Review

Biological Psychiatry

Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder

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ABSTRACT

Autism spectrum disorder (ASD) is a serious neurodevelopmental disorder that affects one in 45 children in the United States, with a similarly striking prevalence in countries around the world. However, mechanisms underlying its etiology and manifestations remain poorly understood. Although ASD is diagnosed based on the presence and severity of impaired social communication and repetitive behavior, immune dysregulation and gastrointestinal issues are common comorbidities. The microbiome is an integral part of human physiology; recent studies show that changes in the gut microbiota can modulate gastrointestinal physiology, immune function, and even behavior. Links between particular bacteria from the indigenous gut microbiota and phenotypes relevant to ASD raise the important question of whether microbial dysbiosis plays a role in the development or presentation of ASD symptoms. Here we review reports of microbial dysbiosis in ASD. We further discuss potential effects of the microbiota on ASD-associated symptoms, drawing on signaling mechanisms for reciprocal interactions among the microbiota, immunity, gut function, and behavior. In addition, we discuss recent findings supporting a role for the microbiome as an interface between environmental and genetic risk factors that are associated with ASD. These studies highlight the integration of pathways across multiple body systems that together can impact brain and behavior and suggest that changes in the microbiome may contribute to symptoms of neurodevelopmental disease.

Keywords: Autism, Gastrointestinal tract, Gut-brain axis, Inflammation, Microbiota, Neurodevelopment

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by impaired social communication and the presence of repetitive, or stereotyped, behaviors. In addition to the spectrum of behavioral abnormalities in ASD, several medical comorbidities are also observed in ASD individuals, including seizures, anxiety, sleep deficiency, and metabolic impairments (1–5). Brain changes in ASD include a reported 67% more neurons in the prefrontal cortex, more than 17% increase in brain weight, and abnormal cortical patterning. Further transcriptomic analysis of postmortem brains from human ASD individuals revealed altered expression of proteins that are important for functional synaptic activity in the prefrontal cortex and cerebellum (6–9). In addition, several brain imaging studies in living patients report correlations between abnormal frontal lobe connectivity, cortical morphology, amygdala activation, and language control centers in ASD individuals compared with neurotypical control subjects (10–13).

The exact causes of ASD are unclear but are believed to involve a combination of genetic and environmental risk factors. It is estimated that the de novo mutations, common variants, and short nucleotide polymorphisms identified across numerous ASD cases altogether account for approximately 50% of the disorder (14,15). As such, many studies highlight the possibility for environmental risk factors and associated medical comorbidities to contribute to core neurobehavioral symptoms of the disorder. Immune dysregulation and gastrointestinal (GI) disturbances are of particular interest in light of numerous studies reporting ASD-associated abnormalities in the peripheral nervous system, enteric nervous system, and neuroimmune system. Postmortem brains of ASD patients show increased microglia and astroglia activation in the cerebellum and cerebral cortex, along with increased levels of proinflammatory cytokines in the cerebrospinal fluid and cortical regions of the brain (16). Moreover, there are ASD-associated genes that encode for features of the immune system, and mutations in those genes are linked with the ASD phenotype, including loss of structural and functional connectivity in brain regions important for sociocommunicative function (17,18). Parallel studies reveal greater prevalence of GI disorders and disturbances in ASD populations compared with control subjects (19,20). Comorbid GI symptoms in subsets of ASD individuals include diarrhea/constipation, abdominal pain, and gastric reflux. Deficient integrity of the gut epithelium and increased intestinal permeability are also reported (21).

These associations of ASD with greater prevalence of immune dysregulation and GI issues motivate explorations of the ASD gut microbiome, which is emerging as a key regulator of intestinal physiology, neuroimmunity, and host behavior. Many studies report dysbiosis of the gut microbiota in ASD individuals. Perhaps most intriguingly, gnotobiotic animal and

SEE COMMENTARY ON PAGE e35

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probiotic studies demonstrate that microbiome changes can directly cause behavioral and neuropathological endophenotypes of human ASD. This avenue of research is critical for determining roles for microbiota dysbiosis and specific bacterial species that may contribute to or modify symptoms of ASD. In this review, we examine links between the microbiome and ASD symptoms, drawing on data from animal experiments showing causal effects of the microbiome on immunity, brain, and behavior. We further explore the notion that the microbiome plays an important role in mediating symptoms of ASD and may be a key consideration for understanding immune and GI dysfunction in subsets of ASD individuals.

GUT MICROBIOTA ON ASD-RELATED ENDOPHENOTYPES IN ANIMAL MODELS

The microbiota plays an important role in regulating normal host physiology, metabolism, nutrition, and brain function. Because mammals are unable to synthesize many key nutrients, the gut microbiota assumes a primary role in digestion, synthesizing essential dietary vitamins and cofactors, such as vitamin B, riboflavin, thiamine, and folate. In addition to roles for the microbiome in regulating digestion, GI physiology, and immunity, increasing research reveals the ability of the gut microbiota to signal across the so-called microbiota-gut-brain axis. Raising animals in the absence of microbial colonization results in abnormalities in a variety of complex behaviors, pointing to the possibility that the microbiota modulates behavioral outcomes in animal models of neurodevelopmental and neurological disorders. Social communication deficits and the presence of stereotyped behaviors are hallmark diagnostic features of human ASD, and other behavioral abnormalities, such as anxiety, seizures, and hyperactivity, are often comorbid. Two independent studies demonstrate that germ-free mice exhibit decreased sociability or propensity to interact with a novel mouse versus a nonsocial object, and reduced social preference to interact with an unfamiliar mouse versus familiar mouse (22,23). This is similarly seen in germ-free rats, which exhibit reduced social investigation of an unfamiliar partner (24). Germ-free mice also display differential gene expression, exon usage, and RNA editing in the amygdala, a key emotional center of the brain mediating responses to social stimuli (25). Interestingly, social-behavioral abnormalities are impaired particularly in male mice, which parallels the male bias that is characteristic of ASD. Moreover, some of the social impairments are corrected by postnatal colonization of germ-free mice with a wild-type mouse gut microbiota at weaning, pointing to the ability to reverse abnormalities in social interactions (26). This is intriguing in light of reports that risperidone, a Food and Drug Administration–approved treatment for autism, does not correct social abnormalities in human ASD or mouse models of ASD (27,28).

Modulation of the maternal environment is also of interest given the neurodevelopmental origins of ASD. Though there are numerous perinatal risk factors that influence maternal-fetal physiology including stress, infection, gestational diabetes, breastfeeding versus formula feeding, maternal age, antibiotic use, and obesity, the changes in the gut microbiota can also be a relevant risk factor. A recent study by Buffington *et al.* (29) showed that high-fat diet–induced maternal obesity alters the offspring gut microbiome and causes social-behavioral deficits that are linked to altered signaling in the mesolimbic reward system. Remarkably, transfer of the gut microbiota from control mice into offspring of high-fat diet–fed mothers completely corrected the impairments in sociability and social novelty seen in the mice, demonstrating a key role for the gut microbiome in regulating mouse social behavior. Furthermore, treatment with the gut bacterium *Lactobacillus reuteri* alone sufficiently restores social behaviors, revealing specificity of social-behavioral modulation in this model to a particular bacterial taxon. The beneficial effect of the microbiome in these studies was associated with its ability to promote hypothalamic levels of oxytocin and activation of neurons in the ventral tegmental area. This novel finding supports the promise of probiotic treatments for social behaviors. Importantly, however, we caution against use of *L. reuteri* for ASD until additional studies examine broader physiological effects of the bacterium on host biology and until such exploratory treatments are validated to be safe and effective in humans.

In addition to social interaction, there is some evidence that manipulation of the microbiome by probiotic treatment can modulate communicative and repetitive behavior in mice. In a mouse model of maternal immune activation, a principal environmental risk factor for autism, mice develop core behavioral features of ASD (impaired social communication and stereotyped behaviors), as well as several neuropathologies and comorbid GI and immunological symptoms relevant to the human disorder (30–32). Altering the postnatal gut microbiota by early life treatment with the human gut bacterium *Bacteroides fragilis* sufficiently ameliorated deficits in the frequency and quality of adult ultrasonic vocalizations and reduced stereotypic burying behavior exhibited by the ASD-like mice. Although the mechanisms underlying the ability of the gut microbiota to modulate ASD-related behaviors are unclear, improvements in GI integrity and alterations in serum metabolites could be involved. Consistent with a possible role for the microbiome in contributing to the symptoms of ASD, it would be interesting to examine the presence and severity of ASD-related behavioral and neuropathological abnormalities in ASD animal models raised on a germ-free background or depleted of gut microbes using treatment with broad-spectrum antibiotics. Such studies would enable dissection of causal mechanisms linking the microbiome to core ASD behaviors and neuropathologies.

Anxiety-like behavior is also observed in subsets of individuals with ASD and is commonly recapitulated in animal models for ASD. The microbiome modulates anxiety-like behavior in mice, as germ-free mice exhibit increased locomotor activity and decreased anxiety-like behavior in several tasks, including open field exploration, the elevated plus maze, light-dark box, and platform step-down test (25,33,34). These behavioral changes are correlated with altered expression of genes involved in second messenger pathways and synaptic transmission, including postsynaptic density protein 95 and synaptophysin in the striatum (35). Moreover, these behavioral changes can be related to learning and memory deficits seen in both germ-free and antibiotic-treated mice (36,37).

Germ-free animals also exhibit several abnormalities in brain gene expression and neurophysiology. For example,