Effect of Intestinal Microbial Ecology on the Developing Brain

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T he mammalian gastrointestinal tract harbors a highly diverse microbial population that plays a major role in nutrition, metabolism, protection against pathogens, and development of the immune system. It is estimated that at least 1000 different bacterial species cohabit the human intestinal tract. Most recently, the Human Microbiome Project, using new genomic technologies, has started a catalog of specific microbiome composition and its correlation with health and specific diseases. Herein we provide a brief review of the intestinal microbiome, with a focus on new studies showing that there is an important link between the microbes that inhabit the intestinal tract and the developing brain. With future research, an understanding of this link may help us to treat various neurobehavioral problems such as autism, schizophrenia, and anxiety.

Evolutionary adaptations of the human host to intestinal microbes have led to their symbiotic and commensal relationship.[1] After the birth of a child, diverse classes of microbes from the environment colonize the newborn's gastrointestinal tract, forming the intestinal microbiome, a complex ecosystem with a number of cells that are greater than all the somatic cells of the human by an order of magnitude and harboring approximately 150 times as many genes as the human genome. Various factors are involved in the development of this complex ecosystem. The infant's gestational age, mode of delivery, type of nutrition, and early use of antibiotics modify the composition of this microbiome and may have significant and long-lasting effects.[2-4]

The use of newly developed nonculture-based technologies is providing new insights into the temporal colonization patterns in infants born at term[5,6] or preterm.[7-9] The combination of emerging microbial genomic technologies with metabolic and immunologic analyses is revealing impor-

tant synergies between microbe and host, and when these synergies are disturbed, it might be possible to better understand how microbe and host interact and to develop new treatment strategies for important diseases (such as inflammatory bowel disease and necrotizing enterocolitis) and autoimmune diseases (such as type 1 diabetes, allergies, and asthma).

ROLES OF THE MICROBIOTA

Metabolic Role

Although often thought of as pathogens, the vast majority of microbes harbored in our intestinal tracts are thought to have beneficial effects. These commensal and symbiotic microbiota have varied roles in the human host; they are directly involved in synthesizing vitamins and cofactors, breaking down complex lipids and polysaccharides, and detoxifying waste particles.[10] Microbes can alter metabolism by extracting 40% to 50% of the available energy from nutrients,[11] thus playing a role in obesity. Through fermentation, the microbiota produce short-chain fatty acids that play important roles

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as colonocyte fuel sources and in the maintenance of epithelial junctional integrity.[12] This in turn may relate to disorders for which a "leaky gut" antecedes the development of disease, such as type 1 diabetes.[13]

Immunologic Role

Over the past couple of decades, the intestinal microbiota have become recognized as key components in the development of the immune system. The intestine is the largest and most complex immune organ of the body. Between 70% and 80% of the body's immune cells are in the gut-associated lymphoid tissue, and they can sense changes in the microbiota through specific gastrointestinal cells and receptors. Lymphocyte accumulation and differentiation in the gastrointestinal tract can be triggered by changes in microbiota composition.[14] Even more important, the interaction between the gastrointestinal cells and the commensal bacteria fosters immunological tolerance, whereas the interaction with pathogens triggers inflammatory responses.[15] This cross talk between microbiota and gut mucosal cells (enterocytes, dendritic cells, lymphocytes, macrophages, and M cells) regulates the production of various cytokines and chemokines. These can be proinflammatory, such as IL-8 and IL-1, or anti-inflammatory, such as IL-10 and transforming growth factor β. Anti-inflammatory cytokines are essential to counteract excessive bowel inflammation.[16] Another important host-protection pathway is the microbiota stimulation of T_H17 cells to eliminate extracellular pathogens.[17] Recent publications[18] elucidated that specific bacterial carbohydrates (such as polysaccharide A) can induce innate and adaptive responses, which are important in homeostasis through Toll-like receptors.

There are several interfaces between the immune responses and the microbiota. Toll-like receptors are a family of pattern-recognition receptors that are able to identify specific microbial cellular components, such as lipopolysaccharide and lipoteichoic acid. These enable the host to differentiate between commensal and pathogenic bacteria.[19] If pathogens cross the intestinal mucosal barrier, Toll-like receptors can trigger further inflammatory and immune responses. In addition, Toll-like receptors have important immunoregulatory and nonimmune functions, including maintenance of epithelial homeostasis, protection from epithelial injury, and mucosal repair.[20] Rakoff-Nahoum et al[20] showed that there was extensive erosion of the intestinal epithelium and mucodepletion of glands 5 days after the administration of dextran sulfate sodium (an agent known to be directly toxic to the colon). This further suggests that Toll-like receptor signaling could be involved in the altered behavior that is induced by intestinal dysbiosis.[20] The mechanisms of these functions are currently topics of intense investigation.

An individual's intestinal microbiota composition could be associated with disease processes. For instance, microbial variation has been associated with the pathogenesis of obesity, the induction of hepatic lipogenesis,[21] and chronic fatigue syndrome.[22] In animal models, changes in gut flora can protect against type 1 diabetes by modulating mucosal oxidative stress and the balance between
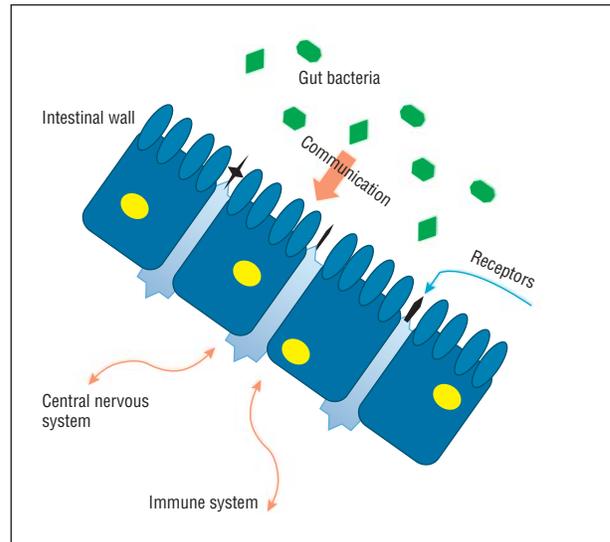

Figure 1. Enteric nervous system, providing bidirectional communication between gastrointestinal cells and the central nervous system. Intestinal epithelial cells mediate interactions between gut bacteria and the central nervous system or the immune system. As bacteria (shown in green) in the intestine come into contact with receptors (shown in black) on the intestinal wall cell surface, the receptors transmit signals to the central nervous system via the vagus nerve pathways (curved arrow to central nervous system) and to the immune system (curved arrow) via Toll-like receptor pathways.

proinflammatory and anti-inflammatory agents.[23,24] Further evidence of the gut microbes' involvement in obesity was evidenced in a study[25] showing that germ-free mice were protected from diet-induced obesity when compared with mice with a normal gut flora because of increased adenosine monophosphate–activated protein kinase activity and elevated levels of fasting-induced adipose factor. The gut microbiota composition is also involved in food allergies owing to its regulatory roles in T_H2, which is thought to interact with the specific glycoproteins, which are associated with allergic responses.[26] For example, infants colonized with *Clostridium difficile* were found to be at higher risk for developing eczema, recurrent wheeze, and allergic sensation.[27]

THE MICROBIOME, THE BRAIN, AND BEHAVIOR

Our brain is intricately connected to our gut through the enteric nervous system, a very complex and extensive system that encompasses between 200 and 600 million neurons.[28] The enteric nervous system provides a bidirectional communication between gastrointestinal cells and the central nervous system[29] (**Figure 1**). Current cutting-edge research has described the ability of enteric microbes to communicate with the brain and has led to the coining of the term *brain-gut-enteric microbiota axis*.[30] This axis is important in brain development, behavior, and gene expression.

Sudo et al[31] were one of the first groups of authors to investigate the role that the microbiome played in the hypothalamic-pituitary-adrenal system. Germ-free–reared mice had exaggerated stress-anxiety behavior with increased corticosterone and adrenocorticotropin levels compared with specific-pathogen–free (SPF) mice.[31]

Germ-free mice colonized with the fecal matter of SPF mice partially normalized their behavior, and animals with probiotics (*Bifidobacterium infantis*) totally reverted to a normal behavior. This research found a decrease in brain-derived neurotrophic factor and in the expression of subunit 2a of *N*-methyl-D-aspartate receptors in the cortex and hippocampus of germ-free animals compared with SPF controls. Thus, brain-derived neurotrophic factor is a neurotrophin factor, which is often associated with change in brain plasticity in response to alterations in intestinal microbial ecology.[31] We can speculate that the normal development of the microbiome is necessary to stimulate brain plasticity. Diaz Heijtz and colleagues[32] found that there was differential expression of synaptophysin and PSD-95, proteins that are specifically involved in synaptogenesis pathways, when changing the gastrointestinal colonization in mice. These proteins were decreased in the striatum of germ-free mice and mice that were adult conventionalized offspring when compared with SPF mice, which suggests that synaptophysin and PSD-95 are subject to modulation by gut microbiota and that, without them, synaptogenesis and synaptic vesicle maturation could be delayed.[32] In summary, gut microbiota modulate a range of neurotrophins and proteins involved in brain development and plasticity.

Other studies have investigated the behavioral phenotypes associated with changes in gastrointestinal microbial ecology. Diaz Heijtz et al[32] found that germ-free mice showed greater exploratory activity and a greater total distance traveled in an open-field activity box when compared with SPF mice, suggesting decreased anxiety-like behavior and increased motor activity. This conclusion was supported by elevated noradrenaline levels, dopamine levels, and serotonin turnover rates in the striatum, which have all been previously associated with anxiety-like behavior.[32] The study[32] also found that germ-free mice that were colonized with normal microbiota early in life displayed similar characteristics as SPF mice, including decreased levels of synaptophysin and PSD-95. These results suggested that changes in microbiota could alter motor activity and anxiety-like behavior.

In a study[33] of germ-free and SPF mice that underwent vagotomy or chemical sypathectomy, a link has been found between the intestinal microbiota and behavior that is dependent on these connections. Bercik et al[33] also found similar results with SPF mice that were treated with antibiotics for 7 days. These mice displayed increased motor activity (stepping down more quickly from an elevated platform and displaying an increased number of transitions between dark and light compartments of an elevated plus maze) and a correlated increased level of brain-derived neurotrophic factor that could explain the behavioral changes observed.[33] Cytokine levels in tissue samples from the colon and small intestine were analyzed, but no difference was found in gut inflammation or specific enteric neurotransmitter levels between the mice that exhibited the altered behavioral phenotype and the mice that did not.[33] Therefore, changes in behavior were not autonomically mediated, and neither the parasympathetic nor sympathetic pathways were involved in the behavioral alterations observed.[33] Such findings suggest that bacterial dysbio-

ses might contribute to the psychiatric disturbances seen in patients with intestinal diseases.

Lyte et al[34] demonstrated behavioral changes in mice after they were infected with *Campylobacter jejuni*. These animals displayed a significant increase in anxiety-like behaviors within several hours of infection, indicating that it was not due to a cytokine-induced immune reaction. Most recently, Goehler et al[35] demonstrated that *C jejuni* activates vagal ascending pathways, which are specific pathways connected to anxiety-like behavior. These experiments, examined together, show that the neural system is able to detect an acute change in gut composition and elicit a reaction.[36] In addition, mice receiving long-term treatment with probiotics (*Lactobacillus rhamnosus*) expressed changes in brain neurotransmitters and displayed a reduction in anxiety and depressive behaviors. This effect was not present in vagotomized mice, thus identifying the vagus nerve as one of the important pathways of bidirectional communication between microbiota and the brain.[37]

Another important concept of the interaction between microbiota and the brain is the possibility of sensitive or critical periods in which these interactions could modulate brain development and overall function.[31-33] In animal models, the early introduction of normal microbes in mice initially exposed to a germ-free environment normalized their behavior compared with controls. But if the introduction of normal microbes occurred after many weeks, the animals failed to normalize their behavior[38] (**Table**).

The observation that most individuals with autism spectrum disorders had gastrointestinal problems lead researchers to explore the hypothesis that gastrointestinal disorders could contribute to worsening behavioral problems, such as decreased concentration, aggression, and/or self-abuse. A recent study by Adam et al[39] found a strong correlation between gastrointestinal symptoms and autism severity, which indicates that children with more severe autism are likely to have more severe gastrointestinal symptoms and vice versa. Thus, it is possible that autistic symptoms are exacerbated by or even partially due to the underlying gastrointestinal problems. Nevertheless, correlation is not causality, and other researchers started to look for mechanistic explanations for this observation. Therefore, there has been an attempt to relate these gastrointestinal problems with an abnormal gut flora, differential activity of digestive enzymes, or altered intestinal permeability.[39,40] Most recently, Adams et al[39] and Iebba et al[40] described changes in the gut flora of autistic patients with higher concentrations of *Lactobacillus* and lower concentrations of *Enterococcus*. Nevertheless, it is important to note that this is an interesting observation but that more research is needed to understand the physiopathology of autism as it relates to an altered microbiome.

SPECULATIONS OF FACTORS AFFECTING MICROBIAL ECOLOGY THAT MAY AFFECT HEALTH

Because the development of the microbiota is affected by so many clinical factors, including mode of delivery, feed-

Table. Microbiota Changes in the Gastrointestinal Tract of Mice and the Effect of These Changes on the CNS[a]

Source	Experimental Design	CNS Effects	Implications of the Study
Sudo et al,[31] 2004	GF mice vs GF mice with recolonized gut vs GF mice with probiotics	GF mice had and exaggerated stress response. GF mice with recolonized gut had less stress response, and GF mice treated with probiotics totally reversed the changes in stress response (to normal stress response).	Microbiota play an important role in the development of stress response. Early in life, there is a critical window when colonization of the gut should occur to ensure normal development of the HPA system.
Diaz Heijtz et al,[32] 2011	GF mice, GF mice with early gut colonization, and SPF mice	GF mice display increased motor activity and reduced anxiety, compared with SPF mice with a normal gut microbiota. GF mice colonized early in life with normal microbiota display similar characteristics as SPF mice, including a decreased levels of synaptophysin and PSD-95.	Normal microbial gut colonization affects development of neuronal circuits involved in motor control and anxiety behavior.
Bercik et al,[33] 2011	SPF mice and GF mice treated with nonabsorbable antibiotics for 7 d	SPF mice treated with antibiotics (with or without vagotomy) transiently altered their microbiota and had increased exploratory behavior and hippocampal expression of BDNF. Colonization of GF mice with microbiota increased exploratory behavior and BDNF levels in the hippocampus.	Changes in microbiota can change motor activity and neurotrophins independently of the autonomic nervous system.
Lyte et al,[34] 1998	Mice infected with *Campylobacter jejuni* vs mice infected with chronic *Helicobacter pylori*	Mice infected with acute subclinical *C jejuni* had rapid activation of vagal pathways and anxiety-like behavior. Mice infected with *H pylori* had abnormal feeding behavior.	There is evidence of rapid and sustained gut-brain communication in response to changes in microbiota. Behavioral changes can be observed after changes in microbiota.
Goehler et al,[36] 2005	Mice infected with *C jejuni*	Infection with *C jejuni* activated neurons in the nucleus of the solitary tract, as well as in brain regions associated with primary viscera-sensory pathways and the central autonomic network.	Peripheral sensory neurons contribute an early signal to the brain regarding changes in the microbiota (such as new potential pathogens).
Bravo et al,[37] 2011	Mice receiving long-term treatment with *Lactobacillus rhamnosus*	Treatment with *L rhamnosus* induced region-dependent alterations in GABA (B1b) mRNA in the brain of these mice compared with controls. Treatment with *L rhamnosus* reduced levels of stress, anxiety, and depression.	Probiotic bacteria have the potential to modulate behavior with regard to anxiety and depression.
Neufeld et al,[38] 2011	GF mice vs SPF mice	GF mice had less anxiety-like behavior compared with conventionally reared SPF mice. Conventionalized adult GF mice failed to normalize their behavior.	Is it possible that a critical window may exist after which reconstitution of microbiota and the immune system does not normalize the behavioral phenotype?

Abbreviations: BDNF, brain-derived neurotrophic factor; CNS, central nervous system; GABA, γ-aminobutyric acid; GF, germ-free; HPA, hypothalamic-pituitary-adrenal; mRNA, messenger RNA; SPF, specific-pathogen–free.

[a] Changes in the environment where animals are reared change the animal's microbiota. Experiments using GF mice (with no bacteria in their gastrointestinal tracts) and conventionalized mice (originally GF mice that have had bacteria reintroduced to their intestinal tracts) showed changes in brain development and animal behavior.

ing mechanism, antibiotic or probiotic treatment, and contact with parents, siblings, and hospital staff, the clinical implications of this recently published link between microbiota and the nervous system pose some intriguing questions (**Figure 2**). For example, what effect does early use of antibiotics have on neonates, especially during critical periods such as the first year of life? Antibiotics are often used in newborns admitted to the intensive care unit; however, routine antibiotic use does not have a statistically significant effect on the incidence of sepsis or mortality with regard to newborns.[41] Cotton et al[42] found that prolonged antibiotic use in preterm neonates can be correlated with a higher incidence of necrotizing enterocolitis. If antibiotic use alters the composition and function of gut microbiota long after the administration of antibiotics has stopped, should we advocate for the repopulation of gut microbiota after antibiotic use or after gastrointestinal diseases, and can this play any role in brain plasticity? Are probiotics good candidates for this sort of treatment? Studies in adults have shown that probiotic use can decrease the incidence of diarrhea, stimulate humoral and cellular immunity, and decrease production of unfavorable metabolites, after antibiotic use.[43] Can these findings be extended to neonates, even preterm neonates? What other factors can change the microbiome? Besides the known benefits of reducing the number of apneas and stabilizing oxygen saturation, respiration, and heart rate, does skin-to-skin contact ("kangaroo care") provide a "natural" source of maternal microbes to repopulate neonatal gut microbiota? Breast milk favors the growth of certain commensal bacterial species and offers approximately 10^9 live bacteria per liter.[44] Could we use breast milk or a changing diet to modulate the microbiome and therefore protect against diseases and protect brain development? Could early breastfeeding prevent diseases through mechanisms related to microbiota changes? When and for how long do these factors exert their effects? Early life exposures may be necessary, especially when considering the possibility of epigenetic programming mechanisms. As developing tissues are exposed to different bacterial species, epigenetics may come into play by influencing gene
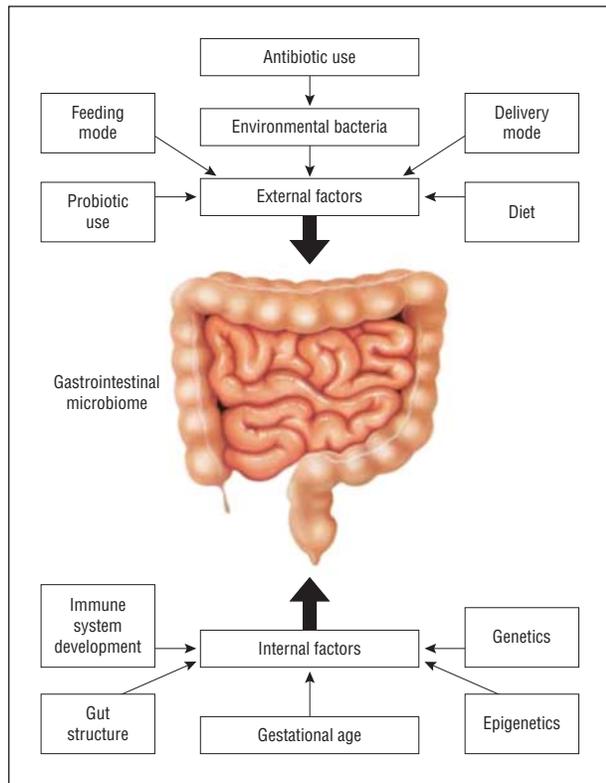
Figure 2. Composition of the gut microbiota in a neonate. The composition of the microbiota is affected by numerous external and internal factors, beginning at birth.

expression, such as DNA methylation and histone modifications.[45] Is there a further epigenetic programming mechanism that changes the human microbiome? Do changes in the composition of commensal bacteria (eg, decreases in *Bacillus fragilis* and its specific bacterial antigens) trigger or exacerbate future central nervous system demyelinating diseases? What effects do changing gut microbiota have on other host diseases, such as autism or obesity? These questions and others need to be explored and answered.

In summary, a description of the complex relationship between resident intestinal microbiota and health and disease is rapidly emerging. Most recent evidence shows that the intestinal microbiota communicate with the brain and could modify cognitive and behavioral functions. The existence of a brain-gut-enteric microbiota axis is an exciting discovery and promises possible, new preventive and therapeutic opportunities.

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