Nutritional Effects on the Gut Microbiome & the Brain-Gut Axis: Unlocking the Therapeutic and Preventative Potential of Nutrition for Gut Dysbiosis Associated Diseases

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Abstract

Diet plays a pivotal role in the overall health of an individual. Not only does it help carry out and regulate certain physiological functions, but it also can determine the composition of the gut microbiome. While the microorganisms that make up the gut microbiome vary between individuals and can be dependent on different environmental factors, there has been evidence to suggest that the type of bacteria that colonize the gut can correlate to better overall health. When the GI microbiome is upset or suddenly changes it results in gut dysbiosis, a condition that correlates to the presence of certain diseases and can worsen clinical symptoms. Diseases linked to gut health range from metabolic disorders, inflammatory bowel diseases, and even disorders of the brain. Many of these diseases are linked to the connection between the brain and the gut, known as the brain-gut axis. This bidirectional communication helps in the digestion of food, intestinal function, and is responsible for GI response to emotion as well as the emotional response to GI upset. By exploiting the interaction between microbiome health and nutrition, diet can be used to alleviate disease symptoms, protect against developing certain conditions, and better maintain overall health. This review will examine the effects of gut dysbiosis, nutrition on the microbiome, the presence of disease linked to disruption of normal microflora, and the way that altering diet can mitigate symptoms or prevent disease.

Introduction

Colonizing the human gastrointestinal (GI) tract is a myriad of different bacterial species that are important in carrying out everyday functions and survival. This complex ecosystem of bacteria, viruses, fungi and protozoa, known as the gut microbiome, is extremely important for human health (Cani, 2018) Consisting of ten more times bacterial cells than human cells, the gut microbiome is "essentially an organ" and in recent years, has become widely studied to determine its composition, and its impact on overall health (Eckburg et al., 2005;Thursby & Juge, 2017)

The composition of the microbiome, much like a fingerprint, varies between individuals. The types of bacteria that colonize an individual's GI tract have been shown to impact things such as; body mass index (BMI), digestion, immune response, allergies, and incidence of disease (Thursby & Juge, 2017) A "healthy" gut microbiome is important for carrying out a multitude of different functions, including: metabolic and endocrine pathway regulation, modulating immune response, production of necessary vitamins, metabolites and neurotransmitters and maintaining overall heath (Marilyn Hair & Jon Sharpe, 2014)

Within the last decade, there has been extensive studies done to determine the mechanism, and implications of a cross-talk between the gut microbiome and the central nervous system (CNS). This communication between the intestinal cells and the brain is known as, the brain-gut axis (GBA). Being a relatively new field of study, there is still much to research and learn about the brain-gut axis, but some studies have already found evidence to suggest this system is crucial for the maintenance of homeostasis and that dysfunction within the system and its players can increase the incidence of certain diseases and severity of symptoms (Rutsch et al., 2020)

Throughout an individual's lifetime, the bacteria colonizing the GI tract can fluctuate mildly or can be wildly altered by different environmental and emotional stressors. The use of antibiotics, addition of dietary supplements, change of environment, aging, and diet can alter the population of the gut and result in a microbial shift (Hasan & Yang, 2019). While these changes can be normal and not cause any issues, an abrupt microbial shift can lead to a loss of homeostasis within the gut known as, gut dysbiosis. This condition has been shown to

correlate with higher risk for certain diseases, both intestinal and extra-intestinal (Carding et al., 2015).

This review aims to present information about the gut microbiome, and the ways in which it can be altered. It will also look at the implications that gut dysbiosis plays on overall health and how disruption of the gut correlates to certain disease states. While there is a myriad of different diseases linked with microbiome health, this review will emphasize a select few diseases including; inflammatory bowel diseases, colorectal cancer, and Parkinson's disease. Lastly, this review will discuss the potential of using nutrition as a way to prevent or alleviate conditions associated with gut health.

Gut Microbiome

Living inside the human gut is a complex and diverse ecosystem consisting of trillions of microorganisms that assist the body in carrying out important physiological functions and maintaining homeostasis (Sender et al., 2016) (Thursby & Juge, 2017). Composed mainly of symbiotic bacteria, the gut microbiome is generally beneficial to the host and is responsible for breakdown of food to important metabolites, protection against harmful opportunistic pathogens, influencing immunologic response and interacting with endocrine and metabolic pathways (Valdes et al., 2018)The types of bacteria that make up the microbiome can be important in maintaining and determining the overall health of an individual.

The microbiome is shaped in part by host genetics and environmental factors. Gender, age, environment, body mass index (BMI), diet, etc. have all been shown to alter gut microflora (Haro et al., 2016), (Goodrich et al., 2014). When these alterations occur, it can be reflected in the health and physiology of the host. Studies done in germ free (GF) mice have shown that gut composition is a potential factor involved in obesity, intestinal motility and development of numerous diseases and disorders(Ge et al., 2017; Magne et al., 2020) Some studies have even started to implicate certain bacteria composition and densities within the gut with personality traits and development of psychiatric disorders (Johnson, 2020)

The predominant bacterial phyla present within the average human microbiome are *Firmicutes* and *Bacteroidetes,* which make up about 75% of the microbiome, followed by *Proteobacteria* and *Actinobacteria.* (Riaz Rajoka et al., 2017)(Matsuoka & Kanai, 2015). The

ratio within the gut of *Firmicutes* and *Bacteroides* fluctuates and has been studied in depth. It is hypothesized that an imbalance in this ratio can be a factor in obesity and other diseases, but there is still no clear connection between an unbalanced ratio and disease (Riaz Rajoka et al., 2017). There is, however, a connection between when there is a reduction of *Firmicutes* and the onset of inflammatory bowel diseases, like Crohn's disease (CD), and inversely, patients with ulcerative colitis (UC) that have higher levels of *Firmicutes* have been shown to respond to treatment better (Matsuoka & Kanai, 2015).

It's inevitable that there will be changes in the microbiome over time, the majority of which will not result in any observable effects, however abrupt shifts in lifestyle or trauma can lead to detrimental changes within the gut. These harsh changes can be so severe that they disrupt the normal microflora, making room for opportunistic, harmful pathogens to dominate, and causing what is known as gut dysbiosis (Carding et al., 2015)

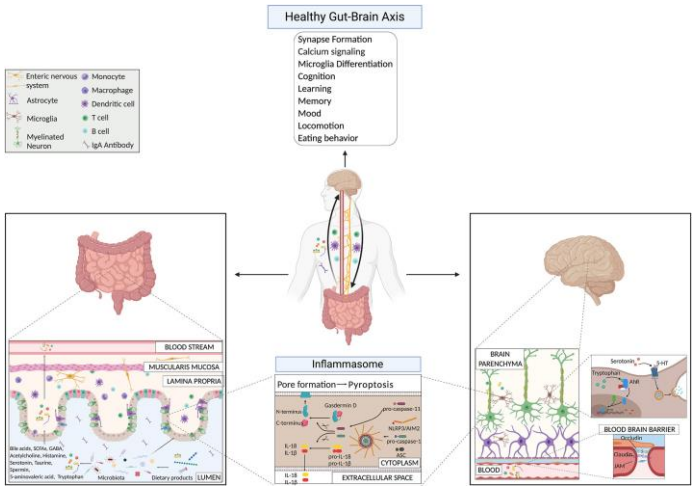
Gut-Brain Axis

In recent years, there has been an influx in studies done about the once controversial idea of a connection between the enteric nervous system (ENS) and central nervous system (CNS) known as the brain-gut axis (GBA). This complex network is a bidirectional communication between the gut and the brain with side involvement from the autonomic nervous system (ANS), hypothalamic-pituitary-adrenal axis and immune signaling proteins. (Rutsch et al., 2020) The ENS consists of over 100 million nerve cells spanning from the esophagus through the GI tract. The connection between the ENS and CNS is responsible for controlling digestion, development of the immune system, ensuring intestinal barrier integrity, and links cognition and digestion. Throughout the last decade, studies in GF mice have shown the importance of the microbiome in proper brain development and signaling (Luczynski et al., 2016)

The role of the microbiome in the gut-brain axis has been demonstrated in studies with GF mice and those undergoing prolonged antibiotic treatment when they were observed to have stunted and decreased neurogenesis of the hippocampus, that can be reversed when treated with probiotics and physical activity (Möhle et al., 2016) The impact of the microbiome on the GBA can also be illustrated by the high incidence of depression in individuals with inflammatory bowel syndromes (Breit et al., 2018)

The bidirectionality of the gut-brain axis can be observed when studying post stroke behavior in individuals. The presence of brain lesions can lead to dysbiosis in the gut, and inversely the state of the gut can influence the outcome of a stroke (Singh et al., 2016).

The gut microbiota is able to carry out its communication with the CNS through signaling pathways including the vagus nerve, immune system, and metabolites produced by the microbes within the microbiome (Rutsch et al., 2020).

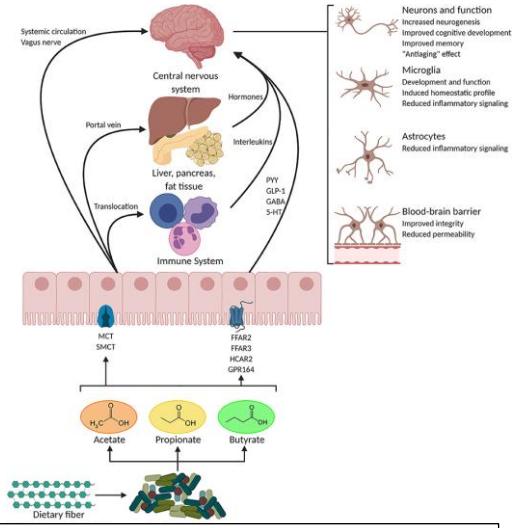


Connection between the gut and the brain is carried out through a number of mechanisms, involving nerves, inflammatory cells, immune cells and mucosal membranes. A healthy gut-brain axis has influences over many factors. Source: (Rutsch et al., 2020)

The vagus nerve provides a connection between the GI tract and the brain. Information from the ENS can influence the vagal nerve to modulate the immune system, digestion, and enteric reflexes. It can also be implicated in neurologic diseases when pathogens from the gut are able to get to the brain and cause inflammation that influences function (Breit et al., 2018).

Lactobacillus, and *Bifidobacteria* bacterial species within the microbiome are responsible for producing neurotransmitters, including acetylcholine, gamma-aminobutyric acid (GABA), and serotonin, which are important in brain signaling (Pokusaeva et al., 2017) Normally, over 90% of serotonin is generated in the gut. In germ free mice, serum levels are significantly reduced, highlighting the role that the microbiome plays in regulating production of serotonin (Yano et al., 2015)

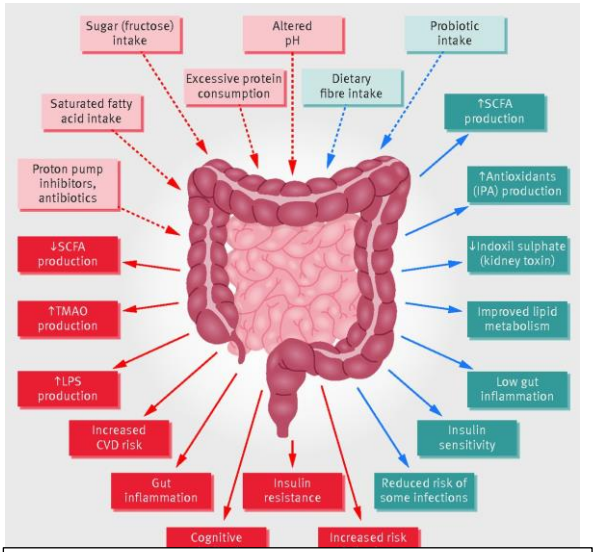
Short Chain fatty acids (SCFA) are carboxylic acids with a 2-6 carbon tail. These metabolites are generated within the gut during the fermentation of starch and fibers (J. Tan et al., 2014) The main short chain fatty acids within the gut include acetate, butyrate and propionate and they are produced by anaerobic bacteria within the gut when they ferment fiber. (Venegas et al., 2019) SCFAs are beneficial metabolites important for signaling, regulating immune response and maintaining homeostasis. When there are higher levels of SCFAs there is reduced inflammation, increased intestinal mucus and less barrier permeability (Hendler & Zhang, 2018).



SCFAs are generated by the fermentation by gut microbiota. These metabolites are important for regulating physiological functions and reducing gut permeability.
Source: (Silva et al., 2020)

Current understanding of the GBA show that dysfunction along the gut-brain axis can be the cause of a multitude of different diseases and disorders; ranging from psychiatric conditions to gastrointestinal issues. Typically, these issues present with an underlying issue of the gut microbiome.

Implication of Gut Dysbiosis in Disease



Dietary intakes and environments effect the composition of the gut microbiome. The outcomes can be either beneficial od detrimental. In red harmful, risks for diseases are highlighted, while beneficial outcomes are shown in green.
Source: (Valdes et al., 2018)

A healthy gut is colonized by diverse and primarily beneficial bacteria. By occupying space in the gut, the healthy microflora creates a barrier that protects against the overgrowth of harmful bacteria and discourages pathogenic bacteria from getting outside the gut and causing disease. This barrier and diversity can be lost due to trauma to the gut, antibiotic use, change in diet, or other environmental factors, resulting in disruption of gut homeostasis, otherwise known as gut dysbiosis (Degruttola et al., 2016).

When the “good bacteria” are replaced with “bad”, mostly gram-negative bacteria, this can lead to immune dysfunction and inflammation due to an increase in nuclear factor kappa B (NF-kB) (*Dysbiosis and Leaky Gut - Illinois Chiropractic Society*, n.d.).

Increased cytokine production in response to inflammation from gut dysbiosis can lead to leaky gut. Leaky gut occurs when gut dysbiosis leads to the disruption of the mucosal barrier that leads to increased permeability and allows for PAMPs from pathogenic bacteria, primarily LPS, to travel outside the gut. When this occurs, it can lead to inflammation throughout the body, including the brain (Mu et al., 2017)

Gut Dysbiosis in GI Disease

Inflammatory bowel diseases such as Crohn’s, IBS, and ulcerative colitis are associated with dysbiosis of the gut. This could be linked to the increase of NF-kB activation, because while NF-kB is needed to maintain homeostasis in intestinal epithelial cells, constitutive activation is linked to gut inflammatory diseases, such as Crohn’s and Ulcerative colitis (T. Liu et al., 2017) Studies have shown a marked difference between the composition and densities of beneficial bacteria of the intestinal microbiome in healthy individuals versus those with inflammatory gut disorders (Schäffler et al., 2016) Using 16S rRNA sequencing to analyze the intestinal microbiome of colonic tissues from individuals with Crohn’s disease, researchers found that there was a “correlation of the mucosa-attached bacterial community with disease activity” (Schäffler et al., 2016) This points to the fact that as the bacterial makeup changes, overall heath can be affected and that disease symptoms can be exacerbated, and vice versa.

One of the most pressing issues of the gut associated with gut dysbiosis is colorectal cancer. This disease is the second leading cause of cancer in the U.S and is a major concern because of its high incidence and mortality rate. Research has shown that there is a correlation between the risk of colon cancer and the health of the microbiome, implicating lifestyle choices in the risk for cancer.

Current research implicates the presence of *Fusobacterium nucleatum* with the over activation of TLR4/MYD88/NFkB, leading to increased risk of colorectal cancer (Yang et al.,

2017). The over activation of these pathways induces expression of pro-inflammatory cytokines and chemokines, leading to increased inflammation that heightens risk and worsens diseases symptoms. The abundance of *F. nucleatum* and its ability to colonize the human gut is influenced by gut dysbiosis caused by diet that induces inflammation (L. Liu et al., 2018).

The implication of microbiome composition having an impact on the presence of colorectal cancer (CRC) is reinforced by a study in which germ-free mice were inoculated with stool samples from individuals with CRC and stool samples of healthy individuals, and found that those given CRC positive stool samples had higher incidence of inflammation, dysplasia, and polyps (Wong et al., 2017)

Neurologic Disorders Associated with Gut Dysbiosis

There has been evidence to suggest that some neurological disorders and diseases such as, Parkinson's, and Alzheimer's, can be linked to dysbiosis in the gut. The ability of the gut composition to have an effect on neurological disorders implicates that gut-brain axis in these diseases' states. Links between the gut and Parkinson's Disease (PD) were noted when a high percentage of patients presented with constipation or GI upset (Baldini et al., 2020)

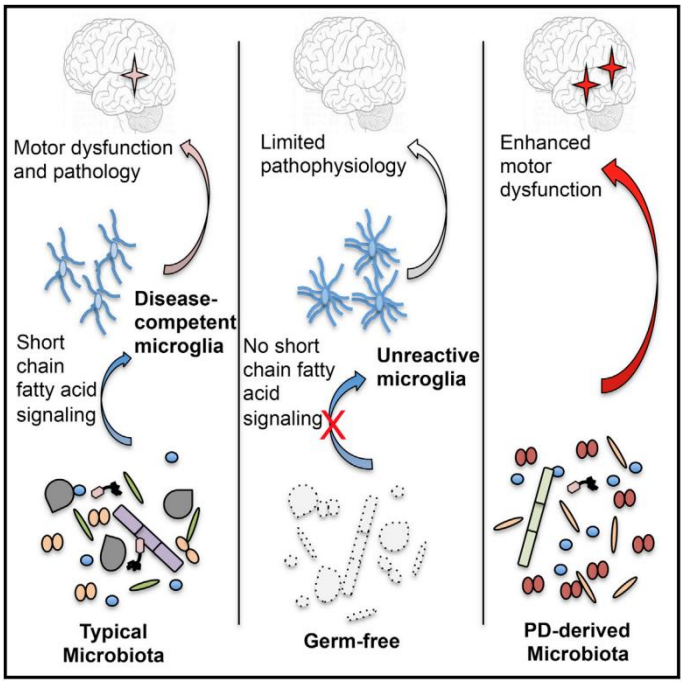
Parkinson's Disease (PD) is a neurodegenerative disorder of the CNS that typically presents with motor deficits. It is one of the leading causes of dementia and affects nearly 10 million people worldwide (Marras et al., 2018)Hallmarks of the diseases include inflammation and the presence of inclusions called Lewy bodies which are made up mostly of alpha synuclein aggregates (Dutta et al., 2019)

Studies have shown that the microbiome of PD patients and other healthy individuals are different – leading researchers to believe that something about the composition of the microbiome plays a role in disease pathology (Chiang & Lin, 2019)

The mechanisms by which disruption in the gut can affect the progression of PD is through SCFA levels, gut barrier dysfunction and the immune response via proinflammatory cytokines. When there is gut dysbiosis the integrity of the gut barrier is reduced, making the gut wall more permeable to different molecules. This permeability potentially allows for pathogenic bacteria to escape the gut and infiltrate spaces where they shouldn't be. If

pathogenic bacteria are able to escape the gut they can get to the brain via the vagus nerve and cause inflammation (Dutta et al., 2019)

One recent study in Parkinson's shows the role of the gut microbiome in PD progression by using GF transgenic mice that overexpress alpha syn. In the GF mice there was a reduction in microglia activation and symptoms associated with PD, but when the mice were treated with microbial produced SCFAs, the clinical symptoms of the diseases were restored. These results lead to the hypothesis that the SCFA play a role in maturation of microglia that sense the alpha synuclein. Further, the study also reported that when PD patient microbiomes were transplanted into the mice the disease conditions worsened, providing some evidence that there is something about the microbiome that leads to disease symptoms (Sampson et al., 2016)



In GF mice there is limited symptoms and pathology of Parkinson's, but when microbiome is restoring the clinical symptoms are restored. The addition of a PD derived microbiome to a GF mouse results in even more clinical symptoms and motor dysfunction.
Source: (Sampson et al., 2016)

This study is important because even though we are still trying to determine the extent of the links between gut health and PD, it shows correlation between microbiome composition and presence of disease and provides a potential therapeutic intervention through regulating SCFA acid production.

Effect of Nutrition on Gut Microbiome Composition to influence health

As stated previously, one of the factors evidenced to modulate the composition of the gut microflora is diet. Over the years there have been a number of studies that have sought out to determine how different diets (Mediterranean, high protein, low carb, etc.) and consumption of different dietary supplements and additives can alter the bacterial populations within the

gut. The current understanding is that diet has a strong effect on the flora that inhabit the gut and can correlate to risk of developing certain diseases (Tilg et al., 2018).

Food additives, emulsifiers, and diets high in fats have been shown to increase inflammation of the intestines, leading to a shift in microbiota and increased risk for inflammatory bowel diseases (Chassaing et al., 2017)(Laudisi et al., 2019). These food additives have also been shown to potentially affect the brain-gut axis by having a connection to an increase in behavioral disorders, like anxiety, in studies done in mice (Holder et al., 2019)

To reduce inflammation and inhibit pathogenic bacteria from colonizing the gut, diets that increase SCFAs have shown to be beneficial. In studies done in animal models on inflammatory bowel diseases, addition of fructooligosaccharides, soluble fiber, or resistant starches increase SCFA production, reducing inflammation within the intestine. (Lewis & Abreu, 2017). Consuming fermented products and probiotics has shown to greatly increase the number of short chain fatty acids and help reduce risk for gut dysbiosis (Markowiak-Kopeć & Śliżewska, 2020)

In a study conducted to discern the risk associated between different diets and *F. nucleatum*-positive colorectal cancer, they found that a diet high in fiber and whole grains correlated to a lower risk for colorectal cancer associated with *F. nucleatum*, but not *F. nucleatum*-negative colorectal cancer, suggesting that there is a link between diet and diseases incidence (Mehta et al., 2017).

New Directions to Study the Interplay of the gut microbiome, intestinal epithelium and central nervous system

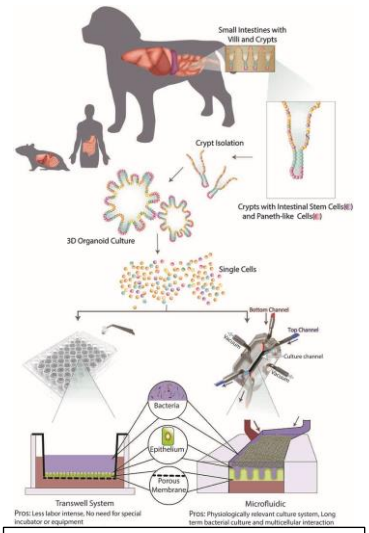
Currently, the majority of microbiome studies are carried out in germ free (GF) mice models. However, as research continues to be done in this field, different animal models are being discovered that may be more appropriate and accurate for microbiome research. Due to the fact that environment and lifestyle play such an important role in shaping the microbiome, dogs are beneficial model because they share environments with, as well as a very similar GI tract and diet with humans. These similarities point at dogs being a valuable model for human microbiome and disease research, because their responses to stressors and treatments will more accurately align with and represent human microbiome responses.

Studies done in companion animal models have shown canines to spontaneously develop many diseases such as cancer, inflammatory bowel diseases (IBD), diabetes, and heart failure, very similarly to humans, adding to the argument that dogs would be a useful model for studying human diseases (Schneider et al., 2018). A study done in canines with naturally occurring type 2 diabetes mellites (T2DM), found similarities in disease states between humans and dogs with T2DM, including the presence of increased LPS in serum, bile acid impairment and increased Gammaproteobacteria (Jergens et al., 2019).

Recently, dogs have also been shown to have microbiomes more similar to humans than murine models and express symptoms associated with gut dysbiosis that parallel that of humans. Much like in humans with IBD, dogs with IBD have been shown to have altered gut microbiota and bacterial distribution when compared to healthy controls. In these dogs, treatment via therapy with glucocorticoids and dietary alterations showed potentially beneficial shifts in bacterial populations and improved barrier functions (Atherly et al., 2019). These findings allow for possible areas of research and avenues of treatment to be potentially carried out in humans.

There is still much to be discovered and understood about the interaction between the gut microbiome and the CNS. Some of the limitations in the knowledge about the GBA are due to the fact that the majority of studies are done in murine models. While these models have offered some insight into the gut-brain communication, they are limited in their resemblance to human anatomy and physiology. Dogs are potentially an extremely valuable model when studying the GBA, because they more accurately mimic human gut microbiome, aging, brain to body ratio and they also can spontaneously develop neurological dysfunction analogous to humans (Ambrosini et al., 2019). Further studies carried out in dogs with canine cognitive dysfunction could be beneficial in determining more about the GBA.

As previously stated, there is a growing concern that murine models are limiting the discovery of therapeutic drugs and treatments because they are not similar enough to humans, with respect to environment, spontaneously occurring diseases, and pathogenesis of diseases (Schneider et al., 2018). An alternative to in vivo models is 3D organoid systems, which are in vitro models that contain cells biopsied from primary tissues or stem cells in a host that can be propagated and made into complex models of systems containing different types of cells that mimic organ systems and tissues (Mochel et al., 2018). These models allow for a deeper understanding of disease pathology through the ability to isolate specific cells from organs, investigate drug safety and efficacy and possibility of more personalized medicine because of its ability to harvest cells from different hosts and preform ex vivo trials on drug efficacy (Clevers, 2016).



Process of creating a 3D environment from a canine model. Cells are biopsied from a host and intestinal stem cells are isolated and propagated.
Source:(Ambrosini et al., 2019)

Organoids developed from intestinal cells can provide more insight on the GI diseases like CRC and IBD (Chandra et al., 2019) Recent work in 3D organoids successfully established a culture system of canine primary intestinal stem cells that have shown to be similar in both physiology and function of intestines in vivo (Ambrosini et al., 2019). This system can be used to compare the function and pathology of intestines when challenged with inflammatory bowel diseases in comparison to healthy intestinal cells (Kingsbury et al., 2018).

Another in vitro model, known as microfluidic systems, can work in tandem with organoid systems to create essentially “organs-on-chips”, which can provide better understanding of the interactions and composition of the gut microbiota (Tauzin et al., 2020) . This technique is potentially more beneficial than current models because it is faster, it can be designed to better mimic the conditions of humans, and is less expensive in the long run (H. Y. Tan & Toh, 2020).

Conclusion

Within the human gut there is a plethora of microorganism whose composition is constantly shifting and is shaped by environment and lifestyle choices. This system of bacteria aides the

body in a multitude of physiological functions and is important for maintaining overall health. If there is a severe disruption to the normal gut flora, known as gut dysbiosis, then host homeostasis is lost and there is potential for metabolic, GI, and neurological disease.

Consumption of certain diets and probiotics can modulate the gut composition, either beneficially or negatively. There is evidence that suggests that diet could be manipulated to help prevent disease or mitigate diseases symptoms. Reduction of inflammation is key to maintain health and preventing disease. This can be accomplished through diet by avoiding processed foods and those high in fat and instead opting for diets high in fiber and whole grains that promote SCFA production and reduction in inflammation.

Moving forward, there is a lot of research still left to be done. It would be beneficial for more research to be done in different animal models, specifically companion animals, because they share our environments, and their GI system, anatomy and physiology is more closely related to humans. Further research could also be done examining different diets on specific diseases states. Lastly, it would be beneficial to have more research on the gut-brain axis and getting a better understanding of how this system is able to carry out its functions and communicate.

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