

Harnessing the microbiota to treat neurological diseases

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Studies over the last decade have transformed our previously simplistic view of microbes, having only a pathogenic role in disease to a more robust understanding that they are critical for maintaining human health. Indeed, our microbiota—the collection of commensal organisms that live in and on each of us—contributes to nearly every facet of host physiology, from ontogeny of the immune system to neurological function to metabolism. Although the specific details of these host–microbe interactions are still being elucidated for most diseases, the coupling of clinical samples with animal models of disease have provided key insights. This review provides some general background on the microbiota, highlights a few examples of how the microbiota influences diseases of the central nervous system, and provides a perspective for how these findings may be clinically translatable.

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Introduction

The pioneers of modern microbiology—the likes of Robert Koch and Louis Pasteur, among others—focused on the idea that host–microbe interactions were antagonistic in nature, with the host immune system living in a constant struggle against the myriad microorganisms it encounters on a daily basis. This concept of the “germ theory” formed the basis of what became the *leitmotif* of 20th-century microbiology research: a molecular dissection of how pathogenic microbes cause disease. Although there has always been a small group of researchers who thought that commensal organisms were similarly important to study,¹ research into how the microbiota—the collection of all the bacteria, viruses, fungi, and Archaea that colonize humans—impacts human health really only began to flourish at the beginning of the 21st century.^{2,3} Aimed at a better understanding of this relationship, myriad studies during the past decade have begun to catalogue the

microbiota at various body sites and in a multitude of disease conditions.²⁻⁶ Diseases in virtually every organ system have been associated with changes in the microbiota. Indeed, the microbiota has been linked to intestinal disorders, disturbances in metabolic function, autoimmune diseases, and psychiatric conditions, and has been shown to influence susceptibility to infection and the efficacy of pharmaceutical therapies. Knowledge of the specific mechanism(s) underlying most of these microbe–disease associations is lacking; it remains unclear whether the disease-associated alterations in the microbiota represent mere biomarkers of disease, a causal relationship, or a combination of the two. Although cause-and-effect relationships are still being elucidated for many diseases, it is clear that humans coexist in an intricate relationship with commensal organisms. Instead of waging a continual battle with each other, the host–microbiota relationship reflects a carefully negotiated state of *détente* in which each side requires the other. Although this

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host–commensal relationship impacts numerous diseases across all organ systems, this review will more specifically describe some exemplar data of how the microbiome affects diseases of the central nervous system (CNS) and provide some thoughts on how these findings can be translated into a novel class of therapeutics.

Overview of the microbiota

There are more than 10 000 different bacterial species present in the collective human microbiota; the intestines alone contain more than 1000 species.⁷ At any given time, the body of any given individual harbors 500 to 1000 bacterial species,⁸ with 100 to 200 bacterial species in the gut alone.⁷ The Human Microbiome Project, a seminal study that catalogued the microbiome in 15 to 18 anatomic sites in ~250 healthy American adults, found that the composition of the microbiota differs by body site, there exists tremendous interindividual variation, and the microbial gene content is fairly conserved irrespective of the body site or individual.⁹ In fact, the effect of the anatomic site on microbial composition is far greater than the effect of heterogeneity between individuals (ie, all samples from a given body region [eg, skin] were more similar to each other than they were to samples from a different body region [eg, stool], even in the same individual).⁹ Taken together, these findings highlight the remarkable personalization of the human microbiome. While the human genome is typically >99.5% identical in different people,¹⁰ the microbiota of two individuals may not overlap at all.

In addition to the tremendous diversity contained within the human microbiome, it is notable that the number of organisms in the microbiota outnumber human cells in the body by ≥ 10 -fold^{11–13} and that the collective set of genes contained within the microbiota (the *microbiome*) is at least 100 times larger than the human genome.^{2,7} Given this numerical superiority of microbes, it is perhaps not surprising that they are critical for many elements of human physiology. For example, commensal microbes process nutrients needed by the host, contribute to proper development of the intestine, provide colonization resistance to many pathogenic organisms, and foster maturation of the immune system.^{12,14–18} Although the intestines harbor the largest number and diversity of organisms, microbial effects—often linked to bacteria present in the gut—can be found in distant sites throughout the body, such as the bone marrow, lungs, and

CNS. It is not clear yet how the gut microbiota communicates with these remote anatomic sites—ie, whether communication takes place via bacterial metabolites that travel in the bloodstream, via migration of whole organisms to the target site, or, in the case of the CNS, via feedback through the vagus nerve.

Identification of the factors that influence the microbiota's composition is critical to an understanding of what leads to and controls intra- and interindividual variation in microbial composition and potential disease phenotypes. Some studies have demonstrated that host genetics have a small but statistically significant effect on the microbiota's composition,^{19–21} but there is some controversy over this finding.^{22,23} Moreover, the microbiota changes with age, with pronounced changes apparent particularly in early infancy and in the elderly.^{24,25} In addition to these factors which are difficult to control, an individual's specific microbial configuration is quickly altered in response to subtle changes in the micro-environments in which the bacteria reside. On a day-to-day basis, these changes usually reflect alterations in the relative abundance of the various microbes. However, some exposures have a greater effect on the microbiota and can shift the microbial population to a new equilibrium via the loss of specific species and/or the acquisition of others; this new microbial equilibrium can be associated with either health or a disease state. Diet is perhaps one of the largest drivers of the intestinal microbiota as it provides nutrients needed not only by our own cells but also by the microbes living in the alimentary tract.^{26,27} Other factors that can alter the microbiota in a dynamic manner include lifestyle choices (eg, choice of household members, presence of pets, living in a rural or urban setting, country of residence) and circadian rhythms.^{2,28} Furthermore, virtually all drugs have the capacity to change the microbiota by altering the chemical landscape in which the microorganisms live (eg, statins, bile acid sequestrants), modulating the host's ability to recognize and react to microbes (eg, immunosuppressants), and/or directly interfering with the microbiota's constituents (eg, antibiotics).^{29–32} While it is clear that the microbiota can be altered through these various mechanisms, it is not yet clear whether these changes are biologically significant.

The case for the gut–brain axis

Although numerous studies have associated the microbiota with a panoply of neurological conditions, the link

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between microbes and CNS disease predates the more recent interest in the microbiota. For example, Hippocrates thought that “madness” resulted from the imbalance of four bodily humors that could be rebalanced by special diets and purgatives, both of which are now recognized to affect the microbiota. Physicians caring for King George III, who alternated between bouts of confusion and angry outbursts, would often examine his stool for insights into the underlying cause of his abnormal behaviors. In fact, many notable British physicians in the 18th century thought that the gastrointestinal system, which was known at that time to have its own nervous system, was central to emotional stability.³³ In the early to mid-20th century, neurosyphilis and schizophrenia were treated with “fever therapy” by intentionally infecting patients with *Plasmodium falciparum*, the causative agent of malaria³⁴; although this example does not involve the gut microbiota per se, it provides additional evidence for links between microbes and neurologic function.

In more recent years, there have been a barrage of cross-sectional case–control studies that have associated the gut microbiota with virtually every neurologic and psychiatric condition imaginable. The issue is that most of these studies simply provide a descriptive account of the microbiota in patients as compared with controls, with little or no insight into whether the microbiota is casually related to disease or whether there exist biological mechanisms that may link microbes with the phenotype. Fortunately, there are a growing number of studies that combine human data with animal studies to tease out some of these more complicated relationships, some examples of which are provided below. In general, the gut and its microbial constituents are inextricably linked with the central nervous system via regulation of metabolic processes, connection via nerves, and education of the immune system.

Microbial regulation of metabolites

It is increasingly recognized that microbial metabolism has pervasive effects on host physiology, and its role in diseases of the central nervous system is no different. For example, modern medicine has used the ketogenic diet to treat seizures for over a century.³⁵ The underlying mecha-

Next-generation microbiome-based therapeutics will have the potential to alter the treatment landscape of many neurologic and psychiatric conditions

nism by which it works remained elusive until investigators recently used a mouse model of seizures to demonstrate that the microbiota is required for the protective effects of the ketogenic diet.³⁶ Moreover, they found that *Akkermansia muciniphila* and *Parabacteroides merdae* were more abundant in mice fed a ketogenic diet, and that these bacteria were sufficient to protect mice fed a normal diet against seizures, likely through the ability of *P. merdae* to decrease enteric γ -glutamylolation which results in increased levels of hippocampal γ -aminobutyric acid (GABA) and glutamate.³⁶ Commensal bacteria can also affect host production of other key neurotransmitters. Serotonin is critically important for a number of neurological and neuropsychiatric conditions, and it has been known for some time

that ~90% of the body’s serotonin is produced by intestinal enterochromaffin cells, with only ~5% produced in the brain.³⁷ Serotonin production in the colon is regulated by spore-forming bacteria, which produce specific metabolites that are sensed by enterochromaffin cells.³⁸ These enterochromaffin cells then increase expression of tryptophan hydroxylase 1 (Tph1), which results in increased serotonin biosynthesis and secretion, both lumenally and basolaterally.³⁸ Although it is not yet clear whether microbiota-regulated intestinal serotonin production influences neurological diseases, the microbiota also affects hippocampal levels of serotonin through unclear mechanisms.³⁹ It is tempting to speculate that these two processes are linked in some way. Beyond affecting the levels of neurotransmitters, some bacterial metabolites can directly cross the blood–brain barrier and directly affect brain physiology. For example, the gut microbiota is necessary to metabolize dietary tryptophan. These metabolites enter the brain where they control microglial activation, TGF α and VEGF-B production, and transcriptional responses of astrocytes, which together regulate pathogenesis of multiple sclerosis.⁴⁰ In addition to directly modulating metabolite levels, the microbiota also plays a key role in maintaining the integrity of the intestinal epithelial layer, defects in which can cause alterations in systemic levels of metabolites. In a murine model of autism, treatment of animals with *Bacteroides fragilis* was able to normalize the barrier integrity and thereby restore levels of autism-induced abnormalities in serum metabolites.⁴¹ Thus,

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the intestinal microbiota is able to directly and indirectly affect metabolite levels that influence host physiology.

Gut-CNS signaling

The intestines have their own enteric nervous system (ENS), which—with greater than 100 million neurons—has more neurons than all other peripheral ganglia combined and at least as many as the spinal cord.⁴² Therefore, it is perhaps not surprising that many neurologic conditions (eg, Parkinson's disease, autism, amyotrophic lateral sclerosis) have gastrointestinal symptoms that often precede the onset of CNS symptoms. While the vagus nerve connects the CNS and ENS, it should be noted that ~90% of the vagal fibers are afferent in nature, a finding that suggests there are more signals going from the intestines to the brain than vice versa.^{43,44} Indeed, vagal nerve stimulation, which mimics signaling from the intestines to the brain, is approved to treat refractory epilepsy and depression, and it is being evaluated as a treatment for a number of other neuropsychiatric conditions. However, the endogenous signals sent via the vagus nerve—and the mechanism by which they are transduced—have remained enigmatic.

It was previously thought that gut stimuli are sensed by the brain via the passive release of hormones. Enteroendocrine cells, which are rare sensory cells in the intestinal epithelium, detect nutrients in the intestinal lumen and secrete slow-acting peptide hormones, such as cholecystokinin and peptide YY, that stimulate neurons throughout the gut and the brain on the time scale of minutes. However, infusion of sucrose into the gut-induced vagus nerve activation in an enteroendocrine cell-dependent manner on a time scale of milliseconds, suggesting that this interaction was more typical of synaptic transmission than the slow nature of neuropeptide signaling.⁴⁵ Further studies revealed that enteroendocrine cells synapse with vagal sensory neurons, which they activate through the release of glutamate.⁴⁵ Conceptually, this finding is similar to an earlier report that serotonergic enterochromaffin cells, a specific type of enteroendocrine cell, synapse with and modulate primary afferent nerve fibers.⁴⁶ Given that these enteroendocrine cells express toll-like receptors, activation of which results in neuropeptide release,⁴⁷ it is possible that these enteroendocrine cells also detect the gut microbiota and signal microbial changes to the brain. These studies lay the foundation for the molecular mechanisms underlying the gut–brain axis, though it remains to be seen which physio-

logical processes require gut–brain signaling that occurs on the order of milliseconds.

Microbiome-induced immunomodulation

The intestines represent the largest immune organ, and the gut microbiota is vital for inducing maturation of the immune system. Immune cells educated in the gut traffic throughout the body, including within the CNS, and can influence disease pathogenesis at these remote sites.^{48,49} Multiple sclerosis is the best studied neurological disease that exemplifies this particular gut–brain connection. Several studies have noted alterations in the microbiome of patients with multiple sclerosis,^{50,51} findings that suggest that the gut microbiome may be linked to disease pathogenesis and which have prompted interventional clinical trials.^{52,53} Using a murine model of multiple sclerosis, oral administration of *Bacteroides fragilis* protected mice from disease.⁵⁴ Strikingly, treatment with just the *B. fragilis* capsular polysaccharide (polysaccharide A, PSA) either before (ie, prophylactically) or after (ie, treatment) the onset of inflammation was sufficient to protect mice.^{54,55} Notably, PSA induces accumulation of interleukin 10-producing regulatory T cells in the cervical lymph node even though PSA was administered orally. Given that CD11c^{high}CD103⁺ dendritic cells, which are typically restrained to the intestines, also accumulated in the cervical lymph node,⁵⁵ it is thought that PSA is taken up by dendritic cells in the intestine and trafficked to the cervical lymph node, where they induce differentiation of naïve T cells into regulatory T cells. Intriguingly, this dendritic cell migration only occurred in the setting of inflammation, potentially due to inflammation-associated alterations in vascular permeability or the cytokine milieu. In contrast to the protective effects seen with *B. fragilis* PSA, other intestinal microbes exacerbate disease. For instance, intestinal colonization with segmented filamentous bacteria not only induces IL-17-producing T cells (Th17) in the gut but also in the CNS, where they promote disease in a mouse model of multiple sclerosis.^{56,57} Taken together, these studies highlight that microbially induced changes in the intestinal immune system can have systemic effects that cross the blood–brain barrier and affect progression of neurological diseases.

Translating microbiome science to the clinic

The numerous microbiome–disease associations identified thus far have generated a great deal of hope that under-

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standing the relevant microbe–host interactions will open the door to unlimited therapeutic applications. Microbiome-based therapies offer several potential benefits. Patients often view such treatment as more “natural” than conventional drug therapy and are therefore more likely to comply with it. Biologically, microbiome-based therapies are more likely to address one of the root causes of disease (microbial dysbiosis) rather than simply affecting the downstream sequelae. Finally, a given microbiome-based therapy may serve as a “polypill” that is effective against several different diseases stemming from similar microbial changes. Despite tremendous interest in therapeutically exploiting the microbiome, there have thus far been few clinical successes along these lines.

The most successful therapeutic application of microbiome science has been the use of fecal microbiota transplantation (FMT), particularly for recurrent *Clostridioides difficile* infections (CDI). FMT involves “transplanting” stool from a healthy individual to a diseased patient, with the idea that the “healthy” microbiota will correct whatever derangement may exist in the ill patient and therefore will alleviate symptoms. Fundamentally, this notion is agnostic as to the specific microbial dysbiosis and holds that any healthy microbiota will be curative. The idea of FMT dates back to at least the fourth century, when traditional Chinese doctors used a “yellow soup” (fresh human fecal suspension) to successfully treat food poisoning and severe diarrhea.⁵⁸ The continued use of FMT through the centuries for the treatment of diarrheal illnesses in both humans and animals, along with the growing appreciation in recent years of the importance of the microbiota, laid the groundwork for using FMT to treat CDI. Since the first major prospective trial assessing FMT for recurrent CDI in 2013,⁵⁹ most of the numerous studies of FMT for CDI have demonstrated remarkable efficacy, with an average clinical cure rate of $\geq 85\%$.^{60,61} The donor stool can be fresh or frozen (use of the latter allows biobanking of samples from a limited number of prescreened donors) and can be administered via nasogastric tube, nasoduodenal tube, colonoscopy, enema, or oral capsules; the cure rate is slightly higher with lower gastrointestinal administration than with upper gastrointestinal treatment.⁶¹ The optimal screening, preparation, and concentration of infused donor stool have not yet been determined. The most common adverse effects of FMT include altered gastrointestinal motility (with constipation or diarrhea), abdominal

cramps, and bloating, all of which are generally transient and resolve within 48 h.^{60,61} At least 80 immunosuppressed patients have undergone FMT with no serious adverse events noted during 3 months of follow-up.⁶¹

The successful use and the favorable short-term safety profile of FMT for CDI have led to its expanded application for other indications. At the end of 2018, 195 active trials (listed at ClinicalTrials.gov) were investigating the efficacy of FMT for a range of diseases, including CDI, inflammatory bowel disease (IBD; ulcerative colitis, and Crohn’s disease), obesity, eradication of multidrug-resistant organisms, anxiety and depression, cirrhosis, and type 2 diabetes. The few published studies regarding indications other than CDI have generally included small sample sizes and have offered mixed results.^{62–64} In contrast to the successes in CDI, the results have been more varied for patients with IBD,⁶⁵ which is perhaps the second-best-studied indication. It is not clear whether these discrepancies are due to heterogeneity in recipients (eg, in terms of underlying disease mechanisms or endogenous microbiotas), the donor material, and/or the logistical details of FMT administration (eg, route, frequency, dose).

Although FMT offers an important proof of concept that microbiome-based therapies can be effective, treatment is difficult to standardize across large populations because of variability among stool donors and among the endogenous microbiotas of recipients. In addition, FMT is fraught with safety concerns, and its mechanism(s) of action are unclear. FMT likely represents the first generation of microbiome-based therapies; subsequent generations will include the use of more refined bacterial cocktails, single strains of bacteria, or bacterial metabolites as the therapeutic intervention. The field of probiotics has a complicated history: many different strains have been tested against a multitude of diseases. Several meta-analyses have combined results across bacterial strains and/or disease indications and have generally concluded that the data are not yet convincing enough to support the use of the tested regimens.^{65–68} It should be noted that the tested organisms have been chosen mainly on the basis of their presumed safety profile rather than in light of a plausible biological link to disease. The hope is that more focused, mechanistic microbiome studies will identify specific commensal organisms—and their underlying mechanisms of action—that are involved in disease pathogenesis and that will serve as the basis for

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the next wave of rationally chosen probiotics, a few of which are currently in clinical trials. The main hurdle in this endeavor has been identifying specific microbes that are causally related to protection from disease.^{69,70} To avoid the issue associated with many over-the-counter probiotic and nutritional supplements being adulterated (ie, contain undisclosed components and/or do not contain the ingredients claimed),^{71,72} the hope is that these next-generation microbiome-based therapies will go through the normal regulatory approval processes to ensure patient safety.

Perspectives

Animal studies have made clear that microbiome-based therapeutics are a viable and effective option for treating a range of diseases, and the early examples in clinical trials—which have mostly concentrated on application of FMT at this point—have yielded promising results. However, our

understanding is still in its infancy of what is an “optimal” microbiome profile and how to specifically shift from one microbial state to another. The ability to generate incredible amounts of data through high-throughput sequencing and high-dimensional metabolite profiling has so far outpaced knowledge in how to integrate these datasets, yet efforts are ongoing to understand how to do so. As we develop a better grasp of how microbes (either individually or collectively) and microbial products impact host physiology, these findings will be able to be translated into next-generation microbiome-based therapeutics that have the potential to alter the treatment landscape of many neurologic and psychiatric conditions. ■

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