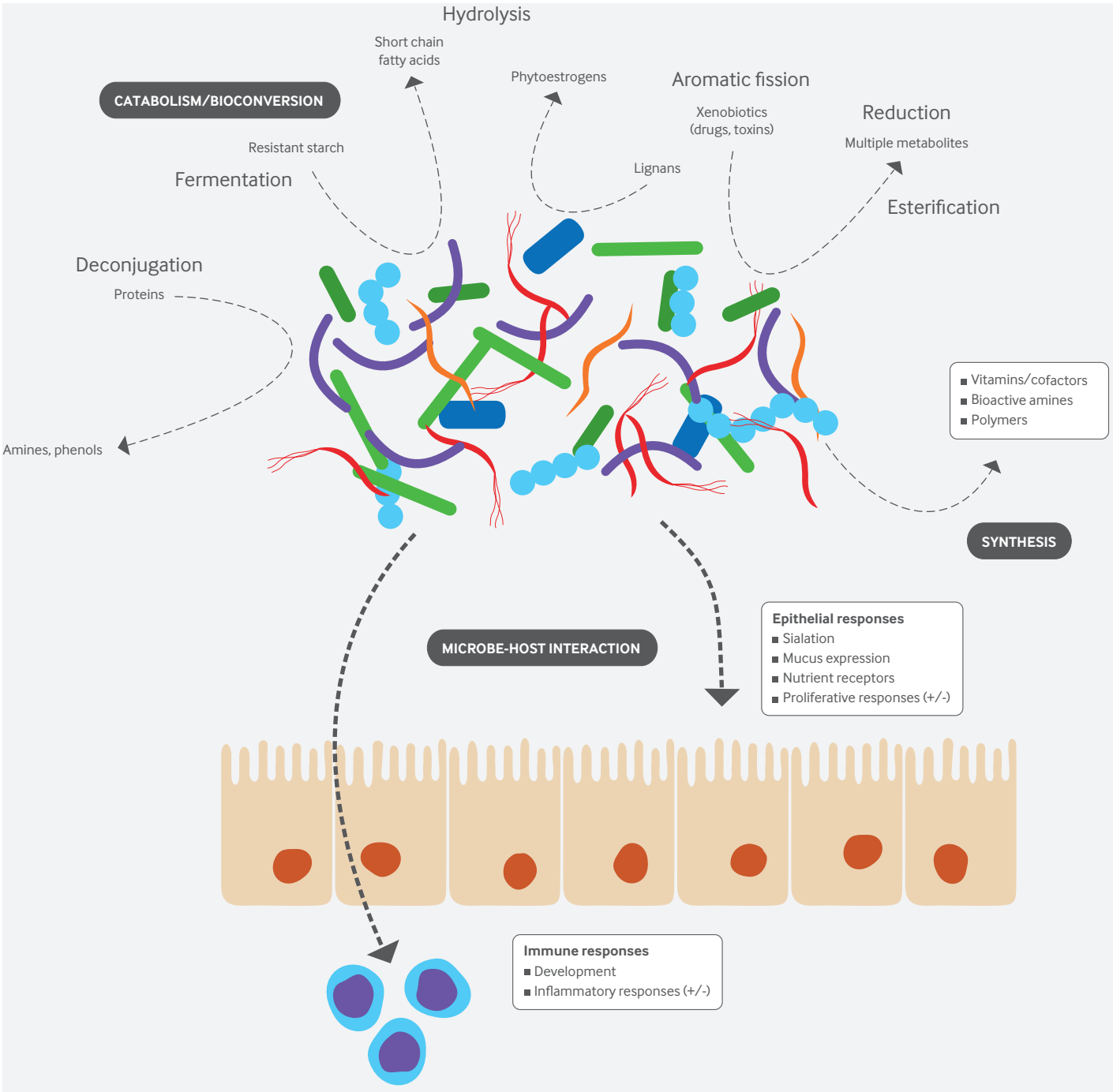

Fig 2| Potential functions of the indigenous microbiota. The microbiota can have effects through the microbes' synthetic or catabolic metabolic activity or through direct host-microbe interactions. Catabolism and bioconversion of dietary or host derived compounds can make nutrients more available to the host or alter the bioavailability of drugs. Some members of the microbiota can synthesize important cofactors or bioactive signaling molecules such as amines. Signaling between the microbiota and the host can trigger alterations in host function, such as altered expression of mucus or alteration of the immune response

delineated, but it probably involves a combination of metabolic activities such as short chain fatty acid production, direct competition for nutrients, and immunologic effects on the host.[34]

Thus, a precise and intricate symbiosis exists between mammalian hosts and their microbial partners, and any disturbance of this symbiosis can have detrimental effects on both partners. For the host, alteration of this symbiosis can lead to a variety of disease states, as will be discussed below.

How is the microbiome studied?

Structure versus function

Several techniques are used to examine various aspects of the indigenous microbiota (fig 3), and many reviews of these techniques are available.[35 36] These techniques can be divided into those that assess the structure (analogous to anatomy) and those that assess the function (analogous to physiology) of the microbiota. Whereas anatomy provides information about the structure of an organism or a part of an organism, physiology provides insight into

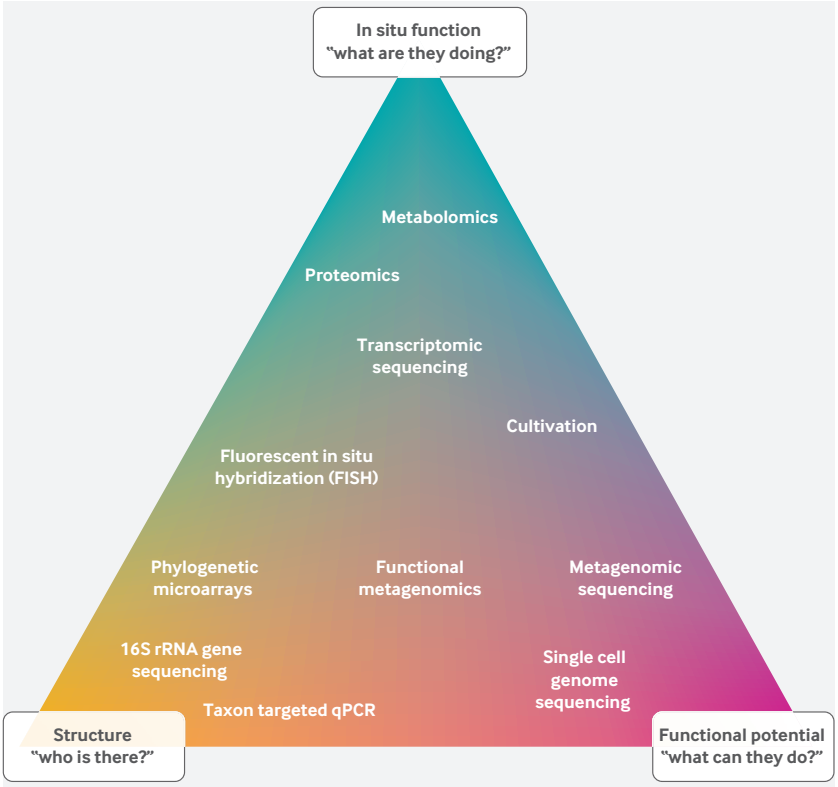

Fig 3| Methods for studying the structure and function of the microbiota. The methods used to discern the structure ("anatomy") and function ("physiology") of the indigenous microbiota can be divided according to which aspect of the microbiota they can interrogate and are positioned accordingly. At the most basic level, methods can simply describe the community structure of the microbiota—that is, which taxa are present and in what relative amounts. Methods that investigate functional potential generally catalog the coding potential of individual members of the microbiota or the entire community (the metagenome). To measure function directly a catalog of the expressed microbial genes (the metatranscriptome) or the proteins or metabolites present in the microbiome environment must be generated. qPCR=quantitative polymerase chain reaction

the function. While function can sometimes be predicted from structure, as anatomy may provide clues about physiology, true assessment of physiology requires the direct measurement of function.

Many of the techniques have leveraged the advances in high throughput nucleic acid sequencing that arose from the Human Genome Project. Given the involvement of the genome centers sponsored by the NIH, it is no coincidence that the Human Microbiome Project echoes the previous effort to study and characterize the human genome. Sequence based techniques (which can obviate the need to isolate and grow microbes) have been invaluable for understanding the role of indigenous microbes in health and disease.[37] However, full assessment of microbial function and the ability to test specific hypotheses requires other techniques. In particular, microbial cultivation is still an essential part of studying microbes.[38-40] Future treatments that target the microbiota (see below) may use specific microbes to replace missing microbes and this can be accomplished only through isolation and propagation of microbes. Therefore, to understand the role that microbes play in health we need to know which microbes are present and what activities they can carry out in their specific environment. The next section will discuss some of the common techniques used to study the microbiome and how they are used to investigate the structure and function of our indigenous microbiota.

Microbial structure

Several techniques can be used to delineate the structure of microbial communities (fig 3)—that is, cataloging which microbes are present in a given community and determining the relative abundance of each type. One of the most common techniques for performing a census of microbes involves the retrieval of sequence data of the gene that encodes the RNA component of the small ribosomal subunit (16S rRNA).[41-43] This sequence dependent method does not depend on microbial cultivation.[44] DNA is extracted from a sample of the microbial community of interest and polymerase chain reaction (PCR) primers targeting broadly conserved regions of the 16S gene are used to amplify most of the microbial species present. These PCR amplicons are then subjected to high throughput DNA sequence analysis. Although a detailed discussion of this analysis is beyond the scope of this review (several excellent reviews are available[45-47]) the analysis can be discussed in broad principles.

Analysis of 16S data ultimately involves grouping the sequences obtained into discrete bins that give rise to a taxonomy. Two different methods are used to accomplish this. In one, all of the DNA sequences in a given analysis are compared with each other and grouped into operational taxonomic units (OTUs; see box 1) generally on the basis of a given predefined degree of sequence similarity. Each OTU can be classified to known bacteria, although OTUs themselves serve as a surrogate for a given microbe in the community, whether or not a formal name can be assigned. The other commonly used method considers each sequence of 16S amplicons individually and compares it to a set database of sequences and thus classifies each sequence in the experiment to a previously defined bin. There are advantages and disadvantages to each of these approaches,[46] but in general the two different approaches yield concordant observations regarding community structure. Perhaps the most important conclusion is that robust biological effects can be observed through 16S analysis and these insights are not dependent on the specific data analysis technique used.

With regard to human health, investigators use 16S analysis to compare people with and without a given disease in a cross sectional manner.[44] In addition, longitudinal analysis can be conducted to monitor the effect of treatments or the development of disease on the structure of the microbiota.[48] However, although this type of analysis is powerful and provides important observations about the potential role of microbes in health, it does not directly assess the function of the microbotia.

Methods have been developed to infer potential function on the basis of a specific microbial community structure,[49] but as with all inferences this tends to be more hypothesis generating rather than specifically determining function. Any inferences need to be considered with appropriate caveats. For example, if a 16S analysis shows the presence of an OTU corresponding to *Escherichia coli*, this result needs to take into account that this could represent

everything from a probiotic, to a benign indigenous *E coli*, to pathogenic *E coli* O157:H7. The 16S gene gives insight into the phylogenetic characterization of a given bacterium in a community, but it does not provide information on the functions encoded by the rest of the genome.

Assaying potential microbial function

As indicated above, obtaining the full genome sequence of a given bacterial species can provide insight into the potential function of a given bacterium. In a similar manner metagenomic sequence analysis has been developed to assess the functional potential of an entire microbial community.[50 51] Starting with community DNA, as with 16S gene analysis, instead of using PCR to amplify this particular phylogenetic marker, the DNA sequence of the entire community is sequenced directly using high throughput techniques.[47] This provides a catalog of all of the genomes present in the microbial community. Analysis of either the metagenome or genomes of specific members of a microbial community provides insight into the potential function of the community. The relative abundance of specific metabolic pathways that are exhibited by the community can help predict the functional capacity of that community.[12] However, this is only a catalog of potential; the next section will review methods that allow direct determination of the actual function of a given microbiome.

Measuring in situ microbial function

The final group of analytic techniques used to study the microbiome directly measure functional output. Using sequence based techniques, metatranscriptomic analysis assays the proportion of a microbial metagenome that is being expressed at a specific point in time under certain conditions.[37] This technique assays the RNA transcripts present in a microbiome by performing sequence analysis of all of the expressed genes through reverse transcriptase mediated RNA sequencing. When these data are considered in conjunction with a corresponding metagenome, the metatranscriptome provides a snapshot of the functionally active genes at a given point in time. Two other techniques are often used to determine directly the effect of transcriptional activity on the metabolic environment of the microbiome. These final two techniques, proteomic and metabolomic analysis, use advanced mass spectroscopy to measure the relative abundance of proteins and metabolites (including peptides, oligosaccharides, and lipids) in a given microbiome.[12-53] This generally includes metabolite species that arise from the host with potential co-metabolism on the part of the microbiota and is thus a true measure of the metabolic environment of a given microbiome.

Conceptual framework for the study of the microbiome

The initial activities of the Human Microbiome Project and other efforts that began about a decade ago focused on establishing the boundaries of what would be considered "normal" in terms of the microbial communities in and on the human body.[8-56] It was hoped that by defining what is normal, associations between deviations from this normal state and disease could be discerned.

These initial efforts saw large variations in the microbiota found in people without apparent clinical disease.[57] This may partly be a product of the methods that were used for these initial studies. The bulk of the work used nucleic acid sequencing, with an emphasis on 16S gene sequence analysis, and limited metagenomic analysis. The variation encountered in these studies reflects what we have come to understand about the relation between the structure and function of a microbial community. It has become clear that multiple communities as defined by 16S analysis can have similar functions.[58] Furthermore, even when functional capacity is examined by looking at metagenomic sequences, the functional redundancy that lies across large taxonomic distances can again yield similarly normal function.

Along with defining the normal status of the microbiota, other studies looked for associations between the structure and function of the microbiome and disease states. The initial efforts, which continue to this day, sought associations between the microbiota and health and disease. Once again, most of the studies analyzed the structure of the microbial community through 16S based analysis. Importantly, much of this work looks at association rather than causation. As will be discussed in more detail below, investigation of the role of the gut microbiota in the pathogenesis of inflammatory bowel diseases (IBD) is illustrative. Early studies performed cross sectional comparisons between patients with and without disease.[59] Differences were seen between the microbiota of affected patients and controls without IBD, but it was unclear whether they were causative or secondary to the presence of the disease. Subsequent studies tried to establish associations at first recognition of the disease but these studies still could not investigate directly.[60]

More recently, studies have attempted to characterize microbiota before the development of overt disease.[61 62] Two studies recruited people at high risk of a specific disease (IBD and type I diabetes) and followed their microbiota longitudinally, comparing those who subsequently developed disease with those who did not. Other studies looked at the effects of specific treatments for specific illnesses on the microbiota, again in an attempt to define a role for the microbiota in the pathogenesis of the disease.[63]

Finally, for diseases where adequate animal models exist, tests of potential causation have been attempted. Examples of each of these strategies for studying the association between the microbiome and health and disease will be briefly presented here. These examples are not meant to be comprehensive but to provide a conceptual framework for readers to understand and evaluate studies that they encounter in the future.

Disease associations with the microbiome

Infectious diseases

Because the study of the microbiome is tied to the field of microbiology, it is appropriate to start with a discussion of infectious diseases. Since the publication of the fulfillment of Koch's postulates for the toxin producing bacterium *Clostridium difficile* nearly 40 years ago,[64] *C difficile* infection has been taught in medical school as

an example of a disease where disruption of the normal microbiota plays a key role in the pathogenesis. Although the association between antibiotic administration and the development of *C difficile* infection has long been appreciated,[65] more recent work has started to define the mechanisms underlying this association. In particular, much work has been done on the microbial functions encoded by the indigenous microbiota that serve to mediate colonization resistance against *C difficile*.

One area is the role that the intestinal microbiota play in bile salt and bile acid metabolism.[66 67] When conjugated bile salts are secreted by the liver into the gastrointestinal tract microbes that can perform de-conjugation and conversion (for example, dehydroxylation) reactions convert these compounds into unconjugated primary and secondary bile acids.[19] Some of these molecular species promote the germination of *C difficile* spores whereas others inhibit the growth of the vegetative form of the organism.[67-69] This understanding of molecular mechanism has led to the exploration of novel treatments. For example, because the pathogenesis of *C difficile* infection, and in particular recurrent infection, is associated with a loss of normal microbial diversity and function, microbiota replacement therapy including fecal microbiota transplantation is an active area of interest.[70]

The intestinal microbiota can also influence several other infections and inflammatory conditions. In patients undergoing allogeneic stem cell transplantation, the status of the microbiota is associated with the risk of developing bacteremia.[71-73] The lungs of patients with sepsis and the acute respiratory distress syndrome have enrichment of gastrointestinal microbes and this seems to drive the pulmonary inflammatory response.[74] Finally, the composition of the gut microbiota may play a key role in influencing the healing of surgical intestinal anastomoses.[75] These observations have implications for treatment and potentially for diagnosis and prognosis.

Inflammatory bowel diseases

Unlike microbe-microbe interactions in the setting of infectious diseases whereby the microbiota can interfere with a classic microbial pathogen, in the IBDs Crohn's disease and ulcerative colitis no classic pathogen has been definitively identified.[76] In this case, the intestinal microbiota itself is thought to be pathogenic and in predisposed hosts contributes to the development of the dysregulated inflammatory response that characterizes these diseases. Multiple studies show that the intestinal microbiota of patients with IBD is distinct from that of people without IBD.[59-78]

Studies of IBD were some of the first studies of disease that extensively used culture independent characterization of the intestinal microbiota to show an association between disease and an altered microbial community. Early studies used both 16S based sequencing methods and fluorescent in situ hybridization to show that the community structure found in patients with disease was distinct from that of controls.[79] Although the associations were strong, the studies were cross sectional, making it difficult to ascribe causation. More recent studies have tried to address causation by examining patients at the initial presentation of disease.[60] Others have studied subtypes of IBD such as the development of pouchitis in patients who have undergone total colectomy with ileal pouch anal anastomosis to examine the microbiota in patients before the onset of overt disease.[62 80] The availability of numerous mouse models of IBD has led to multiple studies that have tried to discern the underlying mechanisms by which the microbiota can contribute to the pathogenesis of IBD.[81-83]

Studies of the genetic susceptibility to the development of IBD highlight the importance of host immunity and the pathogenesis of this disease. Of particular relevance is the fact that genetic variations in the host machinery that interact with microbes are associated with an increased risk of developing IBD.[84 85] Thus, IBD truly is a microbiome related disease because both the host and microbe, and thus the environment created through their interaction, are altered in this condition.

Obesity and metabolic disease

The complex metabolic interplay between the indigenous microbiota of the intestinal tract and the host has led to an examination of the potential role of the microbiome in metabolic conditions such as obesity and diabetes. Nearly a decade ago, landmark studies showed that there was an association between obesity and the intestinal microbiota in both humans and mouse models of disease.[86 87] The close association between host factors and microbial factors in the complex pathogenesis of conditions such as obesity is highlighted through the use of leptin deficient animals in a study that examined the role of the microbiota and obesity.[88] Despite these studies a comprehensive understanding of the precise mechanisms underlying this association remains elusive.[88-90] Furthermore, a recent meta-analysis of multiple studies suggests that the strength of the direct association between the microbiota and obesity may be weaker than previously suggested.[91]

Whatever the size of the effect, it is clear that the microbiota can influence the handling of nutrients by the intestinal tract. Microbially produced products such as short chain fatty acids and bile acids can influence the expression of important metabolic regulatory peptides such as glucagon-like peptide 1 and peptide YY.[92] Recent work has begun to elicit some of the mechanisms by which the microbiota can influence host energy metabolism.[93 94] Other studies have shown that manipulation of the host diet has effects on the intestinal microbiota, setting up a complex system whereby intrinsic and extrinsic associations in the microbiome can alter host metabolism.[95] An interesting line of research that has received much attention is how unintentional alteration of the microbiota—for example, through antibiotic administration—can disrupt the normal balance and skew towards development of the metabolic syndrome and obesity.[96 97]

Recent work has looked at the effects of microbial metabolism on other organ systems. A key example studied the role of the metabolism of trimethylamine *N*-oxide (TMAO), a metabolite that is used to predict the risk of developing cardiovascular disease. Dietary choline was shown to be metabolized by the intestinal microbiota to generate TMAO and modulation of the microbiota to increase

dietary choline blocked enhanced atherosclerosis.[98] This work provides a potential mechanism by which the indigenous microbiota can explain the well established link between certain dietary habits and the development of a given health condition, such as cardiovascular disease.

Lung disease

Recent interest in the study of microbial communities has led to a re-examination of sites that were formerly considered to be free of microbes, such as the upper and lower respiratory tract. Although the lungs were formerly considered to be a sterile site, the use of culture independent methods suggests that the lungs are inhabited by a low biomass of relatively diverse microbes.[99 100] Early studies cast doubt on the importance of this small population of microbes in the healthy lung,[101] but more recent studies indicate that the composition of the lung microbiota can determine basal inflammatory tone even in healthy people.[102]

Furthermore, it is clear that microbial communities are present and biologically important in specific disease states involving the respiratory tract. It has long been known that many patients with cystic fibrosis become chronically colonized with pathogenic organisms, but more recently the lungs of these patients have been found to contain a much more diverse community than had previously been recognized.[103] The importance of this finding for the pathogenesis of lung disease and cystic fibrosis is still be explored, but it is reasonable to assume that microbe-microbe interactions in this environment might be as important as such interactions within the gastrointestinal tract.[104 105] For example, bacteria found in the lungs of these patients may be adapted to degrade the excess mucin seen in cystic fibrosis and this may support the growth of the typical pathogens seen in this environment.[106]

Important work is being done on the role of microbial communities in the pathogenesis of lung diseases such as asthma and chronic obstructive pulmonary disease (COPD).[107-110] Many of the early studies show association rather than causation, but more recent work is examining how the lung microbiota may drive the inflammatory responses central to the pathogenesis of COPD.[111] Further study is likely to provide a clearer indication of the causal role of altered microbial communities in these lung diseases.

In the upper respiratory tract, polymicrobial interactions in acute and chronic rhinosinusitis have been investigated.[112] As with the lower respiratory tract, the role of pathogens and other microbes has been investigated in terms of their ability to modify host physiology. Specific microbes have been found to be enriched in sinusitis. In one study humans with sinusitis had an increase in the abundance of *Corynebacterium tuberculostearicum*, which had not been previously recognized as a potential pathogen.[113] Installation of this organism into a mouse model of sinusitis demonstrated its pathogenic potential.[113] Further examination of the indigenous microbiota of the upper respiratory tract in patients with and without sinus disease suggested that other members of the indigenous sinus community mediate resistance to colonization by this organism.[113]

Other bacteria have been shown to alter local immune responses in the sinuses that are associated with a shift in the resident microbiota.[114] In a manner analogous to the interaction between pathogens and the microbiota in the intestinal tract, the status of the upper respiratory tract microbiota may be associated with susceptibility to both viral and bacterial upper respiratory tract infections.[115] Additionally, acute upper respiratory tract infection with rhinovirus can alter the microbiota, and it has been suggested that this can lead to increased susceptibility to infections elsewhere in the respiratory tract, such as otitis media and pneumonia.[116]

Emerging treatments: the microbiome as a therapeutic target

The microbiome may play a role in a variety of diseases, potentially when a microbial community is deficient in a beneficial function or because of the presence of a detrimental microbial activity. It is therefore tempting to think that restoration of a beneficial microbial structure or function would represent a novel treatment for certain diseases. Several potential strategies have been proposed to accomplish this. Although success to date has been restricted to a few conditions and therapies, the promise for this novel approach to disease treatment and prevention warrants a discussion of what the future may hold.

Figure 4 lists potential strategies for therapeutic microbiome manipulation. The rationale for each strategy and relevant studies that have examined their efficacy are discussed.

Antibiotics

Although collateral damage from therapeutic antibiotics on the indigenous gut microbiota plays a key role in the pathogenesis of *C difficile* infection, antibiotic mediated alteration of the microbiota may serve to alter a disease associated microbial community to restore a healthy state. This strategy was used long before the current interest in the microbiome developed. For example, therapeutic trials of antibiotics have been used for conditions such as hepatic encephalopathy,[117] irritable bowel syndrome,[118] and pouchitis in patients who have undergone colectomy for ulcerative colitis.[119] In these early therapeutic trials, it was assumed that an occult typical bacterial pathogen was not present. Conditions such as bacterial overgrowth or microbial imbalance were posited and the antibiotic was given in the hope of altering this abnormal state. One obvious disadvantage of this approach is that it is generally empiric in nature. As yet, we cannot predict exactly how a particular course of antibiotics will affect a given microbial community. In addition, our elementary understanding of the structure-function relations of the microbiota adds to the current lack of precision with this approach.

A variant of this approach is proposed for the prevention of recurrent *C difficile* infection. Some of the more recent antibiotics developed for the treatment of *C difficile* infection are designed to be more narrowly restricted to the pathogen in the hope of limiting collateral damage to the indigenous microbiota, which is associated with recurrent disease. The use of fidaxomicin, which has less

Approach	Rational	Example
Antibiotics	Target specific members or groups of the microbiota and suppress them, allowing expansion of desirable species	Small intestinal bacterial overgrowth Hepatic encephalopathy
Bacteriophages	Use naturally occurring bacterial viruses to target specific members of a community that are disruptive or are carrying out pathogenic processes	Pathogen targeted therapy to spare the microbiota from collateral damage
Probiotics (single species)	Replace a presumably missing organism and thus a missing function in the form of an organism	*Sacchromyces boulardii* for prevention of antibiotic associated diarrhea
Multispecies/designer communities	As with single species probiotics, use a collection of organisms to replace a missing function in the microbiome	Feces derived communities, multispecies
Prebiotics	Supply a complex food product (often carbohydrates) that is not digestible by the host to stimulate specific members of the microbiota. The prebiotic is meant to be metabolized by the microbiota to compounds beneficial to the host	Inulin, resistant starch
Synbiotics	Supply a complex of a microbe or microbes along with a prebiotic that is meant to be used by these organisms replace a missing function in a microbiome	
Nutritional therapy	Complete redesign of a diet to promote beneficial microbial communities and function	Low FODMAP diet for IBS. Exclusive enteral nutritional therapy for IBD
Community replacement, "microbiota restoration"	To restore a "deficient" microbiota, harvest a presumably normal microbiota from a healthy person and administer it to a patient	Fecal transplantation

FODMAP=fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; IBS=irritable bowel syndrome; IBD=inflammatory bowel disease.

Fig 4 | Potential strategies for therapeutic microbiome manipulation

microbiota disrupting potential, is also associated with lower rates of recurrent disease while maintaining good efficacy against the pathogen.[120] This strategy is restricted to treating *C difficile* infection, but the use of broad spectrum antibiotics should be limited when treating a known bacterial pathogen to spare the microbiota when treating any infection.[121] Therefore, appropriate antibiotic stewardship helps limit the development or selection of antibiotic resistant organisms and can prevent excessive damage to the indigenous microbiota.[122]

Another approach for treating infections that is thought to have minimal effects on the microbiota is the use of bacteriophage therapies. Bacteriophages are bacteriotropic viruses that generally have a restricted host range. Bacteriophage therapies have been developed that target specific bacterial pathogens, and by their very nature they are unlikely to have off-target effects on other members of the microbiota.[123] Although bacteriophages are known to select for bacterial variants that are resistant, these resistant bacteria often have altered surface structures that while leading to phage resistance also attenuate virulence within the host.[124] Much more work is needed before bacteriophages can be developed into therapeutic agents,[125] but there is much interest in exploring novel therapies designed to minimize microbiome disruption.

Probiotics and other live microbial biotherapies

Because many microbiota related conditions are thought to arise from a deficit in beneficial organisms, replacement of "missing" elements of the microbiota is a strategy that also predates recent attention to the microbiome. Probiotics are defined by the World Health Organization as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host."[126] Despite this definition, many putative probiotics have not been developed or validated to fulfill this definition. Even when studies have been conducted to show potential health benefits, a mechanistic basis is often not investigated. This has led to the accusation by some that there is a "non-scientific" aspect to the probiotic field. Furthermore, regulatory agencies such as the US Food and Drug Administration have allowed many probiotics to fall under the dietary supplement rule provided they are not "intended to diagnose, cure, mitigate, treat or prevent a human disease." This has prompted the proposal of new terms for live biotherapeutics that are meant to be used as drugs. However, if the formal WHO definition for probiotic is adhered to, the formal testing and validation needed for a novel drug would be required and thus a new definition might not be needed.[126]

Nonetheless, many studies have used probiotics in therapeutic trials for several conditions. In many cases the organisms used in these trials have been studied for a long time, long before the current interest in the microbiome. Indeed, Élie Metchnikoff, who received the Nobel prize in 1908 for studies on phagocytosis, proposed that microbes could have beneficial as well as harmful effects on their host.[127] He suggested that ingestion of fermented milk products could have a beneficial health effect, and this led to the development of members of the bacterial genera *Lactobacillus* and *Bifidobacterium* as potential probitoics.[128] These organisms are often administered in the form of therapeutic foods, generally fermented milk products such as yogurt and kefir. Studies suggest that these agents can help prevent and treat acute gastroenteritis in children. Effects include limiting the development of antibiotic associated diarrhea and preventing *C difficile* infection.[129 130] Previous results of small trials using typical probiotic agents have been mixed, prompting most published guidelines to recommend against

their use.[131] More recently, a large randomized, double blind, placebo controlled multicenter trial in older people failed to show efficacy of a probiotic mixture of lactobacilli and bifidobacteria in preventing antibiotic associated diarrhea or *C difficile* infection.[132] This provides further support for not recommending the routine use of probiotics to prevent these conditions.

As noted, many of the traditional organisms being proposed as probiotics were isolated from fermented food products. As such, they were chosen for reasons other than theoretic or experimentally proved mechanisms of action. Recent studies of the microbiome, in particular those that include examination of microbial function, have led to the development and preclinical testing of organisms that could be used therapeutically for specific indications.

Returning to *C difficile* infection, recognition of the importance of bile acid metabolism in the pathogenesis of disease has prompted trials of bile acids, bile acid analogs, and organisms that could potentially alter bile acid metabolism within the gastrointestinal tract.[66-133] Although this treatment is still in a developmental stage, the paradigm of developing live biotherapeutics on the basis of rationally chosen mechanisms of action will hopefully become an important strategy for the future development of probiotics.

Prebiotics and diet therapy

Another strategy for beneficially modifying the indigenous microbiota is to alter environmental conditions of the microbiome to supply nutrients that favor the growth and predominance of beneficial microbes and their functions.[15 134] This strategy has largely been applied to modulating the diet to modify the gastrointestinal microbiota. At the most basic level this approach would entail supplying a single food source that is meant to foster beneficial microbes or microbial functions.

Prebiotics are generally non-digestible carbohydrates that are meant to be metabolized by specific microbes to foster their growth.[135] Given the beneficial effects of microbial fermentation products such as butyrate, many strategies are designed to increase the production of this metabolic product and other short chain fatty acids. Because such treatments presume that the appropriate microbes are present, a variation is to administer a "synbiotic" containing both the relevant probiotic organism and prebiotic carbohydrate.[136]

While focusing on a single nutrient has been useful, broader changes in diet that depend at least in part on altering the indigenous microbiota have also been used. Children with IBD, particularly Crohn's disease, have been successfully treated with exclusive enteral nutritional (EEN) therapy.[137] This consists of a precisely defined liquid diet that is used exclusively for all nutrition. It has a remarkable success rate for inducing remission in these children but it is difficult to maintain long term adherence to this diet. Recent studies of the effect of EEN on the intestinal microbiota indicate that it has a statistically significant effect on the structure and function of the bacterial community.[137 138] These changes are associated with functional alterations of the microbiome that go against what previous data suggest would be beneficial, underscoring how little we know about therapeutic manipulation of the microbiota.

Microbial restoration

The replacement or restoration of a dysfunctional community is a logical extension of the probiotic strategy. However, there are some differences. In particular, there has been much interest in the strategy of transplanting an intact microbial community from a healthy person to one with a microbiota associated disease, often referred to as microbiota transplantation.[139-141] Such treatments date to antiquity, particularly transplantation of intact feces or material derived from feces.[142] Recent interest in fecal microbiota transplantation (FMT) for the treatment of recurrent *C difficile* infection has led to several studies of this specific form of microbiome therapy.[143] The first report of FMT for the treatment of antibiotic associated pseudomembranous colitis, presumably due to *C difficile* infection, was published in 1958.[144] More recently clinical trials of FMT for recurrent *C difficile* infection have used different preparations of feces[145-147] and compared different delivery modalities.[148] In one placebo controlled trial the patient's own stool was returned to subjects in the placebo arm.[149]

In general, the remarkable success rate of all forms of FMT for recurrent *C difficile* infection has generated excitement that microbiome replacement might be used in other diseases. However, to date this success has not been replicated in other conditions such as obesity and IBD. Contradictory results have been seen in small (sometimes uncontrolled) trials.[150 151] It has been suggested that this treatment might not be directly translatable to other conditions.[152] It seems that the beneficial effect of FMT in recurrent *C difficile* lies within the spore forming fraction of the intestinal microbiota.[145] The use of FMT as prepared for *C difficile*, which generally favors administration of spore forming organisms, may not necessarily be successful in other conditions.

The therapeutic future: precision microbiome therapy?

In the future therapeutic approaches are likely to become embedded in precision medicine.[153] Precision medicine has largely focused on host variables that can influence health and the response to treatment, such as host genetics, but because the indigenous microbiota can play a key role, the precision medicine paradigm can be expanded to include these microbial variables. This approach would be predicated on a better understanding of the precise causative roles of the microbiota in a given disease as well as knowledge of precise, functional mechanisms that underlie this causation. Given this, diagnostic and prognostic analysis could be performed to delineate the absence of specific beneficial microbial functions in the presence of deleterious ones and integrated with an assessment of patient variables (fig 5). Once this had been defined for a given patient, a customized strategy that might involve several of the potential therapeutic modalities discussed here could be formulated to provide a precision treatment. Although this would be an ideal situation in the future, for this to become reality

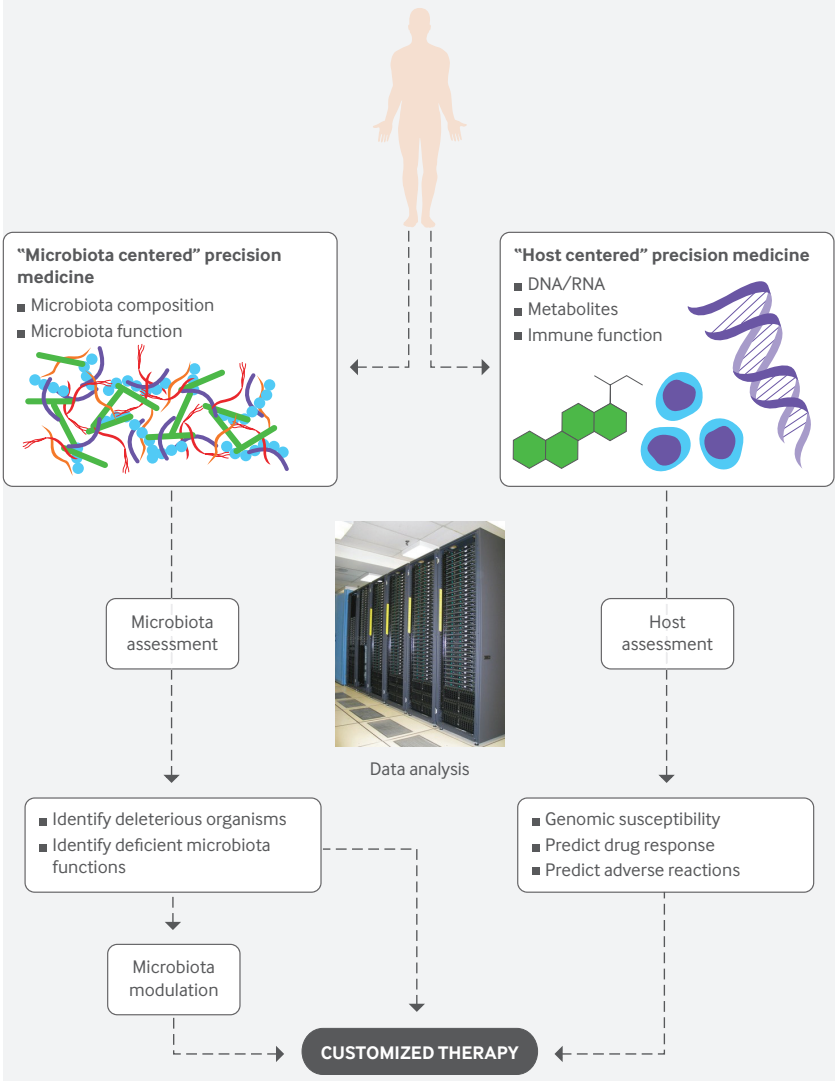

Fig 5| Incorporation of the microbiota into the developing precision medicine paradigm. As currently being developed, precision medicine proposes to determine responses to treatment according to the assessment of human genomics, metabolism, and immune function. On the basis of these data, susceptibility to disease, response to treatment, and potential adverse reactions can be predicted and a customized therapy designed. Because the microbiota can also influence this same susceptibility and response to treatment, we propose that assessment of the microbiota could also be incorporated into a complete precision medicine approach

much work is needed in all of the areas discussed here with regard to understanding basic mechanisms and developing strategies for predictable modification of the microbiome. It is exciting to envisage novel treatments for a wide variety of diseases that are based on targeting the microbiome.

Conclusion

Technical advances in nucleic acid sequencing, data analysis, and culture based microbiology have allowed us to understand new roles for microbes in health and disease. We now know that, rather than existing as individual pathogens, microbes exist as part of complex consortia that have myriad interactions with their mammalian hosts. The insights gained through the study of our indigenous microbiota are leading the way to a better understanding of the mechanisms by which microbes can maintain health and trigger disease. With this greater understanding it is hoped that we will be in a position to develop new ways to prevent and treat a wide range of diseases and to foster health by tending to our microbial symbionts.

QUESTIONS FOR FUTURE RESEARCH

- For health conditions that have been proved to be associated with changes in the microbiota, are the changes observed in microbiota structure or function causative?
- How can we move from detecting altered community structure to elucidating altered community function?
- What principles govern the assembly of the microbiota? Is it possible to alter community assembly, either during initial establishment of the microbiota in infancy or once it has been established?
- To date, much of the focus has been on bacterial inhabitants of the microbiome. What roles do viruses (host and microbiota-tropic), fungi, and other eukaryotic microbes (such as helminths) play in human health and disease? What new technologies need to be developed to accomplish this?
- Can the assessment and alteration of the microbiota be incorporated into the developing precision medicine paradigm?

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