The Roles of the Microbiome in Alzheimer's Disease

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Abstract

The gut and the brain are in constant communication in a complex network known as the brain-gut axis. A growing body of research has found links between the brain-gut axis and Alzheimer's Disease (AD). In this review, we will explore how the mammalian microbiome affects neuroinflammation and increases the permeability of the blood-brain barrier (BBB) in the context of AD. Research shows that the microbiome is associated with neuroinflammation in AD, which is presumably caused by the secretion of cytokines from specialized cells of the brain: microglia and astrocytes. Furthermore, we speculate that metabolic diseases, caused by microbiota dysbiosis, may increase the permeability of the BBB and further develop AD pathology. Researchers have considered whether the therapeutic modification of gut microbiota, through the use of antibiotics and probiotics, may show improvement in AD patients. This review will discuss the various known mechanisms and pathways through which the gut microbiome may be contributing to AD pathology.

Keywords: Alzheimer's Disease, brain-gut axis, microbiome

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disease that destroys brain cells. AD is the most common type of dementia experienced by older adults (Alzheimer's Association, 2020). It is characterized by the gradual deterioration of thinking, memory, and other cognitive abilities. Neuropathological lesions caused by the accumulation of β -amyloid plaques and neurofibrillary tangles in the brain are both considered hallmarks of AD (McCauley & Grush, 2017). Although

extensive research has been conducted to understand the pathological properties of AD, we have yet to determine a definitive underlying cause (McCauley & Grush, 2017). A growing body of research suggests that the brain-gut axis may play a role in the development of AD. The brain-gut axis is the term used to describe the constant bidirectional communication between the gastrointestinal tract (GI tract) and the central nervous system (Kowalski & Mulak, 2019). Gut microbiota

is the collection of microorganisms that reside in the GI tract (Moszak et al., 2020). Its composition is established early on in human development with over 1000 species maintained at stable levels in healthy humans (Seo & Holtzman, 2020). The impacts of intestinal microbiota extend beyond the gut with recent studies linking gut bacteria to various cognitive impairments, including AD (Doifode et al., 2020). Harach et al. (2017) found that amyloid- β precursor protein (APP) transgenic mice (genetically modified mice that overexpress APP) had different microbiota composition compared to non-transgenic wild-type mice. In addition, the study demonstrated that the creation of germ-free transgenic APP mice highly reduced amyloid- β pathology compared to wild-type mice (Harach et al., 2017). This suggests that the absence of microbiota in the gut was enough to decrease AD pathology through the reduction of the amyloid- β precursor protein. Findings such as these suggest that the study of gut microbiota may be a promising area of research for the development of therapeutic interventions for AD. If the intestinal microbiome contributes to AD pathology, then therapeutic interventions targeting the microbiome may potentially improve the condition of AD patients. However, for the development of such interventions, it is crucial that we thoroughly understand the mechanisms and pathways through which the microbiota may be increasing AD pathology. Despite a significant number of

research studies investigating this very topic, the exact pathways and processes involved are still not fully understood. In this review paper, we will identify two main roles of the brain-gut axis in the development of AD - neuroinflammation and increased blood-brain barrier (BBB) permeability.

Neuroinflammation

Numerous studies have indicated that chronic neuroinflammation contributes to AD pathology by accumulating amyloid- β peptides and inducing neuronal death (Cattaneo et al., 2017; Lee et al., 2008; Lin et al., 2018). Neuroinflammation occurs when specialized cells, microglia and astrocytes, secrete proinflammatory cytokines within the central nervous system (CNS) such as interleukin-1, interleukin-6, and tumour necrosis factor- α (Fakhoury, 2018; Lin et al., 2018). These proinflammatory cytokines are proteins that can trigger inflammation (Murphy & Weaver, 2017). Within the brain, the entrance of blood plasma proteins, mediated by a leaky blood-brain barrier (BBB) and the presence of amyloid- β deposits, allow microglia and astrocytes to become active and secrete proinflammatory cytokines (Acharya et al., 2013; Minter et al., 2016). These cytokines facilitate the recruitment of other immune cells of the body to the CNS to eliminate the proteins and trigger neuroinflammation, which is part of an innate immune response (Kim & Joh, 2006).

Microglia are phagocytic cells, and they can exist in two unique phenotypes: M1 and M2. M1 activation leads to the formation of proinflammatory cytokines, while M2 activation generates non-inflammatory cytokines. In the context of AD, microglia are responsible for binding and internalizing soluble amyloid- β fibrils and oligomers by the process known as phagocytosis. However, neuroinflammation seen in AD cannot be minimized due to several mechanisms, such as the increased production of amyloid- β and positive feedback loops between amyloid- β precursor protein (APP) production, a protein that is cleaved by proteolytic enzymes to form pathogenic amyloid- β peptides, and inflammation within the CNS. Furthermore, chronic neuroinflammation can lead to disruption of the BBB, which is provoked by M1 microglia that secrete proinflammatory cytokines.

Astrocytes are cells that contribute to a broad range of functions, including synaptic remodelling, ion homeostasis, neurotransmission, secretion of growth factors, and the regulation of oxidative stress (Fakhoury, 2018). Similar to microglia, astrocytes have the ability to phagocytose amyloid- β oligomers and fibrils in AD and can release elevated levels of proinflammatory cytokines that further contribute to neuroinflammation (Lin et al., 2018). Given the crucial roles of microglia and astrocytes in AD neuroinflammation, several studies have

shown that the gut microbiome can also cause neuroinflammation in AD. However, in this review, only two such studies will be discussed (Cattaneo et al., 2017; Lee et al., 2008).

Lee and colleagues (2008) found that intraperitoneal injection of bacterial lipopolysaccharides (LPS), obtained from a proinflammatory gut microbiota taxon *Escherichia coli* (*E.coli*), promoted memory impairments in male Institute of Cancer Research (ICR) mice and Sprague Dawley rats. LPS is a molecule that is located on the surface of bacteria that can induce an inflammatory response (Murphy & Weaver, 2017). Moreover, the authors chose Sprague-Dawley rats and ICR mice because they have been widely used in previous neurobiological studies (Kim et al., 2017; Whishaw et al., 2003). The authors of this study reported that a higher accumulation of amyloid- β 1-42 peptides was seen in the cortex and hippocampus of these murine species, which caused neuronal death and memory impairments. Additionally, the LPS-treated mice showed a higher expression of inflammatory proteins and amyloidogenic proteins (such as COX2, iNOS, BACE, and C99) in both astrocytes and neurons. These findings suggest that neuroinflammation caused by LPS of the gut bacteria, *E.coli*, can induce AD pathology in murine species.

Furthermore, Cattaneo and colleagues (2017) found that elderly patients with brain amyloidosis, abnormal buildup of the

amyloid- β protein, and cognitive impairment had higher levels of a different pro-inflammatory gut microbiota taxon *Escherichia/Shigella* in their stools compared to *Eubacterium rectale*, a non-inflammatory gut microbiota taxon. The patients showed an increased abundance of *Escherichia/Shigella* in their blood, which led to a higher production of proinflammatory cytokines, such as interleukin-1 β (IL-1 β), chemokine ligand-2, interleukin-6, and nod-like receptor protein 3 (NLRP3), and decreased production of an anti-inflammatory cytokine, interleukin-10. In addition, the authors reported that NLRP3 can induce the formation of protein aggregates, amyloid- β , which is a characteristic hallmark of AD.

Together, these findings suggest that the gut microbiota can trigger neuroinflammation in the brain by the release of proinflammatory cytokines, presumably by microglia and astrocytes, which can contribute to AD progression. However, the exact mechanism by which the gut microbiome causes neuroinflammation in AD remains to be elucidated.

Increased permeability of the blood brain barrier

Apart from neuroinflammation, the gut microbiome can also lead to increased permeability of the blood-brain barrier (BBB) in AD individuals (Kowalski &

Mulak, 2019). In general terms, the BBB is a semipermeable endothelial cell membrane, which borders cerebral microvessels. It plays a role in the regulation of red blood cells and immune cells in the CNS. In addition, it allows neurotoxins to be expelled from the brain to the bloodstream (Zenaro et al., 2017). As previously mentioned, the BBB is disrupted by the secretion of pro-inflammatory cytokines by M1 microglia in AD (Lin et al., 2018). Activated M1 microglia secrete the major proinflammatory cytokine, IL-1 β , which can lead to increased permeability of the BBB. Other functions of the IL-1 β cytokine include the diminished ability of astrocytes to maintain the BBB and the promotion of neutrophil transmigration to the BBB (Zenaro et al., 2017). In addition, a higher permeability of the BBB also contributes to cognitive decline, a common characteristic seen in AD patients (van de Haar, 2016).

Metabolic diseases have been found to be caused by microbiota dysbiosis, which is an imbalance of the gut-resident microbial population (Sanz et al., 2015). As of today, only one study has shown that metabolic diseases can increase the permeability of the BBB, which can further promote AD pathology (Acharya et al., 2013). The authors showed that metabolic diseases, such as diabetes mellitus (DM) and hypercholesterolemia (HC), increase the permeability of the BBB, which allows the entry of blood plasma components, such as

soluble amyloid- β peptides (often present in diabetic mammals), immunoglobulins, and complement proteins, into diabetic and hypercholesterolaemic (DMHC) pig brain tissue. In this study, pigs were chosen as the model organism because their metabolism and physiology are similar to that of humans. Blood plasma components that cross the BBB bind to the surfaces of neurons, microglia, and astrocytes. This causes severe cell death, which is seen in AD, and a chronic neuroinflammatory response. Furthermore, the authors mentioned that immunoglobulin G, also known as IgG antibodies, can bind to cortical pyramidal neurons in brain tissue and disrupt their normal function. However, the choice of IgG to specifically target cortical pyramidal neurons is not well understood.

Although Acharya et al. (2013) did not directly associate an impaired microbiome with increased BBB permeability and consequent AD pathology progression, we speculate that this connection may exist. This is because microbiota dysbiosis, which causes metabolic diseases, increases the permeability of the gut and allows microbiota-derived products such as LPS, β -N-methylamino-L-alanine (BMAA), and microbial amyloids to enter the bloodstream. These microbial products increase BBB permeability, which contributes to amyloid- β accumulation in the brain (Sarkar & Banerjee, 2019). However, the exact mechanism as to how microbiota-derived products can cause an

increased BBB permeability needs to be investigated.

Discussion

It is crucial to find the specific pathways and mechanisms involved in the development of AD for the development of appropriate therapeutic interventions. The scientific literature strongly suggests that gut microbiota may be linked to the pathological development of AD. Gut microbiota promotes neuroinflammation through the secretion of proinflammatory cytokines by activated microglia and astrocytes. In addition, we speculate that metabolic diseases, caused by an impaired microbiome, may increase the permeability of the BBB and promote AD pathology. Research has shown that the ageing process for older adults is correlated with microbiota diversity. More specifically, the ageing process is associated with increases in the level of microbiota species that are connected to inflammation (Xu et al., 2019). It is possible that changes in the microbiome, as an individual ages, may contribute to the development of AD pathology. Hence, age is a huge risk factor for AD (Askarova et al., 2020).

The findings from the literature about the brain-gut axis and development of AD raise the possibility of various therapeutic interventions. For instance, researchers have speculated whether the therapeutic manipulation of microbiota, through the use of antibiotics, may reduce AD's

pathological development. Angelucci et al. (2019) claim that it is possible that the administration of antibiotics may reduce inflammation-causing bacteria. However, this may increase the risk of dysbiosis as antibiotics have broad action rather than selective action. To solve this issue, the researchers believe that the use of probiotics may counter the negative impacts of antibiotics. Only a small number of studies have implemented therapeutic interventions targeting the microbiota. A study demonstrated that the use of probiotics with *Lactobacilli*- and *Bifidobacteria*-based bacteria improves cognitive symptoms in patients with AD (Akbari et al., 2016). When using animal models to investigate the effectiveness of antibiotics in reducing AD pathology, researchers found that antibiotics reduced amyloid- β protein levels in male mice but not in female mice (Dodiya et al., 2019). This suggests that sex differences may impact the effectiveness of treatments. The brain-gut axis and its contributing role in AD is a relatively new field of study with many knowledge gaps that need to be addressed. Future studies should identify the specific gut microbiota species that are associated with AD so that appropriate therapeutic interventions can be developed. It must be noted that AD is considered to be a multifaceted disease. Restoration of the gut microbiome may be tackling one area of this disease and may not be sufficient to address every mechanism and pathway involved in its pathological development.

Note

This paper was written for a course based in the University of Toronto Scarborough.

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