Implication of Gut Microbiome in Brain Chemistry and Behavior

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ABSTRACT

Human microbiome colonization starts during the birth and keeps modulating and establishing itself till adult stage. Advances in research techniques have shown evidences displaying the role of microbiome in brain and behavior development in addition to its involvement in immune, metabolic and physiologic function. Dysbiosis of gut microbiome disturbing the homeostasis with host leads to various diseases. Absence of microbiome in mouse affects the stimulation of nerves with simultaneous changes in behavior and brain chemistry. The perturbed microbiota during neonatal life leads to behavioral shifts which may persist even in adulthood. Short chain fatty acid (SCFA) released after microbial fermentation of undigested food components are able to regulate microglia homeostasis which serve as macrophages of the central nervous system (CNS) and are critical in CNS diseases. Impaired microglia signaling is implicated in neurodevelopmental, neurodegenerative disorders and aging. The administration of antimicrobial agents in mice has revealed altered anxiolytic behavior and correspondingly the brain chemistry, i.e., changes in brain derived neurotrophic factor (BDNF) level. The administration of combinations of probiotic bacteria affect brain function including anxiety, mood, and memory indicating their potential use in therapeutic approach depending upon their strain specific effect. Thus these accumulating reports indicate relevance of gut microbiome in brain chemistry and physiology consequently in brain related disorders.

Keywords: Gut microbiome, Germ-free mice, Short chain fatty acid , Intestinal epithelial cells , Central nervous system , Gamma aminobutyric acid

BACKGROUND

Human microbiome colonization begins at birth continues to develop with the development of the health and immune system which is highly affected by the microbiome. The microbiome development is determined by various factors like prenatal exposure, mode of delivery, solid food introduction, antibiotics and geography. The development of the microbiota begins well before the infant is born. Contrary to what was previously thought amniotic fluid is not sterile. In some cases, bacterial presence in the amniotic fluid is associated with a diseased state and preterm birth [1]. Various areas on and within the human body, serves as niche for microbial communities which results in human microbiome [2]. The gastrointestinal tract harbors 1014 microorganisms spread over 500 to 1,000 species and perform many physiological functions and thus influence human biology profoundly [3]. The mammalian microbiota is highly variable at lower taxonomic levels. The Gastrointestinal tracts (GIT) consist of anaerobic Firmicutes and Bacteroidetes and Proteobacteria, Verrucomicrobia, Fusobacteria, Cyanobacteria, Actinobacteria and Spirochetes and few species of fungi, protozoa and viruses [4]. Though each person has a distinct and highly variable microbiota, however they share a conserved set of gut colonizers (the core gut microbiota) and genes (the core microbiome) among individuals. In terms of concentration of microbiota, the greatest population density is observed in the distal ileum and colon. The metagenome, of the intestinal microbiota has more than 100 times the number of genes of the human genome. Each individual is populated by roughly 15% of the 1,000 or more species of intestinal bacteria that have been described [5]. The human genome codes for approximately 23,000 genes, yet some experts have suggested that the total information coded by the human genome alone is not enough to carry out all of the body's biological functions specifically digestion of complex carbohydrates [6].

The implication of bacteria has been proposed in pathogenesis of various diseases, such as diabetes, malnutrition, inflammatory bowel disease [7,8], inflammatory bowel syndrome, asthma, atopy, autism like disorders and obesity. Advancing evidences show role of microbiome in brain development and behavior. This involvement is adequate enough to affect brain physiology and neurochemistry, thus might be involved in brain related disease [9,10]. Researchers have drawn links between gastrointestinal pathology and psychiatric neurological conditions such as anxiety, depression, autism, schizophrenia and neurodegenerative disorders in animals however the data are more limited humans and required validation [6,11].

This link could be sustained by an abnormally leaky gut barrier leading to increased translocation of bacteria and luminal content thus affecting the gut homeostasis leading to increased growth of pathobionts which can promote inflammation. Bacteria colonizing the gut mucosa have the ability to strongly adhere to intestinal epithelial cells (IECs), to invade IECs by a mechanism involving actin polymerization and microtubule recruitment and to induce granuloma formation *in vitro* [12]. Microbial metabolites have been shown to affect the blood-brain barrier in mice [13]. It has been reported that early life stress in rat causes changes in fecal microbiota composition which effects the adulthood behavior [14] and is a determinant of vulnerability to a variety of disorders that include dysfunction of the brain and gut [15]. It is predicted that behavioral shifts caused by perturbed microbiota during neonatal life may persist even in adulthood. In this review we have described the evidences which confirm the substantial implication of microbiome in brain and behavior development, consequently the related diseases. This would help researchers to further design studies to unravel the potential contribution of gut microbiome in brain related disorders and its use in therapeutic approach.

Microglia homeostasis

Daniel et al demonstrated that SCFA released after microbial fermentation of undigested food components are able to regulate microglia homeostasis which serve as macrophages of the central nervous system (CNS) and are critical in CNS diseases. Microglia signaling is implicated in neurodevelopmental, neurodegenerative disorders and aging. Daniel and colleague observed that in germ-free (GF) mice, microglia are affected and show immature functions, affecting the innate immune responses. Microglia properties are shown to be affected even with temporal destruction of host microbiota. Microglia functional properties are related with microbial complexity, as restricted microbial community relates with defective functions. In one study FFAR2 (SCFA receptor) deficient mice displayed microglia defects similar to GF conditions [16,17]. These observations indicate that host bacteria regulating microglia maturation and function may be involved in pathophysiology of CNS disease.

Effect on germ free mice

The Brain derived neurotrophic factor (BDNF) is essential in the maintenance and survival of neurons, and involves in neuronal circuit formation and cognition. Germ free mice show reduced BDNF expression in their hippocampus and cortex. This reduction is associated increased anxiety and increasing brain fog (the loss of intellectual functions such as reasoning; memory loss; and other neurological abilities) [18,19]. BDNF deficient mice show impaired stimulation of nerves with simultaneous changes in behavior and brain chemistry [20,21]. In experimental infection models having the alteredmicrobiota profiles, also show reduced BDNF expression in the hippocampus and cortex [19,22].

Recent studies have shown that germ free mice exhibit more exploratory and risk taking behaviors as well as more locomotion than specific pathogen free (SPF) mice, that these behaviors are normalized after early, but not late, bacterial colonization [23,24]. Germ free mice have increased turnover of anxiety related neurotransmitters such as noradrenaline, dopamine and 5-hydroxytryptamine (5-HT), altered levels of proteins involved in the regulation of development and function of neural synapses, such as the synaptic vesicle glycoprotein and (PSD95; also known as DLG4) in the striatum. The commensal intestinal microbiota is crucial for passive membrane electrical characteristics involving the enteric nervous system (ENS). This support a potential interacting mechanism for the signaling between the gut microbiota and nervous system [19,25,26].

However, Neufeld et al. [24] showed that female germ free mice show reduced anxiety behavior and simultaneous increased BDNF mRNA expression and decreased5-HT1A auto receptor expression in the dentate gyrus of the hippocampus [24,27], but it is not clear that the changes in BDNF mRNA level in hippocampus gyrus has some significant link with the reduced anxiolytic behavior. Neufeld et al. [27] also observed increased stress hormone (corticosterone) in the plasma of the germfree mice with decreased N-methyl-D-aspartate receptor (NMDA receptor) subunit (NR2B) in the amygdala. With this observation the authors speculated this change may contribute to the anxiolytic like effects.

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In another study, male germ free mice showed increased stress response without basal functional changes in adrenal axis of hypothalamic pituitary. However this was associated with decreased BDNF in hippocampus and cortex and decreased NR1 in hippocampus [28,29]. The exaggerated response of the hypothalamic pituitary to mild stress in germ free mice was normalized after monocolonization of mice with *Bifidobacterium longum* subsp. *infantis* (strain not identified) at 6 weeks of age but not at 14 weeks [29].

Other studies

Others worked with a mouse strain called NOD (non-obese diabetic), and observed induced social avoidance behavior in NOD mice, without changes in overall locomotor activity after daily gastric gavage with water or saline as vehicle for two weeks. However subcutaneous injection of vehicle did not affect behavioral effect. Gastric gavage of antibiotic cocktail depleted the gut microbiota but did not affect the behavior in NOD mice. This indicates that depressive-like behavior is mediated by alterations in gut microbiota. However similar study performed in B6 mice, showed no change in behavior and microbiome of B6 mice. Behavioral differences in genetically different mice are associated with specific gut microbiota composition in specific mouse strain.

Mice with gastric gavage with vehicle also had thinner myelin sheaths in the medial prefrontal cortex than their antibiotic-fed group indicating the involvement of microbiome in myelination. They further observed that after transferring gut microbes of a control NOD mouse donor to B6 mice, resulted in "stressed" behavior [11,30,31]. Taken together, these studies suggest that the development of neuronal circuitry in mice is influenced by the presence of intestinal bacteria and it seems as essential component for full development.

It is suspected the stress might be involved in changing the vaginal microbiota. In a study it was observed that subjecting pregnant mice to stress resulted in decreased lactobacilli bacteria in their vagina, which are the main source of the microbes that colonize the guts of offspring. The altered vaginal microbiota is l transferred to vaginally born pups. This indicated that vaginal microbiota may affect neurodevelopment in offspring. Author and team suggest that vaginally born pups from stressed mums can be treated with the vaginal microbiota of non-stressed mice [32].

Ability of antimicrobial drugs to altered behavior

In healthy subjects, the intestinal microbiota is generally stable over time, in part owing to the presence of a core microbiome [33]. The antimicrobial drugs commonly considered to disturb the intestinal homeostasis due to shifts in microbiota. Transient shift of the gut microbiota composition in mouse induced by non-absorbable antimicrobial components, are associated with altered behaviour, and changes in BDNF levels [34]. The ability of antimicrobial drugs to alter behavior and depressive state serves as an evidence for the role of gut bacteria in nervous system modulation. Another study reported that oral administration of *broad spectrum antibiotic* in adult BALB/c mice, perturbed the microbiota and altered behavior or increased locomotor behavior as tested by the step down and the light/dark box tests [35].

Studieshas shown that gut microbiota involvement is sufficient enough to interfere with neurotransmitter and consequently affecting the behavior and neuron signaling. Gut microbial enzymes like tryptophan decarboxylase can result in increased Tryptamine, by decarboxylating tryptophan. Tryptamine induces the secretion of serotonin (a neurotransmitter) by enterochromaffin cells. Serotonin release from these cells, account for 60% of peripheral serotonin in mice and more than 90% in humans. Significantly less serotonin was observed in blood of germ-free mice which was restored by introducing to their guts spore-forming bacteria. Bifidobacteria treatment can cause higher tryptophan and kynurenic acid levels as shown in rat. Lactobacillus and Bifidobacteria are known to produce gamma aminobutyric acid (GABA) thus affecting its level. Mice with active colitis (related with bacterial dysbiosis), showed reduced dopamine level in feces [36].

Probiotics' impact on nervous system and behavior

Certain probiotic strains may lead to strain specific modulation from the aspects of microbiome gut brain axis. Clinical evidences have reported the role of probiotic in improving the mood, anxiety and stress in patients with IBS and chronic fatigue For e.g. *Lactobacillus reuteri* probiotic [37] reduces anxiety and stress induced increase of corticosterone in mice [38]. The role of this probiotic is justified by its ability to alter GABA-A and GABA-B receptor's expression at the mRNA level in CNS. GABA expression is related to anxious and depressive state in animal [28]. These effects of *L. reuteri* are dependent on vagus nerve indicating the direct interaction of bacteria to brain through parasympathetic innervations [28].

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The administration of combinations of probiotic bacteria has also been shown to affect brain function. For e.g. *Lactobacillus rhamnosus* str. R0011 and *Lactobacillus helveticus* str. R0052 in combination helps to avoid stress induced memory loss [39]. Another example is combination of probiotics *Lactobacillus helveticus* and *Bifidobacterium longum* reduces anxiety in animals (rat). This combination also decreased serum cortisol in patients (human) resulting in beneficial psychological effects [40]. *B. infantis* intervention suppresses stimulation induced increase in peripheral pro inflammatory cytokines and plasma tryptophan [41] both of which are involved in depression [42,43]. *Bifidobacterium infantis* reduced depression in mice during the forced swim test [41,44]. Desbonnet et al. [45] showed that some probiotics for e.g. *B. infantis* resulted in restored few of the early life stress induced changes, maternal separation model. Thus these accumulating reports indicate impact of probiotic intervention on brain and behavior. These reports indicate an established direct link between gut microbiome and nervous system and also the relevance of gut microbiome in brain chemistry and physiology.

CONCLUSION

The accumulated findings described in this review confirm the potential contribution of gut microbiome in brain behaviour and chemistry. Identifying the microbe genus, species or strain, specifically affecting the brain function may lead design therapeutic inventions. This suggests requirement of the extensive analysis of communication between brain and gut microbiome which may help to further understand the pathophysiology underlying the various brain related diseases and to design therapeutic inventions.

CONFLICT OF INTEREST

There are no conflicts of interest.

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