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The Vaginal and Gut Microbiotas: A Review of the Development and Impacts of Bacterial Populations on Inflammation and Psychiatric Disorders

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The Vaginal and Gut Microbiotas: A Review of the Development and Impacts of Bacterial Populations on Inflammation and Psychiatric Disorders

By

Shannon Marie Howes

An Honors Capstone
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Dedication:

This honor's thesis is dedicated to individuals struggling with mental illness and to the medical professionals, family, and friends who support them.

This thesis is also dedicated to Anna Petroff. Without her guidance, patience, and constructive criticism, this research would have never been completed.

Abstract

Numerous studies have proposed a connection between gut bacteria populations, inflammation, and psychiatric disorders, now called the "microbiota-gut-brain axis." This project explores the commensal bacteria commonly found in the human vagina and gut and the pathogenic potential of these and other bacteria as they relate to inflammation and psychiatry. For example, alterations in the abundance of select bacterial phyla is connected to an increase in pro-inflammatory cytokine production, and there is a correlation between those pro-inflammatory cytokines and psychiatric health. This project identifies these bacteria and the pathways through which they influence human health and development. At conclusion, recommendations are given on ways research in the gut microbiota field may be improved.

Introduction

Links between psychiatric disorders, inflammation, and gastrointestinal disease have sparked interest in the relationship between the gut and brain and brought about the term “gut-brain axis.” This term refers to the bidirectional biochemical interactions that occur between the central nervous system and the gut. For example, gastrointestinal disorders are more prevalent in individuals on the autism spectrum than those in control groups [1]. Numerous studies have sought to measure the levels of certain bacterial phyla in individuals suffering from psychiatric or inflammatory disorders compared to controls in an effort to establish a relationship between the two. Many of these studies implicate bacteria as potential agitators that worsen symptoms or as being complicit in causing the disease. The linkages between gut bacteria and neurological functioning are referred to as the “microbiota-gut-brain axis” or, in the case of studies examining genetic compositions of bacteria, the “microbiome-gut-brain axis.” The host and its microbes together makeup what is known as the “holobiont.” The human holobiont is composed of an

approximately equal number of bacterial and human cells [2]. Because bacteria are so abundant in humans, understanding their functions and how they can impact the body and mind is critical to human health.

The Initial Colonization of the Gut Microbiota and the Role of the Maternal Vaginal Microbiome in Gut Microbiome Development

Introduction

In order to understand the complexity of gut microbial populations, it is critical to examine where gut bacteria come from. Bacteria are organisms and as such, must come from existing organisms. Those existing populations come from an individual's mother, who already has an established microbiome originating from her own mother that is then further influenced by environmental factors in everyday life. It was once believed that the fetus was sterile and that initial colonization of an individual occurred during natural birth as the infant passed through the vagina, which is rich in bacteria, and that bacteria was only present *in utero* in cases of intrauterine infection [3]. The maternal vaginal bacteria do contribute to the early colonization of the gut, but the more recent understanding is that the first bacteria to colonize an individual's gut come from the placenta, amniotic fluid, and meconium before birth [4]. Therefore, a review of fetal sources of bacteria and of the maternal vaginal microbiome and factors impacting it is critical to understanding the development of an individual's microbiota and early infant health, which can have lasting impacts on an individual.

Fetal Colonization

The first colonization of the infant gut comes from the placenta, amniotic fluid, and—if it is released prior to birth thereby coating the fetus in the enclosed womb environment—the meconium, which is the infant's first bowel movement [5]. Of course the source of these bacteria

is still the mother, but the exact mechanism of how her bacteria colonize the placenta is unknown. Interestingly, placental populations most resemble those of the mother's mouth, so researchers hypothesize that there may be a way for bacteria in the mother's mouth to travel through the blood to the placenta, but this theory is only speculative [5] The placenta provides blood and nutrients to the infant from the mother via the umbilical cord. Bacteria found in the placenta include *Fusobacteria, Firmicutes, Tenericutes, Proteobacteria,* and *Bacteroidetes* [5]. Additional findings indicate that the dominant bacterial species in the placenta is *E. coli*. The amniotic fluid that is ingested once the fetal gut has developed has bacteria. This ingestion of amniotic fluid is able to directly colonize the gut and contributes to the population of bacteria found in the meconium. The meconium may act as a source of colonization if it is released prior to birth or otherwise accesses the infant's mouth. In one study, 77.08% of neonates had bacteria in their meconium, and the bacteria found included *Staphylococcus, Enterobacteriaceae, Enterococcus, Lactobacillus,* and *Bifidobacterium* [6]. Colonization during the fetal stage is one factor that determines the earliest compositions of an infant's gut, but the birth canal further colonizes it in natural birth.

An Overview of the Vaginal Microbiome

The vaginal microbiome is of particular importance because it is one of the first colonizers of vaginally delivered neonates' guts. Before puberty, the vaginal microbiome is primarily composed of anaerobic bacteria [7]. The vaginal microbiome after puberty is typically dominated by *Lactobacillus crispatus, L. iners, L. gasseri, or L. jensenii* [8]. *Lactobacillus* species produce lactic acid, which results in a low vaginal pH and aids in protection against gram-negative pathogenic bacteria and HIV [8]. Protection from pathogenic bacteria is important because they can elicit powerful pro-inflammatory responses by activating toll-like receptors to

release cytokines [7]. If pathogenic bacteria are present, they may be passed to neonates born via the birth canal. Vaginal bacterial diversity is lowered during pregnancy, and the vaginal microbiota is dominated by *Lactobacillus* species, *Clostridiales, Bacteroidales,* and *Actinomycetales* [9]. It is believed that the increased levels of *Lactobacillus* in pregnant women is correlated with increased levels of estrogen [10]. A study by Lewis et al. found the composition of the vaginal microbiome varies between different ethnicities. This study found African American women born in the U.S. have greater microbial diversity and a lower abundance of *Lactobacillus* than Caucasian women born in the U.S. This difference in populations between ethnicities can contribute to differences in neonatal microbiotas and could potentially impact infant health. The implications for neonatal health outcomes stress the importance of a healthy population of bacteria in the vagina.

Maternal Bacterial Vaginosis and its Effects on Neonates

Bacterial vaginosis is a condition characterized by abnormal bacterial populations in the vagina. Vaginosis populations have a lowered abundance of *Lactobacillus* species and a greater population of anaerobic bacteria [11]. Findings also show that bacterial vaginosis in pregnant women has been linked to premature birth, chorioamnionitis (inflammation of fetal membranes), low birth weight, an increased risk of NICU admission, and miscarriage. In addition, there is a 60% increase in risk of neonatal sepsis (bacterial blood infection) occurring when the mother has bacterial vaginosis. This evidence further supports the claim that the vaginal microbiome is key to infant health.

Maternal Stress and its Impacts on the Maternal Vaginal Microbiome and Infant Health

External factors, such as maternal stress, can also influence the vaginal microbiome. An experiment performed on mice found that exposure to prenatal stress resulted in an altered

maternal vaginal microbiota [12]. These changes impacted the abundances of amino acids, which form proteins necessary for biological processes, in offspring. It is important to note that the gut microbiotas of vaginally-delivered offspring were influenced in a sex-specific manner [12]. Microbiomes being altered in a sex-specific manner may offer explanations as to why certain illnesses are more prevalent in one sex than the other. Maternal stress can also cause long-term alterations to the hypothalamic-pituitary-adrenal axis and central nervous system, which play prominent roles in psychiatric and neurodevelopmental health [13]. As is evidenced, the vaginal microbiome can determine early infant health if the infant is delivered through the birth canal.

Gut Microbiota Development – Method of Infant Delivery

As noted, infants delivered vaginally acquire additional microbes from the birth canal, and those delivered via cesarean section acquire them from the hospital environment [14]. Full-term infants delivered vaginally have greater gut microbial diversity than preterm infants delivered via cesarean section [15]. *Bifidobacterium*, *Bacteroides*, and *Escherichia coli* are found in neonates delivered vaginally [14]. In a mouse experiment on prenatal stress, it was found that inoculating mice delivered via C-section with maternal vaginal fluid resulted in gut microbiota profiles similar to mice delivered vaginally [12]. This inoculation can have profound impacts on the development of the gut microbiome. The method of delivery is a key player in determining early gut microbial composition.

Gut Microbiome Development – Breastfed vs Formula-Fed Infants

Differences have also been found between formula-fed infants and those given breast milk from their mothers. *Bifidobacterium, Lactobacillus, Staphylococcus, Bacteroides, Streptococcus, Enterococcus,* and *Clostridium* are found in breast milk [14]. *Bifidobacteria* hold the majority in breastfed infant intestinal microbiota [16]. Infants fed their mother's milk rather

than formula or donor milk had an overall greater diversity of gut bacteria [15]. Formula-fed infants have lower levels of *Bifidobacterium,* which inhibit the growth of pathogens [14]. It was also found that those fed formula or donor milk had a dominance of *Enterobacteriales.* Diverse gut microbiota with members of *Bifidobacterium, Clostridiales,* and *Lactobacillales* dominating may protect premature infants from necrotizing enterocolitis [14]. Ergo, the method of feeding impacts an infant's gut microbial development and health.

Gut Microbiome Development – Maternal & Neonatal Antibiotic Usage

Another impact on early infant health and the developing microbiome is antibiotic usage. Premature infants whose mothers were given antibiotics while pregnant with them or who received antibiotics directly after birth had a narrower diversity of bacteria when compared to those who were not exposed to antibiotics [14]. The gut bacteria found in infants exposed to antibiotics consisted only of *Enterobacter, Escherichia, Enterococcus,* and *Staphylococcus* [14]. Lowered microbial diversity has been associated with diseased states and could therefore be dangerous to infant health.

Gut Microbiome Development – The Birthing Environment

The birthing environment plays a role in early gut colonization as well. Environmental factors include contact with medical personnel and time spent in the NICU, but the exact impact on the infant's gut microbiota has yet to be determined [14].

Summary of Gut Microbiota Development

The development of the microbiome begins prior to birth. The placenta, amniotic fluid, and meconium all contribute to fetal gut microbiome colonization. If the infant is delivered via the birth canal, vaginal bacteria play a major role in early gut microbiota development. Dysbiosis of the vaginal microbiota can in turn negatively impact infant health. This finding

makes vaginal health critical to infant health and perhaps even future health outcomes. Infants born via C-section have different bacteria because they do not go through the vagina, and their microbiomes tend to be shaped more by the environment into which they are born. Exposure to antibiotics before or after birth, the type of food, and the environment in which the infant is born also impact early development. Understanding the initial colonization of the gut microbiome aids in the understanding of later development and potential consequences of dysbiosis.

Typical Gut Microbiota & Variables Impacting Composition

Introduction

A healthy population of gut bacteria is conducive to an individual's well-being. One of the greatest difficulties faced by researchers is establishing what constitutes an ideal gut microbiome. This problem exists because gut bacterial populations vary considerably from person to person. Factors that can impact the development and maintenance of the gut microbiome include the environment, age, genetics, gender, diet, and antibiotic use. Despite these variables, there are bacterial phyla that appear consistently in subjects from a variety of studies. These observations point towards a basic composition of common gut bacteria.

Overview of Commensal Gut Microbiota Populations

The gut contains a diverse array of bacteria with varying populations that are dependent upon the area of the gut examined. The colon contains 10^{14} bacteria and has the highest abundance of bacteria of any human organ [2]. The lower small intestine harbors 10^{11} bacteria [2]. The upper small intestine and stomach each have 10^{7} bacteria [2]. These microorganisms aid in digestion, metabolism, and immunity. Human gut microbiomes vary based on geographic location, diet, genetics, gender, age, and usage of antibiotics, but there is a 40% similarity in intestinal microbial gene composition amongst at least half of the individuals in the general

population [17]. Both gram-positive and gram-negative bacteria are found in the gut. Gram-negative bacteria have a cell wall with a thin layer of peptidoglycan and are generally associated with pathogenicity. Gram-positive bacteria have thick layers of peptidoglycan. More than 90% of the bacteria in the gut belong to the phyla *Bacteriodetes* and *Firmicutes* [18]. Bacteriodetes are gram-negative, and Firmicutes are mostly gram-positive. Other than *Bacteroidetes* and *Firmicutes,* major commensal gut bacterial phyla include *Actinobacteria*, *Proteobacteria*, and *Fusbobacteria* [16]. Major genera include *Bacteroides (*phylum *Bacteriodetes), Prevotella (Bacteroidetes)*, and *Ruminococcus (Firmicutes)* [19]. *Bifidobacterium* (phylum *Actinobacterium*) and *Lactobacillus* (phylum *Firmicutes*) are commonly found in the intestines [16]. The exact abundances of these bacteria vary from individual to individual. Three different enterotypes have been proposed to classify human populations based on their gut bacteria: Enterotype I is enriched in *Bacteroides,* Enterotype II has a greater population of *Prevotella* and *Desulfovibrio*, and Enterotype III has high levels of *Ruminococcus* [20]. Which enterotype an individual has is determined both by lifestyle and biological factors.

Environment

One factor in determining the composition of the gut microbiome is exposure to green spaces. Urban environments deprived of green spaces have less microbial diversity than the natural environment [21]. This lack of bacterial diversity in the external environment results in lowered exposure to diverse microbes and in turn leads to a less diverse gut microbiome. Studies comparing the prevalence of inflammatory bowel disease, which is connected to gut microbial composition, in urban and rural environments found that there is a higher incidence of inflammatory bowel disease in urban environments due to the lowered exposure to green spaces [13]. Differences in environments can also be seen at an international level, which may be

explained by cultural differences that impact time spent outdoors or the geographic location. In a Japanese study, researchers found that Japanese adults have higher levels of the genera *Blautia* and *Bifidobacterium* and lower levels of *Bacteroidetes* than adults in other countries [22]. It is important to note that cultural differences in diet could also contribute to differences between nations.

Diet and Obesity

Diet is another major determinant in gut microbiota composition. Protein-rich diets are associated with enterotype I, and carbohydrate-rich diets are associated with enterotype II [23]. In mouse models, diets high in fat (Western diets) increased the prevalence of *Proteobacteria* and decreased the abundance of *Bacteroidetes* [23]. Diets high in fiber and carbohydrates have been found to lower the abundance of pathogenic bacteria in the families *Enterobacteriaceae* and *Bacteroidaceae* [23]. Children from Burkina Faso, where high fiber diets are prominent, had a lower ratio of *Firmicutes* to *Bacteroidetes* than European children [24]. Burkina Faso subjects also had higher levels of short-chain fatty acids [24]. One possible problem with this particular study is the difference in environments: a rural African village as opposed to a European "environment typical of the of the developed world [24]." The differences in exposure to green spaces could have introduced a variable other than diet to this study.

Dietary impacts on the gut microbiome can also be seen by examining gut populations in obese individuals. Obese subjects have a different microbiota composition than lean subjects [25]. When microbiota transplants from obese humans to germ-free mice were performed, the mice gained weight [25]. These differences are reflected when comparing the prevalence of bacterial species in lean mice to those in obese mice. Obese mice have a higher ratio of *Firmicutes* to *Bacteroidetes* [26]. Obese humans mirrored these findings and have a lower microbial diversity

than non-obese individuals [26]. This finding is fascinating because *Firmicutes* produce butyrate, which is considered anti-obesogenic, and *Bacteroidetes* produce propionate, which is obesogenic [26]. In addition, future obesity could be predicted by examining the gut microbiome. Six month old infants with a low abundance of *Bifidobacterium* and an increased abundance of *Staphylococcus aureus* were found to be at an increased risk of being overweight when they reached the age of seven [26]. This finding supports a connection between obesity and the gut microbiome.

Age

Age is another important factor in microbiota composition. A Japanese study examined 367 healthy subjects between zero and 104 years old to see what differences could be found in their microbiotas [22]. *Actinobacteria*, the phylum to which *Bifidobacterium* belongs, decreased substantially over time after weaning [22]. This outcome is unsurprising given that a high abundance of *Bifidobacterium* is associated with infants being breastfed [16]. After age 3, the gut microbiome becomes more stable and is reflective of the adult microbiome [14]. Bacterial diversity increases from this post-weaning age until the twenties [22]. Changes occur again upon individuals reaching senior status. *Bacteroidetes* and *Proteobacteria* increased after the age of 70, and the elderly also have much higher levels of *Bacteroidetes, Betaproteobacteria,* and *Deltaproteobacteria* [22]. This evidence demonstrates that the gut microbiome varies by age.

Genetics

Genetics is believed to play a role in the composition of the microbiome, but more research must be done before concluding the exact impacts of genes on microbiota composition. There is some debate about the degree to which genetics influences the microbiota. Part of a study conducted by Turnbaugh et al. found that adult monozygotic twins are not more similar

than dizygotic twins in terms of microbial populations, which suggests that genetics may not be a major component in determining microbial makeup [27]. On the contrary, some child studies comparing monozygotic and dizygotic twins have found that monozygotic twins are more similar in terms of gut microbial composition than dizygotic twins or unrelated individuals [14]. One possible explanation for this discrepancy is the age of the subjects. Adult twins will likely live separately and therefore have different environmental exposures and diets, which can influence the gut microbiota's composition. On the other hand, children will likely be living in the same household and have similar diets and other lifestyle factors. Still to the contrary, one study examining data from the Human Microbiome Project found the gut microbiome was an exception to relatives having more similar microbiomes than when compared to non-relatives [28]. More research is needed to determine the magnitude of the genetic role in microbiome compositions.

Gender/Sex

Gender plays a role in gut microbial composition. A study of pre-term infants found that in the first ten days of life, females had a more diverse microbiota than males [15]. The same study found that male premature neonates had microbiomes dominated by *Enterobacteriales*, and females tended to have *Clostridiales* and *Lactobacilliales* in the majority [15]. Because these differences are found very early in life, the causes of this discrepancy are most likely biological and not the result of differences in cultural gender-roles. In post-menopausal women and similarly aged men, there are differences found between the genders at the genus level [29]. *Bilophila* was found in greater abundance in women, and *Veillonella* and *Methanobrevibacter* were greater in men [29]. As evidenced by these studies, gender is an important factor in determining gut microbiome composition.

Summary of Typical Gut Microbiota & Variables Impacting Composition

The gut microbiome contains a variety of bacteria. This gut bacterial habitat is dominated by *Firmicutes* and *Bacteroidetes.* Because there are many factors that influence the composition of a gut microbiota, it is difficult to establish what constitutes an "ideal" gut microbiota. Lifestyle factors, such as exposure to green spaces and diet, and biological factors, such as age, gender, and genetics, influence the bacterial populations within the gut. Knowing what factors can affect the gut microbiome can give important insights into the role the gut microbiome plays in human health and could potentially lead to treatments for a variety of illnesses.

Inflammatory Response

Introduction

The gut microbiome is able to influence human health through inflammation. Inflammation is an immune response that can be invoked by bacteria the body views as pathogenic. This response is considered healthy except when exaggerated or chronic. The mechanism by which gut bacteria cause inflammation depends upon the type of bacteria. The products of these responses to gut bacteria are not necessarily confined to the gut environment, which helps with understanding how gut bacteria are able to impact other areas of the body.

Inflammation – Gram Negative

An example of how bacteria can lead to inflammation is the toll-like receptor 4 (TLR4) response to lipopolysaccharide (LPS). Gram-negative bacteria have LPS in their outer membranes. The lipid A portion of LPS is considered highly toxic. The response of the immune system to LPS may depend upon the shape of its Lipid A, which varies depending upon the species of bacteria. Conically-shaped LPS activate TLR4, but cylindrically shaped LPS activates

TLR2 and can act as an antagonist to the actions of TLR4 [30]. LPS binding protein binds to the lipid A moiety—whether LPS is still bound to the bacteria or free--and then forms a ternary complex with CD14 [30]. CD14 may be free in the plasma or membrane-bound to myeloid cells [30]. CD14 then presents LPS to the MD-2 and TLR4 complex [30]. Lipid A binds to MD-2, which triggers TLR4's signal transduction [30]. TLR4 is able to signal to internal cellular components because it is a transmembrane protein. The steps that occur after TLR4 signal initiation are beyond the scope of this paper, but ultimately lead to NF-κB activating the production of pro-inflammatory cytokines, such as IL-6 [31].

Inflammation – Gram Positive

It is a high ratio of the mostly gram-positive *Firmicutes* to the gram-negative *Bacteriodetes* that results in increased levels of interleukin 6, TNF-alpha, and other markers of inflammation (Chrobak, Nowakowski, & Dudek, 2016). This finding indicates that gram-positive bacteria are likely also playing a pro-inflammatory role. Gram-positive bacteria's lipoteichoic acids and peptidoglycan are the suspected agonists of cytokine release as described by Moreillon and Majcherczyk [33]. Trimers and more complex substructures of peptidoglycan have been found to elicit the release of tumor necrosis factor from peripheral blood monocytes. Peptidoglycan binds to CD14, but instead of using TLR4, it uses TLR2. The mechanism after TLR2 signaling is initiated is similar to that of the TLR4 signaling cascade. It is important to note that it takes a concentration of 100-1000 times as much lipoteichoic acid or peptidoglycan to evoke the same response as a given amount of LPS. The strength of inflammation is independent of pathogenicity. Also of note is that the peptidoglycan substituents found to be inflammatory are kept "hidden" from host recognition and must be exposed by endogenous wall

remodeling, hydrolysis, or bacterial lysis. Through this mechanism, gram-positive bacteria are able to cause inflammation.

Inflammation – Flagellin

Another way for host immune cells to detect bacteria is through the presence of flagellin. Flagellin is a protein found in flagella, whip-like projections on some bacteria used for motility. TLR5 is responsible for the detection of flagellin as described by Sang et al. [34]. The TLR5 pathway activates NF-κB and activator protein 1, which leads to the production of pro-inflammatory cytokines, such as IL-8.

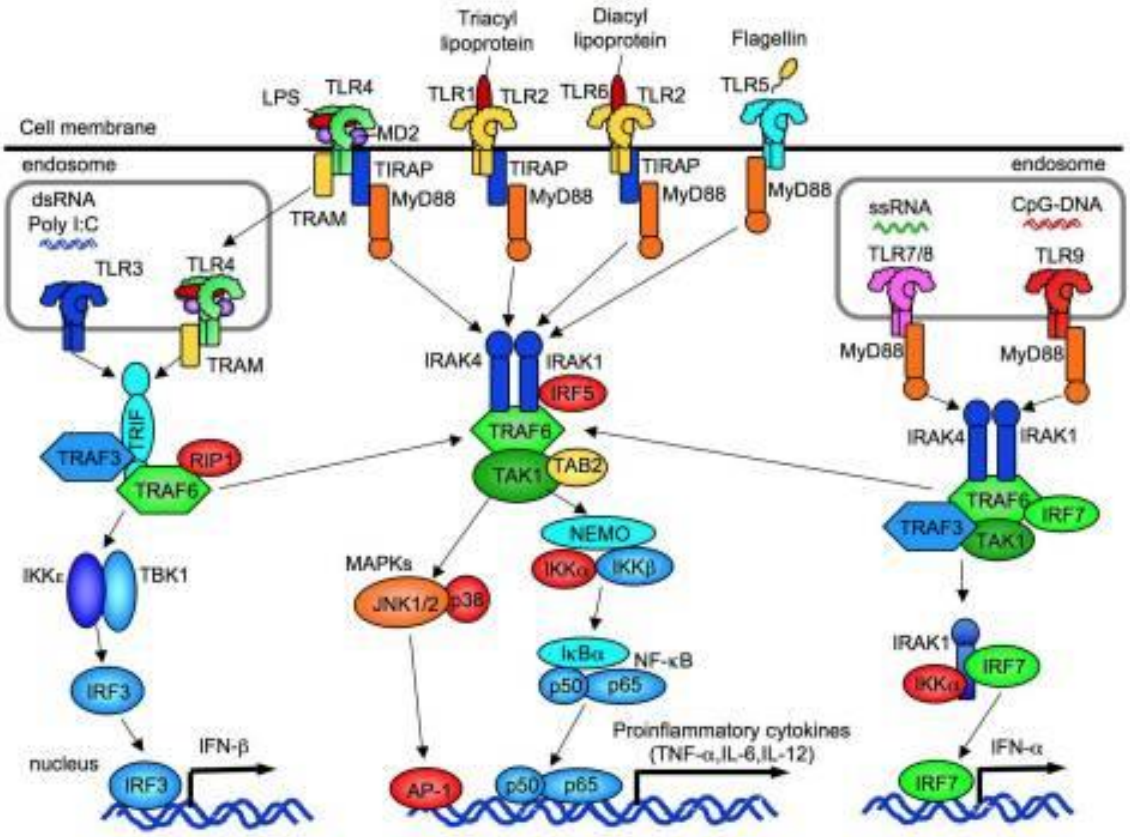

Figure 1. This image illustrates the activation and signaling pathways of TLR4, TLR2, TLR5, and other toll-like receptors not discussed in this paper [31].

Inflammatory Bowel Diseases

Further supporting the role of bacteria in inflammation, dysbiosis of gut microbiota has been implicated in inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis. In adults with active ulcerative colitis or Crohn's disease, the abundance of *Faecalibacterium prausnitzii* is lower than in healthy controls [35]. *F. prausnitzii* produces butyrate, a short-chain fatty acid that down-regulates production of pro-inflammatory cytokines [36]. There is a substantial reduction in the population of *Roseburia hominis*, which also produces butyrate, in both of these inflammatory bowel diseases as well [36,37]. Another distinction between healthy guts and those with inflammatory bowel disease is that significantly higher levels of *Lactobacillus* and *E. coli* were found in the colonic mucosae of ulcerative colitis patients [38]. Inflammatory bowel disease increases the risk of developing colorectal cancer, so differences between those with colorectal cancer and those without may also provide insight into which bacteria may result in harmful inflammatory responses. A study examining subjects with colorectal cancer found that those afflicted have a higher abundance of the genera *Fusobacterium* and *Porphyromonas* [39]. As expected due to the relationship between inflammatory bowel disease and colorectal cancer, this study also found colorectal patients have lower concentrations of *Faecalibacterium.* Gastrointestinal inflammation is more prevalent in subjects afflicted with psychiatric disorders than in controls. Post-mortem research on the correlation between schizophrenia and gastrointestinal inflammatory disorders found that 88% of deceased schizophrenics had enteritis, 50% had gastritis, and 92% had colitis [32].

Summary of Inflammatory Response

The gut microbiome is capable of causing an inflammatory response. When the presence of certain bacterial components is detected by the immune system, toll-like receptor signaling

cascades cause the production of pro-inflammatory cytokines and other markers of inflammation. Chronic inflammation is correlated with diseased states and can be detrimental to health. An example of harmful inflammation can be seen in inflammatory bowel disease, which research shows is likely linked to the lowered abundance of butyrate-producing bacterial species[36]. It is evident that there is a relationship between the bacteria present in the gut and the inflammatory response, and this information may be useful in treating chronic inflammation.

Microbiota-Gut-Brain-Axis Pathway

Introduction

With an understanding of how dysbiotic gut bacteria result in the production of pro-inflammatory cytokines, it is possible to see how the gut microbiome is able to influence psychiatric functioning. This pathway begins in the gut and requires the circulatory system to reach the brain. Examining bacterial products, such as hormones, gives an even clearer picture of how the bacteria in the gut can influence cognition. The theory that gut bacteria are able to affect psychiatric functioning is supported by evidence of gut dysbiosis in those suffering from depression, autism, and psychosis.

Leaky Gut

Bacterial products produced in the gut are able to affect other areas of the body due to a phenomenon commonly known as "leaky gut." "Leaky gut" refers to an increased permeability of the intestinal epithelium, which separates the contents of the intestines from the rest of the body. There is an inner and an outer layer of mucus atop the intestinal epithelium that serve to separate the intestinal epithelium from direct contact with microbes in the intestinal lumen [40]. Gut bacteria inhabit the outer mucosal layer [40]. The mucosal layers and single layer of epithelial cells form a selectively permeable barrier that regulates what substances are able to leave the

intestinal lumen and enter into the bloodstream to circulate throughout the body [41]. Pro-inflammatory cytokines, such as those secreted in response to gut bacteria, have been found to increase intestinal permeability [40]. This effect of pro-inflammatory cytokines on intestinal permeability is evidenced by patients with inflammatory bowel disease having a mucosal layer that is more easily penetrated by bacteria [40]. If bacterial products are able to make contact with the intestinal epithelium, it could elicit an immune response and result in the release of pro-inflammatory cytokines [42]. Individuals with schizophrenia or autism have been found to have increased intestinal permeability, which supports a link between "leaky gut" and psychiatric dysfunction [42].

HPA Axis

A key component to how the gut microbiome is able to influence neurological development and psychiatric health is found in the hypothalamic-pituitary-adrenal (HPA) axis. Circulating inflammatory cytokines, which can be produced in response to the presence of gut bacteria and enter circulation because of increased intestinal permeability, cause the hypothalamus to produce corticotropin-releasing hormone according to a review on the matter [43]. Corticotropin-releasing hormone then prompts the pituitary gland to make adrenocorticotropic hormone. Adrenocorticotropic hormone stimulates the adrenal glands to produce cortisol. Cortisol is then able to inhibit the production of pro-inflammatory cytokines and to encourage the production of anti-inflammatory cytokines [44]. This finding offers a potential target in regulating the impacts of cytokines.

Bacterial Products

With an understanding of how bacterial products produced in the gut are able to affect other areas of the body, the products and their effects become pertinent. Approximately 95% of

serotonin, which plays key roles in cognitive functioning, is made in the gut [45]. Gut bacteria also produce the short-chain fatty acids acetate, propionate, and butyrate, which play key roles in reducing inflammation. Acetate and propionate are produced by bacteria belonging to the phyla *Bacteroidetes*, and butyrate is produced by bacteria in the phyla *Firmicutes* [26]. Cytokines, which can be produced in response to the presence of certain bacteria, are able to alter the permeability of the blood-brain barrier, which controls the passage of substances between blood and the brain [46]. In addition, short-chain fatty acids produced by some gut bacteria are able to cross the blood-brain barrier [46]. This ability to cross the blood-brain barrier is necessary in order to affect neurological functions. These products and others are given along with their functions in **Table 1** and **Table 2**.

Name	**Taxonomic Classification**	**Gram +/-**	**Product(s)**
Bifidobacterium	Genus	+	GABA[4]
Lactobacillus	Genus	+	GABA[4], Acetylcholine[32]
Faecalibacterium prausnitzii	Species	+	Butyrate[36]
Roseburia hominis	Species	-*	Butyrate[36]
Streptococcus	Genus	+	Serotonin[32]
Escherichia	Genus	-	Serotonin[32], Norepinephrine[32]
Enterococcus	Genus	+	Serotonin[32]
Clostridium	Genus	+	Butyrate, Propionic Acid[47]
Bacillus	Genus	+	Dopamine[32], Norepinephrine[32]
Serratia	Genus	-	Dopamine[48]
Desulfovibrio	Genus	-	Propionic Acid[47]
Bacteroidetes	Phylum	-	Acetate[26], Propionate[26]
Firmicutes	Phylum	mostly +	Butyrate[26]

Table 1: This table displays bacteria commonly found in the gut, their taxonomic classifications, gram-staining results, and their products. **Roseburia hominis* can be either gram-negative or gram-variable.

Product	Classification	Function/Effects
Acetate	SCFA	Anti-inflammatory[49], Obesogenic[26]
Acetylcholine	Neurotransmitter	Attention, Learning, Memory
Butyrate	SCFA	Anti-inflammatory[49], Anti-obesogenic[26]
Dopamine	Neurotransmitter	Reward/Motivation, Emotion, Movement
GABA	Inhibitory Neurotransmitter	Behavior
Norepinephrine	Neurotransmitter	Stress Response ("Fight or Flight")
Propionic Acid, Propionate	Carboxylic Acid, SCFA	Anti-inflammatory[49], Anti-obesogenic[26]
Serotonin	Neurotransmitter	Sleep, Appetite, Mood, Cognitive Functions

Table 2: This table lists the products of bacteria with their classifications and functions. SCFA = Short-Chain Fatty Acid.

Summary of the Microbiota-Gut-Brain Axis Pathway

Gut bacteria are able to impact the brain through the circulatory system. Increased intestinal permeability allows for cytokines and bacterial products to enter the bloodstream. From there, products are able to reach other areas of the body including the brain. The exact impacts vary based on the functions of the product. Combining knowledge of the abundance of

gut species in dysbiotic states with the roles of corresponding bacterial products allows for a greater understanding of how gut bacteria are able to influence neurological functioning.

Microbiota Composition in Psychiatric Disorders

Introduction

With the pathway between the gut and brain established, it becomes clear that gut bacteria can play a role in psychiatric function. Examining the differences in the gut microbiota found between healthy subjects and those afflicted with psychiatric disorders can point towards potential targets for treatment. Although further research is needed to establish causal roles for specific gut bacteria, these findings provide insight into the links between the gut and brain. The disorders discussed include major depressive disorder, psychosis, and autism.

Depression and Anxiety

Dysbiosis of gut microbiota has been observed in patients experiencing major depressive disorder (MDD). Major depressive disorder is characterized by a lack of motivation in daily life and depressed moods. There is evidence that the gut microbiome could play a causal role in MDD. In one study, fecal samples were taken from healthy patients and from patients diagnosed with MDD [50]. Germ-free mice then received a fecal microbiota transplant from either healthy samples or MDD samples [50]. Mice that received a MDD sample exhibited symptoms of depression and anxiety [50]. It was also found that MDD patients had a higher abundance of *Actinobacteria* and a lower abundance of *Bacteroidetes* [50]. A different study found that patients with active MDD had a lower abundance of *Actinobacteria* and a higher abundance of *Fusobacteria*, *Bacteroidetes,* and *Proteobacteria* [51]. A key difference between these two studies is that the fecal microbiota transplant study's MDD subjects were primarily drug-naïve, and the conflicting study reported widespread atypical antipsychotic, SSRI, SNRI, and benzodiazepine

usage amongst its MDD subjects [50,51]. A review of existing studies on antidepressants found that some antidepressants have antimicrobial activity, which could explain the inconsistencies in studies that do not control for antidepressants [52]. Regardless of whether or not bacterial populations increased or decreased in abundance, key differences were observed between healthy patients and those with MDD.

In a less direct way, another connection can be drawn between gut microbiota and depression: inflammation. Pro-inflammatory cytokines may release neuroendocrine hormones that activate the hypothalamic-pituitary-adrenal axis [53]. Higher levels of IL-6, tumor necrosis factor-α, and acute phase proteins have been found in depressed patients [54]. The injection of a pro-inflammatory cytokine, interferon-α, resulted in depression [55]. Microbiota may also alter the permeability of the blood brain barrier [54]. These findings reinforce the connection between depression and gut bacteria.

Psychosis

Psychosis is a psychiatric disorder characterized by a loss of touch with reality. Compared to controls, subjects experiencing their first episodes of psychosis have been found to have a greater abundance of *Lactobacillus, Halorubrum, Deferribacter, Tropheryma, Halothiobacillus, Saccharophagus,* and *Ochrobactrum* [56]. These subjects also had a lower abundance of *Anabaena, Gallionella,* and *Nitrosospira* [56]. *Lactobacillus* group bacteria (*Lactobacillus, Leuconostoc, Pediococcus,* and *Weissella*) were found to increase with symptom severity [56]. *Bacteroides* spp., *Lachnospiraceae,* and *Ruminococcaceae* became less abundant as functionality increased [56]. The prevalence of bacteria varying with psychiatric functioning supports a connection between psychosis and the gut microbiome.

Autism

Autism is a neurodevelopmental disorder characterized by impaired social functioning. With no clear cause, autism is thought to be the result of a combination of factors. One factor researchers have taken interest in is the gut microbiome. When compared to healthy controls, individuals with autism spectrum disorder have a higher incidence of inflammatory bowel disease, diarrhea, constipation, gastric reflux, and abdominal pain [1]. Because of the high rate of comorbidity between autism and gastrointestinal issues, researchers have looked to the microbiota of those on the autism spectrum. Autistic patients have an increased *Firmicutes* to *Bacteroidetes* ratio, which has been shown to result in an increase of inflammatory markers [1,32]. There is a greater abundance of LPS and pro-inflammatory cytokines in children with autism, which supports the theory of a link between gut microbiota populations and autism [42]. *Lactobacillus, Desulfovibrio*, *Sutterella*, and *Clostridium* are increased in autistic patients, and the abundance of *Lactobacillus* and *Desulfovibrio* were positively correlated with autism severity [1]. Autistic patients have decreased levels of propionate, butyrate, and acetate according to this same report. This observation contradicts other reports that state short-chain fatty acids, such as propionic acid, are overproduced in those with autism [47]. This finding of increased propionic acid makes sense given that propionic acid is produced by *Clostridium* and *Desulfovibrio*, which are more prevalent in those afflicted with autism [1,47]. There is also a reduced abundance of *Coprococcus, Prevotella,* and *Veilonellaceae*, which are necessary for the degradation of carbohydrates and fermentation [1]. In one mouse study, treatment with *Lactobacillus reuteri* restored social behaviors [1]. Treating these mice in early life with *Bacteroides fragilis* reduced autistic behaviors. Regarding children with regressive autism, the phylum *Firmicutes* was increased, and the phyla *Bacteroidetes, Actinobacteria,* and *Proteobacteria* were decreased [47].

Even with present evidence, more research must be done to establish the exact role the gut microbiome plays in autism.

Summary of Gut Microbiota Composition & Psychiatric Disorders

The gut microbiome is an area of active interest in psychiatric research. This interest is due to the prevalence of chronic inflammation and gastrointestinal dysfunction being comorbid with psychiatric disorders. There is potential for new treatment methods using the composition of the gut microbiome, but more research is necessary.

Limitations of Research & Methods for Future Studies

Introduction

In order for studies on the gut microbiome to be meaningful, the methods by which research is conducted must be scrutinized. Although human studies are not uncommon, mouse studies are more easily performed and constitute a significant portion of studies on the gut microbiome. These studies are often used to inspire human studies or to gain insight into factors that could negatively impact the health of the holobiont. Regardless, mouse study findings cannot always be translated to human studies, and this case is especially so when dealing with something as delicate as the gut microbiome. Reading through numerous reviews and experimental studies also makes it clear that greater consistency in procedures is needed. Inconsistencies in the types of bacteria examined makes it difficult to find studies that can be directly compared. Small sample sizes can also make findings questionable. Careful consideration should be given when determining how to best examine the microbiome so that results may be efficiently communicated.

The Advantages and Disadvantages of Mouse Studies

Germ-free mice are popular model organisms for studying the impacts of changes to the human microbiome. In general, germ-free mice have been found to be more prone to inflammatory disorders and behaviors associated with abnormal psychiatric profiles than mice with a typical mouse microbiome, which supports the overarching theory of the influence of the microbiome on inflammation and psychiatric health. Humanized gnotobiotic mice are of particular interest in gut microbiota studies because they are born germ-free and then inoculated with human gut microbiota [57]. This is done in hopes of replicating the human microbiome without the added difficulties associated with using human subjects. Nguyen et al. finds in their review that the advantages of using mouse models include the low cost, genetic consistency as a result of inbreeding, comparable anatomy to humans, and fewer restrictions on medical interventions and experiments than using humans [57]. This review of lab animal usage also found that the homogeny of lab mice populations has its drawbacks: two lab mice will be far more similar than two humans because mice will have similar genetics, diets, and be raised in similar environments, whereas humans will have far more variability. Because everything is so consistent in lab mouse populations, changes made to the mice's microbiotas will likely impact all of the mice in a similar way, but because humans have far more lifestyle and biological variables that cannot be as easily controlled, changes made to human microbiomes may have different impacts from subject to subject. There are even disadvantages to using humanized gnotobiotic mice; some microbial species of lower abundance are lost after being transferred to the mouse models, and although low in number, these bacteria are believed to play a vital role in the gut microbiome [57]. This is just one of many struggles encountered by microbiota researchers.

Inconsistencies Amongst Studies in Bacteria Examined

One of the greatest hindrances encountered in studying the microbiome is the inconsistency of methods between studies. Some studies focus on bacteria that are low in abundance but could potentially have large impacts, such as the psychosis study discussed. Other studies focus on alterations in the abundance of major commensal bacteria that can be found in almost every subject. This leads to fewer studies with comparable results because the bacteria tested for are entirely different. This is further complicated when studies examine bacteria at different taxonomic levels because a bacterial phylum could be found less abundant as a whole, but a genus or species within that phylum may be significantly increased in prevalence. This could lead to the appearance of inconsistent findings. Without several studies examining the same populations of bacteria, it is difficult to establish a causal role for gut bacteria in psychiatric disorders and inflammation. In addition, many studies have small sample sizes, which makes it difficult to find statistically significant data.

The Additional Complication of Medication in Psychiatric Studies

Examining correlations between psychiatric disorders and gut bacteria provides additional challenges. One such challenge is trying to control for medication usage while still having a large enough sample size to be statistically significant. Studies have shown that certain antidepressants may have antimicrobial properties, so those receiving treatment for depression may have altered gut bacteria that do not accurately represent bacteria influencing their depressive states [52]. Taking patients off of medications for the sake of studies could prove detrimental to their health and is unethical. In addition, taking patients off of these medications will not necessarily restore the gut microbiome to its state before medication was introduced. The best way to handle this is to examine which psychiatric medications have beneficial or detrimental effects on gut bacterial populations. Subjects on these medications can then either be

excluded from the study or put into a subgroup to be compared to those who are drug naïve or on different medications. Whatever route is taken when examining groups with medications as a variable, it is imperative for the study to discuss the possible influences medications may have had on results.

Summary of Limitations of Research & Methods for Future Studies

Research on the gut microbiome is gaining traction, but it still has a long way to go before specific bacterial populations can be implicated as having a causal role in inflammation and psychiatric disorders. Although mice can provide a basis for conducting studies on humans, findings in the mouse gut microbiota may not translate to the human gut microbiota. It is imperative that researchers bear this in mind when examining studies on model organisms. Studies performed to examine bacterial populations and how they relate to the diseased state should test for bacteria that have been observed in other studies in order to strengthen or weaken evidence of causal roles. Testing for bacteria not previously examined can lead to new insights regarding the gut microbiome, but these findings must be considered with caution because of the inability of researchers to control every variable that contributes to microbial composition.

Conclusion

The gut microbiome plays a key role in regulating inflammatory responses and in influencing psychiatric health. This conclusion is supported by studies examining pathways through which these influences occur and by studies examining gut bacterial populations and their secretions in relation to inflammation and psychiatric disorders. Many findings are based on mouse studies, but these studies are not without their disadvantages. In addition, studies tend to examine the microbiome at different taxonomic levels, which can cause complications when trying to compare results of multiple studies. Variables that may be difficult to control without

severely hindering sample size, such as medication usage, provide additional challenges. Regardless, the evidence presented by current studies still overwhelmingly supports connections between the microbiome and inflammation and psychiatry. In order to draw more precise conclusions and establish detailed causal roles, more research is necessary.

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