ADVANCES IN TRANSLATIONAL SCIENCE

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Analysis of the Human Gut Microbiome and Association With Disease

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Advances in DNA sequencing technology and bioinformatics methodology have led to a revolution in our understanding of the microorganisms that inhabit the human body. Distinctive populations of organisms belonging to each of the 3 domains of life, Archaea, Bacteria, and Eukarya, inhabit each body site.[1] The communities of microorganisms are referred to as the *microbiota*. When considering the organisms and all of their related genomes, the term *microbiome* is used. The human gut microbiome is particularly unique in that it is home to an enormous number of bacteria, approximately 100 trillion bacteria cells, outnumbering the human cells by an estimated 10 fold and the human genome by 150 fold. We have co evolved to exist with our gut microbiota largely in a mutualistic rela tionship where we as hosts rely on these organisms for a number of key functions related to nutrition, education of the immune system, and prevention of infection by pathologic species. In turn, we provide our gut microbiota with a unique niche in which to live where we provide a source of nutrition to the microbiota in the form of mucus. Alteration of the microbiota composition asso ciated with disease, known as dysbiosis, has been described in the gut for numerous disease processes including inflammatory bowel diseases (IBDs), metabolic disorders, cancer, and infection, particularly with *Clostridium difficile* infection (CDI), to name a few.

Advances in Methods to Study the Gut Microbiome

The expression of ribosomal genes are required for all life forms. The 16S genes are found in all bacteria and archaea, the latter of which are prokaryotic microorganisms that, in the mammalian gut, are primarily methanogens responsible for the production of methane. However, similar to human beings, microeukaryotes such as fungi and yeast have the 18S ribosomal gene. Over the past decade, dramatic technical advances in DNA sequencing technologies, sometimes described as high throughput, massively parallel, or deep sequencing, have allowed scientists to determine the DNA sequence of specific regions in either the 16S or 18S gene that, when matched to a ribosomal DNA sequence database, permits the identification of microor ganisms that reside within a sample from which DNA was iso lated[1] (Figure 1). Furthermore, the number of sequence reads for a specific organism is roughly proportional to its abundance within a sample. The use of DNA sequencing technology is a major advance in the characterization of complex microbial communities because it is a culture independent method that avoids the difficulty of growing the majority of obligate anaerobic microorganisms in the gut microbiota. By using even newer technologies capable of sequencing billions of DNA base pairs in a single run at an affordable cost, shotgun metagenomic sequencing can be performed in which community DNA is sequenced in totality, permitting not only an evaluation of mi crobial community structure but also allowing an evaluation of the genomic representation of the community (Figure 1).[1] The latter can be used to help understand the functions encoded by the genomes of the gut microbiota.[1] Shotgun metagenomic sequencing also can be used to characterize the abundance of viruses, or the virome, biological entities that lack ribosomal genes yet are among the most abundant organisms in the biosphere. Although currently not feasible as a routine clinical test, profiling of the gut microbiota in fecal samples may ultimately have utility in the clinical setting with the caveat that the microbiota in fecal samples is somewhat distinct from the microbial communities adherent to the intestinal mucosa. As the technology of DNA sequencing continues to advance, costs continue to decrease significantly. Together with the automation of bioinformatic tools made available online, the accessibility of these technologies for more widespread clinical and research use is becoming apparent.

The Role of the Gut Microbiota in the Maturation of the Mucosal Immune System and Its Link With Immune-Mediated Diseases

Interactions between the microbiota and the intestinal mucosa play a critical role in the maturation of the host immune system. For example, certain bacteria and their products play a role in the development of tolerogenic anti inflammatory T regulatory cells while others lead to the development of more proinflammatory Th17 cells as well as the programming of B lymphocytes to produce secretory IgA that is secreted at mucosal surfaces. Further emphasizing this host microbial mutualism, the intestinal epithelium interacts with the gut microbiota through the production of nutrients in the form of mucus to support bacterial metabolism, as well as antimicrobial peptides that help to shape the structure of the gut microbiota. Genetic loci associated with the development of IBD include pathways that impact each of these processes, showing that alterations in the delicately balanced homeostatic relationship between the

Abbreviations used in this paper: CDI, *Clostridium difficile* infection; FMT, fecal microbial transplantation; IBD, inflammatory bowel disease.

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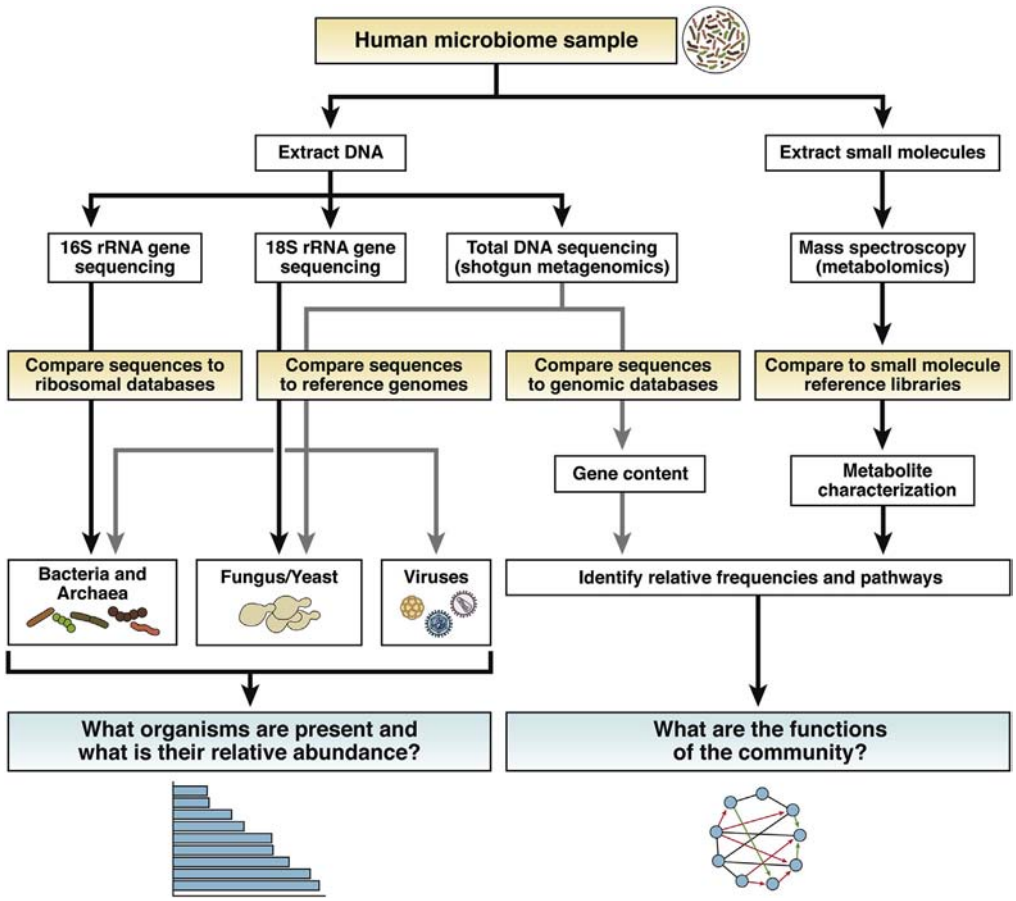

Figure 1. Analytic approach to study both the structure and function of the human gut microbiome. rRNA, ribosomal RNA.

gut microbiota and the host can lead to unrestrained inflam mation, the hallmark of IBD.[2]

The interaction between host genetics and environmental factors in the development of complex immunologically medi ated diseases such as asthma, IBD, type 1 diabetes, and others, have led to the notion that the rapidly increasing incidence of these diseases over the past few decades is caused, in part, by an alteration in the microbial environment. Indeed, many aspects of our environment have been changed dramatically over the past few decades concurrent with the increasing incidence of these disease processes. Elements of the modern lifestyle that have been postulated to result in changes in the gut microbiota include improved sanitation, vaccinations, increased antibiotic use, decline in parasite infections, caesarean section, decline in *Helicobacter pylori*, smaller family size, refrigeration, less crowded living conditions, sedentary life styles, food processing, and dietary changes.

Dysbiosis of the Gut Microbiota: Cause or Effect?

By using the latest DNA sequencing technologies, sci entists now are able to characterize alterations in the composi tion of the gut microbiota associated with various disease processes, otherwise known as *dysbiosis*. Dysbiosis could have value as biomarkers of disease such as atherosclerosis or in pre dicting response to drugs. However, more meaningful would be a demonstration that the dysbiotic microbiota play a role in disease pathogenesis and that restoration of the normal healthy micro biota is an effective therapy. The only disease process in which this has been shown to be the case is CDI, in which the con sumption of antibiotics dramatically, but transiently, alters the composition of the gut microbiota, providing a niche in which *C difficile* can expand. Dramatic alteration of the gut microbiota by direct transfer of an entire community from a healthy donor, a process known as fecal microbial transplantation (FMT), is highly effective in the treatment of refractory disease. The fecal microbiota typically have been administered via enema, colo noscopy, or a nasogastric, nasoduodenal, or nasojejunal tube. The major distinction between probiotics, defined by the World Health Organization as "live microorganisms which when administered in adequate amounts confer a health benefit on the host,"[3] and FMT is that FMT entails transfer of entire communities in the same relative abundance as occurs within a healthy host. Although not proven, this ability of a complex community of microorganisms to engraft in the host also may contribute to the dramatic efficacy of FMT for refractory and recurrent CDI, with approximately 90% success rates.[4] Supporting this notion, the failure of individual probiotics to form resilient gut communities would explain why they have not been beneficial in the treatment of CDI.[5]

Despite its effectiveness in the treatment of CDI, FMT may be associated with unforeseen long term health consequences. For example, recent studies have linked the composition of the human gut microbiota to the risk of metabolic syndrome, car diovascular disease, and alteration of drug metabolism. As such,