Review

Brain-Gut-Microbiota Axis in Alzheimer's Disease

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Disturbances along the brain-gut-microbiota axis may significantly contribute to the pathogenesis of neurodegenerative disorders. Alzheimer's disease (AD) is the most frequent cause of dementia characterized by a progressive decline in cognitive function associated with the formation of amyloid beta (Aβ) plaques and neurofibrillary tangles. Alterations in the gut microbiota composition induce increased permeability of the gut barrier and immune activation leading to systemic inflammation, which in turn may impair the blood-brain barrier and promote neuroinflammation, neural injury, and ultimately neurodegeneration. Recently, Aβ has also been recognized as an antimicrobial peptide participating in the innate immune response. However, in the dysregulated state, Aβ may reveal harmful properties. Importantly, bacterial amyloids through molecular mimicry may elicit cross-seeding of misfolding and induce microglial priming. The Aβ seeding and propagation may occur at different levels of the brain-gut-microbiota axis. The potential mechanisms of amyloid spreading include neuron-to-neuron or distal neuron spreading, direct blood-brain barrier crossing or via other cells as astrocytes, fibroblasts, microglia, and immune system cells. A growing body of experimental and clinical data confirms a key role of gut dysbiosis and gut microbiota-host interactions in neurodegeneration. The convergence of gut-derived inflammatory response together with aging and poor diet in the elderly contribute to the pathogenesis of AD. Modification of the gut microbiota composition by food-based therapy or by probiotic supplementation may create new preventive and therapeutic options in AD.
(J Neurogastroenterol Motil 2019;25:48-60)

Key Words
Alzheimer disease; Amyloid; Blood-brain barrier; Gastrointestinal microbiome; Inflammation

Introduction

The brain-gut axis reflects the bidirectional, constant communication between the central nervous system (CNS) and the gastrointestinal tract. There is also a growing body of evidence that the intestinal microbiota influences the brain-gut interactions in different points of time (from early life to neurodegeneration), as well as at different levels (from the gut lumen to the CNS).[1] The importance of microbiota impact on the brain led to broadening the term to "brain-gut-microbiota axis." The mechanisms of this communication include neural, immune, endocrine, and metabolic signaling.[2] The neural network controlling gastrointestinal function—the enteric nervous system (ENS)—has the ability either to work independently or to be influenced by the CNS via sympathetic (prevertebral ganglia) and parasympathetic (the vagus nerve) signaling. The results of animal studies using germ-free mice point to the key role of gut microbiota in early brain development and adult neurogenesis.[1,2]

In the elderly, hyperstimulation of the immune system results in chronic, low-grade state of inflammation ("inflammaging").[3] It may be associated with persistent inflammatory state of the gut mucosa evoked by age-related alterations in the gut microbiota composition characterized by its decreased diversity and stability.[1] This leads to

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ⓒ 2019 The Korean Society of Neurogastroenterology and Motility
J Neurogastroenterol Motil, Vol. 25 No. 1 January, 2019
www.jnmjournal.org

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the gut barrier breakdown, further increase of proinflammatory cytokines and bacteria-derived products in the circulation, the blood-brain barrier impairment and neuroinflammation.[4] Apart from protecting against infection, the immune system influences neural function and development. The results of studies in germ-free mice confirm microbiota impact on microglia maturation. This could be mediated by short chain fatty acids (SCFAs) which are products of bacterial metabolism. Similarly, specific products of microbial tryptophan metabolism modulate astrocyte activity via aryl hydrocarbon receptors. Microbiota influences peripheral immune cell activation and cytokine profile, which affect systemic and CNS inflammation and injury, but also neurodevelopment.[2] A recently identified network of lymphatic vessels in the meningeal spaces connects peripheral lymphatic tissues to the CNS.[5]

In addition, the gut microbiota may affect the CNS function via direct synthesis of various neurotransmitters and neuromodulators like serotonin, dopamine, or SCFAs.[6,7] Importantly, the gut microbiota signaling may modulate the function of intestinal enterochromaffin cells, which produce different hormones and neurotransmitters including serotonin.[6] Disturbances along the brain-gut-microbiota axis may significantly contribute to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD).[7]

AD is the most frequent cause of dementia characterized by a progressive decline in cognitive function.[8] The key feature of the disease is deposition of amyloid beta (Aβ) followed by formation of plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein.[9] Those deposits trigger neuroinflammation leading to synapse loss and neuronal death.[4] It is still not well-known what triggers amyloid plaque formation, but the gut microbiota plays certainly an important role in the process. Regarding tau, it is a highly soluble protein modulating the stability of axonal microtubules. According to the tau hypothesis, altered and aggregated forms of this protein appear to act as toxic stimuli contributing to neurodegeneration.[9]

AD is classified based on the age of onset into early-onset (EOAD) starting before the age of 65 and late-onset (LOAD) beginning above that age. The EOAD accounting for 1-5% of all cases is in majority associated with the mutations in *APP*, *PSEN1*, and *PSEN2* genes with autosomal dominant inheritance.[8,10] Depending on the mutations, the consequences are: an increase in total Aβ production, an increase in more amyloidogenic and prone to aggregation Aβ42 production or a change in amino acid sequence resulting in increased aggregation properties. The increased amount or aggregation propensity of Aβ is sufficient to cause AD, while Aβ aggregation is critical for the pathogenesis of the disease.[10] The

majority of AD cases are its LOAD type, where different genes contribute to susceptibility for the disease.[10] Those genes code proteins which are involved in amyloid precursor protein (APP) metabolism, immune response, inflammation, intracellular trafficking, or lipid metabolism, indicating the potential pathogenetic factors.[8] Other, non-genetic, risk factors for LOAD encompass cerebrovascular disease, brain injury, hypertension, type 2 diabetes, and obesity.[8]

The review presents recent data on the role of brain-gut-microbiota axis dysregulation in the pathogenesis of AD based on the results from animal studies and available clinical observations. Potential therapeutic implications of the gut microbiota modulation in AD are also briefly discussed.

Amyloid Plaque Formation

Central Nervous System

The amyloid plaques are composed mainly of Aβ which is a cleavage product of APP.[11] This transmembrane protein is involved in various biological processes such as neuronal development, signaling, or intracellular transport.[12,13] APP is processed by secretases in the non-amyloidogenic pathway (α- and γ-secretase) or amyloidogenic pathway (β- and γ-secretase).[11,12] The amyloidogenic pathway creates Aβ peptides of different lengths, among which most frequent are Aβ40, and less abundant, but more neurotoxic Aβ42 peptides that form the core of the plaque.[4] Those peptides can aggregate to form oligomers, protofibrils, and fibrils that deposit into senile plaques, the intermediate forms being the most neurotoxic (Fig. 1).[4,12,14] Monomeric and oligomeric structures are bonded to the ends of the initial seed, which finally breaks generating in this way new amyloid seeds that makes the process self-propagating.[14] The process of seed formation (nucleation phase) is the most time-consuming step, thermodynamically unfavorable and may not occur in physiological conditions.[15] In vitro, the time that precedes protein aggregation can be greatly shortened by the addition of exogenous seeds.[14]

Interestingly, Aβ has recently been recognized as antimicrobial peptide (AMP), a part of the innate immune system.[16,17] In addition, while monomeric Aβ shows little antimicrobial activity, its capability of aggregation allows to form antimicrobial pore-forming structures.[17] The process of amyloid formation includes myeloid differentiation primary response 88 (MyD88) pathway activated by toll-like receptor 2 (TLR2). MyD88 is a universal adaptor protein used by almost all TLRs, except for TLR3, to activate transcrip-
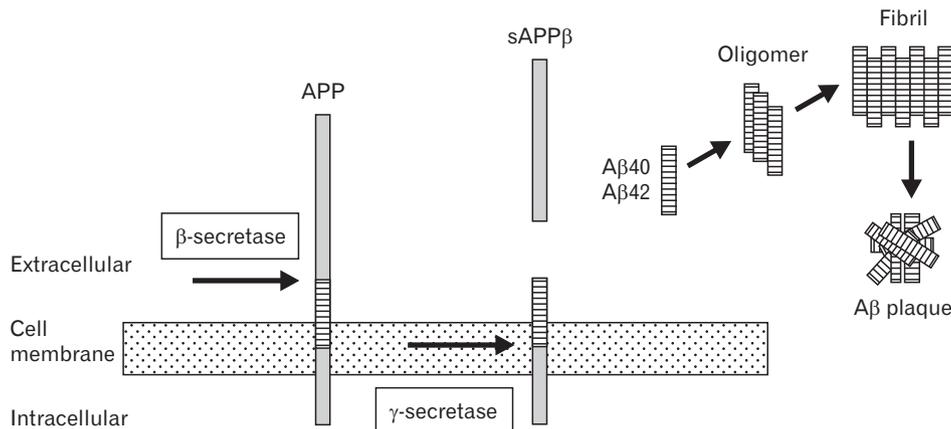
Figure 1. Amyloid beta (Aβ) plaque formation. Aβ is a cleavage product of amyloid precursor protein (APP). APP is a transmembrane protein which undergoes cleavage via amyloidogenic pathway involving 2 enzymes: β-secretase and γ-secretase. β-secretase cuts APP at a position outside the cell and γ-secretase cuts APP at a position inside the cell membrane. Misfolded proteins (Aβ40 and Aβ42) act as seeds that accelerate the protein aggregation into oligomers, fibrils, and amyloid plaques. The fibril then breaks forming new seeds allowing for self-propagation of the process. sAPPβ, soluble amyloid precursor protein β.

tion factors such as nuclear factor kappa B (NF-κB).[18] It has been shown that MyD88 deficiency ameliorates β-amyloidosis in an animal model of AD.[19] The generated TNF-α in conjugation with TNF-α converting enzyme becomes α-secretase, splitting APP. Then, NF-κB produced in this process together with Aβ converting enzyme activates β- and γ-secretases forming Aβ.[18] The normally protective Aβ function and harmful properties in dysregulated state is consistent with observations concerning other human AMPs.[16]

Enteric Nervous System

The ENS is the intrinsic nervous system of the gastrointestinal tract. Its neurons are organized in microcircuits allowing for modulation of gastrointestinal function independently of the CNS, although the systems are interconnected and influence one another. This connection also allows for the disease spreading.[20] In Parkinson's disease (PD) gastrointestinal dysfunction is present in almost 80% of the patients, preceding motor dysfunction. In fact, α-synucleinopathy of the ENS has been suggested to be an early indicator of PD pathology.[21] The regular APP expression in the ENS supports the theory of the ENS involvement also in AD. The APP transgenic mice develop accumulation of Aβ in the enteric neurons leading to a decrease in enteric neuron abundance, dysmotility, and increased vulnerability to inflammation.[20] Preliminary data confirm that changes in the ENS in APP overexpressing transgenic mice correlate with the disease expression.[22]

The Role of Gut Microbiota in the Development of Alzheimer's Disease

Bacterial Amyloids

The gut microbiota is a source of a significant amount of amyloids. The best studied bacterial amyloid is curli produced by *Escherichia coli*.[23] The production of amyloid proteins helps bacterial cells to bind to each other forming biofilms and to resist destruction by physical or immune factors.[23,24] Although bacterial amyloids differ from the CNS amyloids in their primary structure, they share similarities in their tertiary structure.[25,26] The exposure to bacterial amyloid proteins in the gut may cause priming of the immune system, consequently enhancing immune response to endogenous production of neuronal amyloid in the brain.[24] In the pioneer study by Chen et al[27] rats exposed to curli-producing *E. coli* displayed increased neuronal alpha-synuclein (α-syn) deposition in both the gut and brain, and enhanced microgliosis and astrogliosis compared to rats exposed to bacteria without the ability to produce curli.[27] Moreover, in the brain of animals exposed to curli-producing bacteria an increased expression of TLR2, Il-6, and TNF was found.[27] Friedland and Chapman[24] have even proposed a new term "mapranosis" to describe the process ("-osis") of microbiota-associated proteopathy and neuroinflammation. Through molecular mimicry bacterial amyloids may act as prion proteins, eliciting cross-seeding, in which one amyloidogenic protein (curli, tau, Aβ, α-syn, and prion) causes

another (eg, host proteins with a different primary structure) to adopt pathogenic β-sheet structure. Cross-seeding of amyloidogenic proteins by bacterial amyloids has been documented both in vivo and in vitro.[28,29]

Lipopolysaccharides

An experimental study in an animal model has shown that injection of bacterial lipopolysaccharide (LPS) into the fourth ventricle of the brain reproduces many of the inflammatory and pathological features seen in AD.[30] Moreover, the injection of LPS into the peritoneal cavity of mice has led to prolonged elevation of Aβ in the hippocampal region resulting in cognitive defects.[31] The results of in vitro studies confirmed that bacterial LPS promotes amyloid fibrillogenesis.[32] It is also known that LPS is capable to induce a more pathogenic β-pleated sheet conformation of prion amyloids.[33]

Recently, LPS presence has been detected in the hippocampus and neocortex brain lysates from AD patients.[34] Most of LPS is aggregated in the perinuclear region[35] significantly reducing output of DNA transcription products.[36] Moreover, LPS colocalizes with Aβ1-40/42 in amyloid plaques and around blood vessels.[37] The plasma concentration of LPS in AD patients is also significantly higher than in healthy people.[38] Other bacterial products such as E.coli pili protein[37] or nucleic acids[39] were also found in human brain and were more prevalent in AD patients.

LPS activates TLRs expressed in microglial cells of the innate immune system, which recognize common damage or pathogen associated molecular patterns.[40] Through interactions with CD14 and MD-2 proteins, LPS activates TLR4 receptor promoting inflammatory response.[25] The TLR4 activation by CD14 also mediates the inflammatory response to Aβ[41] and S100A8/A9 proteins.[42] The other LPS-activated receptor, TLR2, is also triggered by Aβ and bacterial amyloids.[25] These interactions support the concept of molecular mimicry of those particles.[26]

Gut Inflammation and Gut Barrier Dysfunction

Intestinal inflammatory process causes migration of polymorphonuclear cells from the circulation to the gut mucosa or even further to the gut lumen, in the case of mucosal architecture disturbance. The process of intestinal inflammation can be indirectly measured by assessing stool calprotectin concentration. This small calcium-binding protein which is a heterodimer of S100A8/A9 contributes to 60% of cytosol protein content of neutrophils and has antimicrobial properties.[43] The S100A8 and S100A9 proteins have intrinsically amyloidogenic amino acid sequences and can form amyloid oligomers and fibrils, which closely resemble amyloid

polypeptides such as Aβ and α-syn, and in vitro monomeric and dimeric S100A9 may induce Aβ fibrillization.[44,45] S100A9 secreted by macrophages and microglia during amyloid plaque formation also induces its expression in neuronal cells and these may further activate microglia via TLR4 and receptor for advanced glycation end products (RAGE) pathways.[44] Calprotectin levels are significantly increased in the cerebrospinal fluid and the brain of AD patients, which promotes its amyloid aggregation and co-aggregation with Aβ.[44] The elevated fecal calprotectin level was found in nearly 70% of AD patients in one study, and it was assumed that it could translocate into circulation and contribute to neuroinflammation.[46] Analogical changes in the intestinal epithelial barrier integrity and gut immune system activation expressed by elevated fecal calprotectin have also been reported in PD patients.[47] It is possible that this intestinal source of calcium binding proteins may contribute to amyloid fibril formation in the gut or directly in the brain. Gut inflammation and dysbiosis is directly associated with gut barrier dysfunction and increased intestinal permeability ("leaky gut") may contribute to the process of neurodegeneration.[48,49]

The intestinal barrier is composed of the mucus layer, intestinal epithelium, and lamina propria. Interruption of this barrier leads to increased permeability causing translocation of bacteria (process known as atopobiosis) and harmful substances into the bloodstream.[50-52] A very high number of bacteria localized in the colon is physically separated from the host by a thick, impenetrable mucus layer. Contrary, in the small intestine the mucus allows particles as large as bacteria to penetrate, although high concentrations of antibacterial products prevent bacteria from reaching the cell surface. The microbiota composition determines mucus layer properties influencing its permeability.[53] The abundance of mucin-degrading bacteria Akkermansia muciniphila improves the gut barrier function, reduces obesity and systemic inflammation.[54,55] Some probiotic strains such as Lactobacillus plantarum, E. coli Nissle, and Bifidobacterium infantis enhance intestinal barrier increasing expression of proteins forming tight junctions.[52] Other bacterial products, exotoxins, disrupt epithelial cell integrity. Different pathogenic E. coli strains, Salmonella, Shigella, Helicobacter pylori, Vibrio, or Clostridium mediate changes in tight junctions.[51] The exotoxin of Bacteroides fragilis disrupts adherence junctions by cleavage of cell adhesion molecule—E-cadherin.[56] Damaged tight junction structures and increased intestinal permeability connected with shifted microbiota profile were also found in a mouse model of amyotrophic lateral sclerosis—another neurodegenerative disorder connected with amyloid deposition.[57]

In addition to alterations in the gut microbiota composition,

the increased amount of bacteria in the small intestine also influences permeability, as seen in small intestinal bacterial overgrowth (SIBO).[58] There are some preliminary results showing the increased SIBO prevalence in AD patients.[59]

Neuroinflammation

Neuroinflammation expressed by activation of microglia, reactive astrocytes and complement in the vicinity of amyloid plaques is a well-known feature of AD.[60] The increased inflammatory response is also detected in blood and cerebrospinal fluid of AD patients.[60]

The physiological clearance of Aβ is very efficient.[61] In the early stages of AD low Aβ concentration activates microglia through CD14 and TLR promoting phagocytosis and amyloid clearance.[4] The process of oligomerization significantly increases amyloid retention.[61] Excessive microglial stimulation and increased neuroinflammatory signaling through NF-κB, proinflammatory cytokines and reactive oxidative and nitrosative stressors lead to neuronal and glial cell death.[62] Another consequence of neuroinflammation is downregulation of triggering receptor expressed on myeloid cells 2 which further impairs phagocytosis leading to the accumulation of Aβ42.[63]

An altered threshold for microglial activation seen in neurodegeneration and aging may be a consequence of repeated or chronic systemic infection.[64] Repeated systemic exposure to LPS in mice induced microglial priming and prolonged cytokine production. Subsequent intracerebral injection of LPS in previously infected mice resulted in exaggerated inflammatory response.[65] It is possible that microglial cells primed with bacterial amyloid may be more responsive to Aβ in the brain.[26]

Amyloid Spreading

The Aβ seeding and propagation is well documented with experiments based on animal models. The brain infusion with Aβ extract from AD brain leads to amyloid formation[66] and the seeding of amyloid in one brain region spreads to neuroanatomically connected regions of the brain.[15,67] When a small amount of insoluble, aggregation-prone Aβ42 is seeded it acts as a template promoting otherwise soluble and abundant Aβ40 oligomerization and spreading.[68] The amount of the soluble target peptide in the brain is the most important feature for toxic species formation.[68] Interestingly, intraperitoneal injection of Aβ extracts also leads to amyloid deposition in the brain.[69] The potential mechanisms of amyloid spreading include neuron-to-neuron or distal neuron spreading, direct blood-brain barrier crossing, or via other cells as astrocytes, fibroblasts, microglia and immune system cells.[70]

Neuronal Transport

An amyloid protein—α-syn, forming intracellular deposits constituting a hallmark of PD—was found in the myenteric neurons of the gut wall.[58] The protein may gain access to neuronal cells from the gut lumen via epithelial microfold cells (M cells) and dendritic cells in the Peyer's patches of the small intestine. The dorsal motor nucleus of the vagus nerve is one of the first affected brain regions containing α-syn deposits.[26] These data suggest that misfolded proteins spread along the gut-brain axis.[58] The accumulation of misfolded proteins in neuronal cells and subsequent cell death result in release of misfolded proteins into the intracellular space. Moreover, living cells may release the proteins via exocytosis. These proteins are then taken up by other neurons leading to local transmission of misfolded proteins. Absorbed misfolded proteins may then induce templated conformational changes in susceptible proteins of the cell. The process may spread across neuronal network via synapses.[71] Propagation of the misfolded tau protein from the outside to the inside of the cell, subsequent intracellular protein misfolding, aggregation and transfer to other co-cultured cells were observed in vitro.[72] In addition to extracellular Aβ deposition, the protein accumulates inside neurons. This process is observed early in the disease course, preceding neurofibrillary tangles formation and extracellular Aβ deposition.[73] Regarding previously mentioned in vivo observations of Aβ spreading across neuronal networks, the potential role of neuronal transport in amyloid misfolding propagation can be assumed.

Compromised Blood-Brain Barrier

The blood-brain barrier formed by brain endothelial cells and pericytes separates the CNS from blood-derived molecules, pathogens, and cells.[74] In normal conditions soluble Aβ is transported from the blood to the brain via RAGE and via low-density lipoprotein receptor-related protein 1 in the opposite direction.[12,75]

In post-mortem studies in AD patients, the blood-brain barrier damage and accumulation of blood derived products in the brain were demonstrated.[17] This process was confirmed by MRI studies of the living human brain, which showed age-dependent blood-brain barrier breakdown in the hippocampus associated with learning and memory.[74] The breakdown was worse in mild cognitive impairment and correlated with pericyte injury shown by cerebrospinal analysis.[74] The pericyte injury is accelerated by the ε4 allele of the apolipoprotein E gene, the major genetic risk factor for LOAD.[76]

Gut Microbiota in Animal Models of Alzheimer's Disease

The contribution of gut microbiota to the pathogenesis of AD

is well depicted in animal models of AD (Table 1).[77-89] In 2016, for the first time, Minter et al[77] reported that antibiotic-induced perturbations in the gut microbiota diversity influence neuroinflammation and amyloidosis in a murine model of AD. The results of another study, in which sequencing bacterial 16S ribosomal RNA from

Table 1. Current Data From Animal Studies on the Role of Microbiota in the Pathogenesis of Alzheimer's Disease

AD model	Main findings	Reference
APP/PS1 mice	Antibiotic-treated Tg mice display alterations in the gastrointestinal microbiome composition (expansion of Lachnospiraceae) and circulating inflammatory mediators; antibiotic-treated male Tg mice display reduced Aβ deposition but increased soluble Aβ levels, reduced reactive gliosis surrounding Aβ plaques and significantly altered microglial morphology	Minter et al,[77] 2016
	Early post-natal antibiotic treatment results in long-term alterations of gut microbial genera (expansion of Lachnospiraceae) and reduction in brain Aβ deposition in aged Tg mice; plaque-localized microglia and astrocytes reduced in antibiotic-exposed mice	Minter et al,[78] 2017
	A remarkable shift in the gut microbiota profile in Tg mice compared to WT mice; a drastic reduction of cerebral Aβ pathology in germ-free Tg mice compared to control mice; colonization of germ-free Tg mice with microbiota from conventionally-raised Tg mice increased cerebral Aβ pathology, while colonization with microbiota from WT mice was less effective in increasing cerebral Aβ levels	Harach et al,[79] 2017
	A significant increase in the abundance of Helicobacteraceae and Desulfovibrionaceae at the family level, *Odoribacter* and *Helicobacter* at the genus level, and lower *Prevotella* abundance in Tg mice compared to WT mice	Shen et al,[80] 2017
	AD pathology associated with a shift in the gut microbiota composition towards profiles that share features with autism and inflammatory disorders	Bäuerl et al,[81] 2018
	Prebiotic supplementation with oligosaccharides from *Morinda officinalis* (OMO) maintained the diversity and stability of the microbial community; OMO ameliorated brain tissue swelling and neuronal apoptosis and downregulated the expression of Aβ	Xin et al,[82] 2018
3×Tg-AD mice	Probiotic treatment influenced plasma concentration of inflammatory cytokines and gut hormones, and induced also a reduction in brain damage and accumulation of Aβ aggregates	Bonfili et al,[83] 2017
5×FAD mice	Changes in fecal microbiota composition along with age; reduced trypsin amount in fecal proteins; human APP expressed not only in the brain but also in the gut tissue	Brandscheid et al,[84] 2017
ApoE⁻/⁻ mice	Active invasion of *Porphyromonas gingivalis* and infection-induced complement activation in ApoE⁻/⁻ mice brains	Poole et al,[85] 2015
AD mouse model (ICV injection of Aβ)	Oral administration of *Bifidobacterium breve* strain A1 prevented Aβ-induced cognitive dysfunction and suppressed Aβ-induced changes in gene expression in the hippocampus; *B. breve* A1 did not affect the gut microbiota, but significantly increased plasma acetate levels; non-viable *B. breve* A1 and acetate partially ameliorated behavioral deficits	Kobayashi et al,[86] 2017
AD rat model (IP injection of D-galactosea)	*Lactobacillus plantarum* MTCC 1325 restored acetylcholine level, attenuated Aβ plaque formation, and ameliorated cognitive function	Nimgampalle et al,[87] 2017
AD rat model (intrahippocampal injection of Aβ)	*Lactobacillus* and *Bifidobacterium* ameliorated memory and learning deficits and oxidative stress	Athari et al,[88] 2018
Transgenic flies: *Drosophila*	Enterobacteria infection exacerbates progression of AD by promoting immune hemocyte recruitment to the brain; genetic depletion of hemocytes attenuates neuroinflammation and alleviates neurodegeneration	Wu et al,[89] 2017

[a]D-galactose administration induces brain aging.

AD, Alzheimer's disease; APP, amyloid precursor protein; PS1, presenilin-1; APP/PS1 mice, double transgenic mice harboring mutations in APP and PS1 genes; Tg, transgenic; Aβ, amyloid beta; WT, wild type; 3×Tg-AD mice, triple transgenic mice displaying both plaque and tangle pathologies; 5×FAD mice, mice carrying 5 familial AD mutations in APP and PS1 transgenes; ApoE⁻/⁻ mice, apolipoprotein E-deficient mice; ICV, intracerebroventricular; IP, intraperitoneal.

fecal samples of APP transgenic mice was performed, revealed significant differences in the gut microbiota composition compared to that of control wild type mice.[79] These changes included an increase in Rikenellaceae and a decrease in *Allobacillum* and *Akkermansia*.[79] The reduced abundance of *Akkermansia* has previously been associated with obesity and type 2 diabetes,[55] which are known risk factors for developing dementia.[90] Moreover, its relative abundance negatively correlated with the amount of Aβ42 in the brain.[79] In germ-free APP transgenic mice cerebral Aβ was significantly reduced. In addition, reduced microgliosis and changes in cytokine profile were observed. Recolonization of the germ-free mice with conventionally raised APP transgenic mice microbiota increased cerebral Aβ pathology, and this increase was less effective when wild type mice microbiota was used.[79] Similarly, germ-free mice overexpressing α-syn, used as a model of PD, developed reduced α-syn inclusions and microglial activation compared to the controls.[91] These features were restored by reintroduction of microbiota (especially those derived from PD patient donors) or by addition of SCFAs—products of bacterial metabolism. Interestingly, recolonization with bacteria derived from PD patients enhanced physical impairments, which was connected with different SCFA profile produced by this bacterial strains.[91]

Clinical Data on the Gut Dysbiosis in Alzheimer's Disease

Many human studies implicated microbiota presence in the brain in the etiology of AD,[71] although most of the studies were conducted post-mortem, diminishing the evidence for their causative role in AD pathology (Table 2).[34-39,60,92-102] These pathogens include *Chlamydophila pneumoniae*,[93] *Borrelia burgdorferi*, and other spirochetes[94] or herpes simplex virus type 1.[103] Also *H. pylori* infection was linked with AD.[96] AD patients with *H. pylori* infection had lower Mini-Mental State Examination scores corresponding with more serious cognitive impairment.[97] A recently published study has revealed an increase in the abundance of *Helicobacter* and *Odoribacter* and a decreased level of *Prevotella* in APP transgenic mice.[80] Also, in PD patients *H. pylori* infection was linked with disease severity and progression.[58] The significantly increased levels of *H. pylori*-specific IgG antibody in the cerebrospinal fluid and serum of AD patients were found.[97] Moreover, the titer of this antibody correlated with the degree of severity of AD.[97] However, in a recent population-based cohort including 4215 participants, the association between *H. pylori* serology and dementia risk was not confirmed.[104] A potential mechanism of bacterial translocation

to the brain is transmigration of infected monocytes and T cells through the compromised blood-brain barrier.[17] Another possibility is that the entry point for pathogens might be the olfactory nerves, as their plasma membrane is the only barrier between the nasal cavity and the brain.[26] Moreover, poor dental hygiene has been linked to AD.[99] Although the data are limited, some studies demonstrated elevated serum antibodies to bacteria associated to periodontitis in AD patients.[100,101]

The influence of gut microbiota on brain function is being constantly investigated, and the mechanisms of the brain-gut-microbiota axis contribution to pathogenesis of stress-related conditions or brain disorders is being discovered.[105] In irritable bowel syndrome, where altered microbiota is one of key pathophysiological factors of the disease,[106] some preliminary results indicate that there is also increased risk for either AD or non-AD dementia development.[107] Other conditions, where the gut microbiota influence has been implicated, include autism, schizophrenia or multiple sclerosis.[1,2,48,105]

A recently conducted study revealed that the increased abundance of proinflammatory *Escherichia/Shigella* and decreased abundance of anti-inflammatory *Eubacterium rectale* were possibly associated with peripheral inflammation in patients with cognitive impairment and brain amyloidosis.[60] In another study fecal microbiota compositions of AD and non-AD patients were compared.[95] Fecal microbiota profile in AD patients was characterized by the reduced microbial diversity, decreased abundance of *Firmicutes* and *Bifidobacterium* and increased abundance of *Bacteroidetes*. The relative bacterial abundance correlated with the increase of cerebrospinal fluid markers of AD pathology.[95] Neurodegenerative disorders usually reveal in advanced age, when the gut microbiota composition is influenced by variety of factors. Poor diet is associated with reduced microbial diversity, contributing to the increased local and systemic inflammation in the elderly. As mentioned in the introduction, the phenomenon is adequately named as "inflammaging".[2,3] Multiple comorbidities influence microbiota composition directly or by used medications such as antibiotics, metformin or proton pump inhibitors.[3]

Microbiota Modulation as a Therapeutic Target in Alzheimer's Disease

A better understanding of the role of gut microbiota in the pathogenesis of AD and the close association between gut dysbiosis, increased intestinal permeability, and neurological dysfunction creates opportunity for potential therapeutic interventions.[108] The results of numerous studies confirm the beneficial effect of probiotics

Table 2. Recent Clinical Data on the Role of Microbiota in the Pathogenesis of Alzheimer's Disease

Type of study	Number of subjects (M/F)	Main findings	Reference
Post-mortem brain samples	10 AD 10 C (sex not specified)	LPS from periodontopathic *Porphyromonas gingivalis* present in AD brains	Poole et al,[92] 2013
	10 AD (all F) 8 C (all F)	Bacterial LPS present in AD brain lysates; mean LPS levels varied from 2-fold increase in the neocortex to 3-fold increase in the hippocampus in AD over age-matched controls	Zhao et al,[34] 2017
	7 AD (all F) 7 C (all F)	> 75% of all LPS signals associated with brain cell nuclei in AD (random association of LPS with Aβ deposits in the controls); LPS abundance greater than 7-fold in AD neocortex and > 21-fold in AD hippocampus	Zhao et al,[35] 2017
	15 AD (all F) 12 C (all F)	LPS accumulates in neocortical neurons of AD brain and impairs transcription in human neuronal-glial primary co-cultures	Zhao et al,[36] 2017
	24 AD (9/15) 18 C (10/8)	*Escherichia coli* K99 and LPS levels greater in AD brains; LPS colocalized with Aβ1-40/42 in amyloid plaques and around vessels in AD brain	Zhan et al,[37] 2016
	14 AD (3/11) 12 C (10/2)	Increased bacterial populations in AD brain tissue showed by 16S rRNA sequencing	Emery et al,[39] 2017
	5 AD 5 C (sex-matched)	Typical intracellular and atypical extracellular *Chlamydia pneumoniae* antigens present in the frontal and temporal cortices of AD brain; *C. pneumoniae*, amyloid deposits, and neurofibrillary tangles present in the same regions of AD brain	Hammond et al,[93] 2010
	10 AD 4 C (sex not specified)	*Borrelia burgdorferi* specific DNA found in senile plaques	Miklossy et al,[94] 2016
Living subjects	25 AD (8/17) 25 C (7/18)	Gut microbiota alterations characterized by reduced microbial diversity, decreased abundance of *Firmicutes* and *Bifidobacterium*, and increased abundance of Bacteroidetes	Vogt et al,[95] 2017
	18 AD (7/11) 23 sALS (16/7) 18 healthy (12/6)	Significantly higher levels of LPS in sALS and AD plasma specimens; a significant positive correlation between LPS plasma levels and degree of blood monocyte/macrophage activation in the disease groups	Zhang et al,[38] 2009
	40 Amy (+) (20/20) 33 Amy (−) (15/18) 10 C (4/6)	Increased abundance of proinflammatory gut microbiota taxon—*Escherichia/Shigella*, and a reduction in anti-inflammatory taxon—*Eubacterium rectale* possibly associated with a peripheral inflammatory state in Amy (+); a positive correlation between proinflammatory IL-1β, inflammasome complex (NLRP3), and CXCL2 with the abundance of *Escherichia/Shigella* and a negative correlation with the abundance of *E. rectale*	Cattaneo et al,[60] 2017
	50 AD 30 C (sex not specified)	The prevalence of *Helicobacter pylori* infection amounted to 85% in AD and 47% in C	Kountouras et al,[96] 2006
	27 AD 27 C (sex not specified)	*H. pylori*-specific IgG antibody levels significantly increased in cerebrospinal fluid and serum of AD; Antibody titer correlated with the degree of AD severity	Kountouras et al,[97] 2009
	53 AD (sex not specified)	Infection of *H. pylori* associated with a greater cognitive impairment in AD	Roubaud-Baudron et al,[98] 2012
	38 cognitively normal healthy subjects (12/26)	Association between periodontal disease and brain Aβ load showed using ¹¹C-PIB PET imaging	Kamer et al,[99] 2015

Table 2. Continued

Type of study	Number of subjects (M/F)	Main findings	Reference
	35 AD (9/26) 46 MCI patients (24/22) 77 C (32/45)	Serum IgG antibody levels against *Fusobacterium nucleatum* and *Prevotella intermedia* significantly increased in AD compared to the controls	Sparks Stein et al,[100] 2012
	110 AD (35/75) 109 C (36/73)	Serum IgG levels to common periodontal microbiota associated with risk for developing incident AD; high titer of anti-*Actinomyces naeslundii* IgG associated with increased risk of AD; high titer of anti-*Eubacterium nodatum* associated with lower risk of AD	Noble et al,[101] 2014
	30 AD in probiotic group (6/24) 30 AD in control group (6/24)	Probiotic supplementation for 12 weeks induced a significant improvement in Mini-Mental State Examination score	Akbari et al,[102] 2016

M, male; F, female; AD, Alzheimer's disease (patients); C, controls; LPS, lipopolysaccharide; Aβ, amyloid beta; 16S rRNA, 16S ribosomal RNA; sALS, sporadic amyotrophic lateral sclerosis; Amy (+), cognitively impaired patients with brain amyloidosis; Amy (−), cognitively impaired patients with no brain amyloidosis; NLRP3, NOD-like receptor family pyrin domain containing 3; CXCL2, C-X-C motif chemokine ligand 2; ^{11}C-PIB PET, Pittsburgh Compound-B positron emission tomography; MCI, mild cognitive impairment.

by enhancing intestinal epithelial integrity, protecting against barrier disruption, reducing proinflammatory response, and inhibiting initiation or propagation of neuroinflammation and neurodegeneration.[3,109] For example, it has been shown in vitro that *Enterococcus faecium* and *Lactobacillus rhamnosus* reduce TNF-α production and supplementation of these probiotic strains in animal studies reduced oxidative stress markers and induced antioxidant enzymes in the brain.[110] Several other reports from animal studies support the therapeutic potential of Lactobacilli and Bifidobacteria.[86-88,102,111] It has been suggested that attenuation of LPS-induced neuroinflammation and memory deficit by probiotics could be mediated via anti-inflammation through inhibition of acetylcholinesterase and antioxidant activities.[111] Also, in a clinical study, supplementation with Lactobacilli and Bifidobacteria-based probiotics improved significantly Mini-Mental State Examination scores in AD patients.[102]

Antibiotic treatment offers another option for the gut microbiota modulation and can be applied to treat SIBO and intestinal colonization by pathogenic strains. Surprisingly, in PD patients treatment of SIBO with rifaximin resulted not only in the improvement in gastrointestinal symptoms, but also motor fluctuations.[112]

Fecal microbiota transplantation is used in many animal models exploring pathogenetic mechanisms of neurodegenerative disorders. Its therapeutic potential has been reported in single cases of patients with PD, multiple sclerosis and autisms, but not AD so far.[113] Supposedly, fecal microbiota transplantation from healthy, young donors could restore the gut microbiota diversity and stability in the elderly.

However, one of the most effective approaches to modify the gut microbiota is dietary intervention. Food-based therapies may influence the gut microbiota composition or directly affect neuronal functioning in both the ENS and the CNS.[114,115] Healthy diet characterized by high intake of plant-based foods, probiotics, antioxidants, soy beans, nuts, and omega-3 polyunsaturated fatty acids, as well as low intake of saturated fats, animal-derived proteins, and refined sugar, has been shown to inhibit inflammatory response, reduce insulin resistance, and decrease the risk of neurocognitive impairment and eventually the risk of AD.[63,116]

Conclusions and Perspectives

There is increasing evidence for the gut microbiota contribution to the pathogenesis of AD (Fig. 2). The gut microbiota as the source of a large amount of amyloid, LPS, and other toxins, may contribute to systemic inflammation and disruption of physiological barriers. Bacteria or their products can move from the gastrointestinal tract and the oronasal cavity to the CNS, especially in the elderly. Bacterial amyloids may act as prion protein cross-seeding misfolding and enhancing native amyloid aggregation. Moreover, gut microbiota products may prime microglia, enhancing inflammatory response in the CNS, which in turn results in pathologic microglial function, increased neurotoxicity and impaired amyloid clearance. Taking into account Aβ role as the antimicrobial peptide, infectious or sterile inflammatory factors may enhance Aβ formation through TLRs. The modulation of the gut microbiota composition can be used as a potential therapeutic target in AD.

Up to now, the data on the role of gut microbiota in AD and other neurodegenerative disorders are based on preclinical or cross-
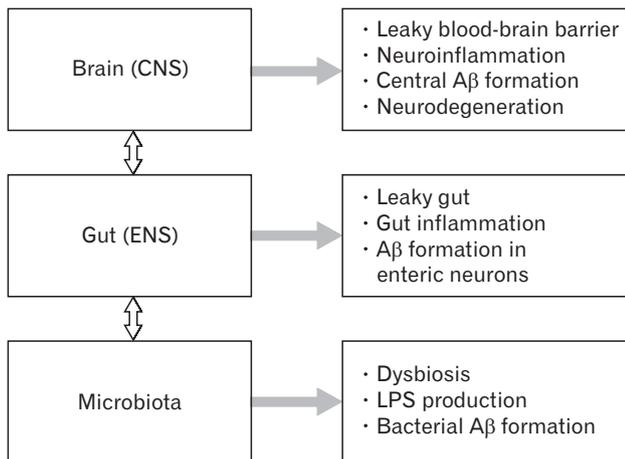
Figure 2. Disturbances of the brain-gut-microbiota axis in Alzheimer's disease. Disturbances along the brain-gut-microbiota axis, including the central nervous system (CNS) and the enteric nervous system (ENS), contribute to the pathogenesis of Alzheimer's disease. The gut microbiota is known to upregulate local and systemic inflammation due to lipopolysaccharides (LPS) from pathogenic bacteria and synthesis of proinflammatory cytokines. Alterations in the gut microbiota composition may induce increased permeability of the intestinal barrier and the blood-brain barrier further enhancing inflammation at the gut, systemic and CNS levels. Amyloid beta (Aβ) formation takes place in the ENS and the CNS. In addition, a large amount of amyloids is secreted by the gut microbiota.

sectional human studies.[48,108] Some limitations in translating basic research results to humans are related to the host-specific interactions with microbiota. To enhance and forward AD research large-scale epidemiological studies investigating the complex interactions between genes, microbiota, diet, and aging should be conducted. Some methodological issues concerning the evaluation of the microbiota composition and function including metabolomic profiling techniques need also to be considered. Moreover, the involvement of other microbiotas, apart from the microbiota in the gut and the oral and nasal cavities, in the pathophysiology of neurodegenerative disorders has not been explored so far.[48,117] Future studies should also encompass the role of other parts of the human microbiome including mycobiome and virome. Finally, the effect of numerous confounding factors such as diet, concomitant diseases and drugs require a careful attention in the analyses.[48] Recently, in light of the multi-dimensional nature of AD pathology, a potential reevaluation of the current definition of aging, cognition and their relationship to a variety of biological, social and environmental factors including the gut microbiota has been suggested.[118] An interdisciplinary approach to that complex field of host-microbiota interactions should eventually result in a strategic breakthrough in the treatment and,

more importantly, in the prevention of AD.

References

1. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. J Physiol 2017;595:489-503.

2. Quigley EMM. Microbiota-brain-gut axis and neurodegenerative iseases. Curr Neurol Neurosci Rep 2017;17:94.

3. Frasca D, Blomberg BB. Inflammaging decreases adaptive and innate immune responses in mice and humans. Biogerontology 2016;17:7-19.

4. Köhler CA, Maes M, Slyepchenko A, et al. The gut-brain axis, including the microbiome, leaky gut and bacterial translocation: mechanisms and pathophysiological role in Alzheimer's disease. Curr Pharm Des 2016;22:6152-6166.

5. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. Nature 2015;523:337-341.

6. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015;161:264-276.

7. Bhattacharjee S, Lukiw WJ. Alzheimer's disease and the microbiome. Front Cell Neurosci 2013;7:153.

8. Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol 2014;88:640-651.

9. Jouanne M, Rault S, Voisin-Chiret AS. Tau protein aggregation in Alzheimer's disease: an attractive target for the development of novel therapeutic agents. Eur J Med Chem 2017;139:153-167.

10. Schellenberg GD, Montine TJ. The genetics and neuropathology of Alzheimer's disease. Acta Neuropathol 2012;124:305-323.

11. De Strooper B. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. Physiol Rev 2010;90:465-494.

12. Chen GF, Xu TH, Yan Y, et al. Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacol Sin 2017;38:1205-1235.

13. Priller C, Bauer T, Mitteregger G, Krebs B, Kretzschmar HA, Herms J. Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci 2006;26:7212-7221.

14. Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature 2013;501:45-51.

15. Eisele YS. From soluble Aβ to progressive Aβ aggregation: could prion-like templated misfolding play a role? Brain Pathol 2013;23:333-341.

16. Kumar DK, Choi SH, Washicosky KJ, et al. Amyloid-β peptide protects against microbial infection in mouse and worm models of Alzheimer's

disease. Sci Transl Med 2016;8:340ra72.

17. Welling MM, Nabuurs RJ, van der Weerd L. Potential role of antimicrobial peptides in the early onset of Alzheimer's disease. Alzheimers Dement 2015;11:51-57.

18. Allen HB. Alzheimer's disease: assessing the role of spirochetes, biofilms, the immune system, and amyloid-β with regard to potential treatment and prevention. J Alzheimers Dis 2016;53:1271-1276.

19. Lim JE, Kou J, Song M, et al. MyD88 deficiency ameliorates β-amyloidosis in an animal model of Alzheimer's disease. Am J Pathol 2011;179:1095-1103.

20. Chalazonitis A, Rao M. Enteric nervous system manifestations of neurodegenerative disease. Brain Res 2018;1693(Pt B):207-213.

21. Nair AT, Ramachandran V, Joghee NM, Antony S, Ramalingam G. Gut microbiota dysfunction as reliable non-invasive early diagnostic biomarkers in the pathophysiology of Parkinson's disease: a critical review. J Neurogastroenterol Motil 2018;24:30-42.

22. Semar S, Klotz M, Letiembre M, et al. Changes of the enteric nervous system in amyloid-β protein precursor transgenic mice correlate with disease progression. J Alzheimers Dis 2013;36:7-20.

23. Cherny I, Rockah L, Levy-Nissenbaum O, Gophna U, Ron EZ, Gazit E. The formation of Escherichia coli curli amyloid fibrils is mediated by prion-like peptide repeats. J Mol Biol 2005;352:245-252.

24. Friedland RP, Chapman MR. The role of microbial amyloid in neurodegeneration. PLoS Pathog 2017;13:e1006654.

25. Zhao Y, Dua P, Lukiw WJ. Microbial sources of amyloid and relevance to amyloidogenesis and Alzheimer's disease (AD). J Alzheimers Dis Parkinsonism 2015;5:177.

26. Friedland RP. Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. J Alzheimers Dis 2015;45:349-362.

27. Chen SG, Stribinskis V, Rane MJ, et al. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged Fischer 344 rats and Caenorhabditis elegans. Sci Rep 2016;6:34477.

28. Lundmark K, Westermark GT, Olsén A, Westermark P. Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: cross-seeding as a disease mechanism. Proc Natl Acad Sci USA 2005;102:6098-6102.

29. Zhou Y, Smith D, Leong BJ, Brännström K, Almqvist F, Chapman MR. Promiscuous cross-seeding between bacterial amyloids promotes interspecies biofilms. J Biol Chem 2012;287:35092-35103.

30. Hauss-Wegrzyniak B, Vraniak PD, Wenk GL. LPS-induced neuroinflammatory effects do not recover with time. Neuroreport 2000;11:1759-1763.

31. Kahn MS, Kranjac D, Alonzo CA, et al. Prolonged elevation in hippocampal Aβ and cognitive deficits following repeated endotoxin exposure in the mouse. Behav Brain Res 2012;229:176-184.

32. Asti A, Gioglio L. Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? J Alzheimers Dis 2013;39:169-179.

33. Saleem F, Bjorndahl TC, Ladner CL, Perez-Pineiro R, Ametaj BN, Wishart DS. Lipopolysaccharide induced conversion of recombinant prion protein. Prion 2014;8:221-232.

34. Zhao Y, Jaber V, Lukiw WJ. Secretory products of the human GI tract microbiome and their potential impact on Alzheimer's disease (AD):

detection of lipopolysaccharide (LPS) in AD hippocampus. Front Cell Infect Microbiol 2017;7:318.

35. Zhao Y, Cong L, Jaber V, Lukiw WJ. Microbiome-derived lipopolysaccharide enriched in the perinuclear region of Alzheimer's disease brain. Front Immunol 2017;8:1064.

36. Zhao Y, Cong L, Lukiw WJ. Lipopolysaccharide (LPS) accumulates in neocortical neurons of Alzheimer's disease (AD) brain and impairs transcription in human neuronal-glial primary co-cultures. Front Aging Neurosci 2017;9:407.

37. Zhan X, Stamova B, Jin LW, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology 2016;87:2324-2332.

38. Zhang R, Miller RG, Gascon R, et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). J Neuroimmunol 2009;206:121-124.

39. Emery DC, Shoemark DK, Batstone TE, et al. 16S rRNA next generation sequencing analysis shows bacteria in Alzheimer's post-mortem brain. Front Aging Neurosci 2017;9:195.

40. Lukiw WJ. Bacteroides fragilis lipopolysaccharide and inflammatory signaling in Alzheimer's disease. Front Microbiol 2016;7:1544.

41. Fassbender K, Walter S, Kühl S, et al. The LPS receptor (CD14) links innate immunity with Alzheimer's disease. FASEB J 2004;18:203-205.

42. He Z, Riva M, Björk P, et al. CD14 Is a co-receptor for TLR4 in the S100A9-induced pro-inflammatory response in monocytes. PLoS One 2016;11:e0156377.

43. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. Clin Exp Gastroenterol 2016;9:21-29.

44. Wang C, Klechikov AG, Gharibyan AL, et al. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. Acta Neuropathol 2014;127:507-522.

45. Zhang C, Liu Y, Gilthorpe J, van der Maarel JR. MRP14 (S100A9) protein interacts with Alzheimer beta-amyloid peptide and induces its fibrillization. PLoS One 2012;7:e32953.

46. Leblhuber F, Geisler S, Steiner K, Fuchs D, Schütz B. Elevated fecal calprotectin in patients with Alzheimer's dementia indicates leaky gut. J Neural Transm 2015;122:1319-1322.

47. Mulak A, Budrewicz S, Panek-Jeziorna M, et al. Fecal biomarkers of gut inflammation and intestinal barrier dysfunction in Parkinson's disease. Gastroenterology 2017;152:S924.

48. Marizzoni M, Provasi S, Cattaneo A, Frisoni GB. Microbiota and neurodegenerative diseases. Curr Opin Neurol 2017;30:630-638.

49. Sochocka M, Donskow-Łysoniewska K, Diniz BS, Kurpas D, Brzozowska E, Leszek J. The gut microbiome alterations and inflammation-driven pathogenesis of Alzheimer's disease – a Critical Review. Mol Neurobiol 2018 (In press)

50. Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol Rev 2015;39:567-591.

51. König J, Wells J, Cani PD, et al. Human intestinal barrier function in health and disease. Clin Transl Gastroenterol 2016;7:e196.

52. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterol

2014;14:189.

53. Jakobsson HE, Rodríguez-Piñeiro AM, Schütte A, et al. The composition of the gut microbiota shapes the colon mucus barrier. EMBO Rep 2015;16:164-177.

54. Alkasir R, Li J, Li X, Jin M, Zhu B. Human gut microbiota: the links with dementia development. Protein Cell 2017;8:90-102.

55. Everard A, Belzer C, Geurts L, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc Nati Acad Sci USA 2013;110:9066-9071.

56. Choi VM, Herrou J, Hecht AL, et al. Activation of *Bacteroides fragilis* toxin by a novel bacterial protease contributes to anaerobic sepsis in mice. Nat Med 2016;22:563-567.

57. Wu S, Yi J, Zhang YG, Zhou J, Sun J. Leaky intestine and impaired microbiome in an amyotrophic lateral sclerosis mouse model. Physiol Rep 2015;3:e12356.

58. Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. World J Gastroenterol 2015;21:10609-10620.

59. Kowalski K, Jasinska M, Mulak A. Small intestinal bacterial overgrowth in Alzheimer's disease – a pilot study. [abstract book] Archives of International Society of Microbiota 2017;4:91.

60. Cattaneo A, Cattane N, Galluzzi S, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiol Aging 2017;49:60-68.

61. McIntee FL, Giannoni P, Blais S, et al. *In vivo* differential brain clearance and catabolism of monomeric and oligomeric Alzheimer's Aβ protein. Front Aging Neurosci 2016;8:223.

62. Li CQ, Zheng Q, Wang Q, Zeng QP. Biotic/abiotic stress-driven Alzheimer's disease. Front Cell Neurosci 2016;10:269.

63. Pistollato F, Sumalla Cano S, Elio I, Masias Vergara M, Giampieri F, Battino M. Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. Nutr Rev 2016;74:624-634.

64. Perry VH, Teeling J. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. Semin Immunopathol 2013;35:601-612.

65. Püntener U, Booth SG, Perry VH, Teeling JL. Long-term impact of systemic bacterial infection on the cerebral vasculature and microglia. J Neuroinflammation 2012;9:146.

66. Kane MD, Lipinski WJ, Callahan MJ, et al. Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. J Neurosci 2000;20:3606-3611.

67. Hamaguchi T, Eisele YS, Varvel NH, Lamb BT, Walker LC, Jucker M. The presence of Aβ seeds, and not age per se, is critical to the initiation of Aβ deposition in the brain. Acta Neuropathol 2012;123:31-37.

68. Sowade RF, Jahn TR. Seed-induced acceleration of amyloid-β mediated neurotoxicity *in vivo*. Nat Commun 2017;8:512.

69. Eisele YS, Obermüller U, Heilbronner G, et al. Peripherally applied Aβ-containing inoculates induce cerebral β-amyloidosis. Science 2010;330:980-982.

70. Espargaró A, Busquets MA, Estelrich J, Sabate R. Key points concerning amyloid infectivity and prion-like neuronal invasion. Front Mol

Neurosci 2016;9:29.

71. Frost B, Diamond MI. Prion-like mechanisms in neurodegenerative diseases. Nat Rev Neurosci 2010;11:155-159.

72. Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. J Biol Chem 2009;284:12845-12852.

73. Bayer TA, Wirths O. Intracellular accumulation of amyloid-beta – a predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. Front Aging Neurosci 2010;2:8.

74. Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron 2015;85:296-302.

75. Deane R, Du Yan S, Submamaryan RK, et al. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med 2003;9:907-913.

76. Halliday MR, Rege SV, Ma Q, et al. Accelerated pericyte degeneration and blood-brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease. J Cereb Blood Flow Metab 2016;36:216-227.

77. Minter MR, Zhang C, Leone V, et al. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci Rep 2016;6:30028.

78. Minter MR, Hinterleitner R, Meisel M, et al. Antibiotic-induced perturbations in microbial diversity during post-natal development alters amyloid pathology in an aged APP$_{SWE}$/PS1$_{\Delta E9}$ murine model of Alzheimer's disease. Sci Rep 2017;7:10411.

79. Harach T, Marungruang N, Duthilleul N, et al. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci Rep 2017;7:41802.

80. Shen L, Liu L, Ji HF. Alzheimer's disease histological and behavioral manifestations in transgenic mice correlate with specific gut microbiome state. J Alzheimers Dis 2017;56:385-390.

81. Bäuerl C, Collado MC, Diaz Cuevas A, Viña J, Pérez Martínez G. Shifts in gut microbiota composition in an APP/PSS1 transgenic mouse model of Alzheimer's disease during lifespan. Lett Appl Microbiol 2018;66:464-471.

82. Xin Y, Diling C, Jian Y, et al. Effects of oligosaccharides from *Morinda officinalis* on gut microbiota and metabolome of APP/PS1 transgenic mice. Front Neurol 2018;9:412.

83. Bonfili L, Cecarini V, Berardi S, et al. Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. Sci Rep 2017;7:2426.

84. Brandscheid C, Schuck F, Reinhardt S, et al. Altered gut microbiome composition and tryptic activity of the 5xFAD Alzheimer's mouse model. J Alzheimers Dis 2017;56:775-788.

85. Poole S, Singhrao SK, Chukkapalli S, et al. Active invasion of *Porphyromonas gingivalis* and infection-induced complement activation in ApoE-/- mice brains. J Alzheimers Dis 2015;43:67-80.

86. Kobayashi Y, Sugahara H, Shimada K, et al. Therapeutic potential of *Bifidobacterium* breve strain A1 for preventing cognitive impairment in Alzheimer's disease. Sci Rep 2017;7:13510.

87. Nimgampalle M, Kuna Y. Anti-Alzheimer properties of probiotic, *Lactobacillus plantarum* MTCC 1325 in Alzheimer's disease induced albino rats. J Clin Diagn Res 2017;11:KC01-KC05.

88. Athari Nik Azm S, Djazayeri A, Safa M, et al. *Lactobacillus* and *Bifido-bacterium* ameliorate memory and learning deficits and oxidative stress in Aβ (1-42) injected rats. Appl Physiol Nutr Metab 2018;43:718-726.

89. Wu SC, Cao ZS, Chang KM, Juang JL. Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in *Drosophila*. Nat Commun 2017;8:24

90. Walker JM, Harrison FE. Shared neuropathological characteristics of obesity, type 2 diabetes and Alzheimer's disease: impacts on cognitive decline. Nutrients 2015;7:7332-7357.

91. Sampson TR, Debelius JW, Thron T, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell 2016;167:1469-1480.e12.

92. Poole S, Singhrao SK, Kesavalu L, Curtis MA, Crean S. Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. J Alzheimers Dis 2013;36:665-677.

93. Hammond CJ, Hallock LR, Howanski RJ, Appelt DM, Little CS, Balin BJ. Immunohistological detection of *Chlamydia pneumoniae* in the Alzheimer's disease brain. BMC Neurosci 2010;11:121.

94. Miklossy J. Bacterial amyloid and DNA are important constituents of senile plaques: further evidence of the spirochetal and biofilm nature of senile plaques. J Alzheimers Dis 2016;53:1459-1473.

95. Vogt NM, Kerby RL, Dill-McFarland KA, et al. Gut microbiome alterations in Alzheimer's disease. Sci Rep 2017;7:13537.

96. Kountouras J, Tsolaki M, Gavalas E, et al. Relationship between *Helicobacter pylori* infection and Alzheimer disease. Neurology 2006;66:938-940.

97. Kountouras J, Boziki M, Gavalas E, et al. Increased cerebrospinal fluid *Helicobacter pylori* antibody in Alzheimer's disease. Int J Neurosci 2009;119:765-777.

98. Roubaud-Baudron C, Krolak-Salmon P, Quadrio I, Mégraud F, Salles N. Impact of chronic *Helicobacter pylori* infection on Alzheimer's disease: preliminary results. Neurobiol Aging 2012;33:1009.e11-e9

99. Kamer AR, Pirraglia E, Tsui W, et al. Periodontal disease associates with higher brain amyloid load in normal elderly. Neurobiol Aging 2015;36:627-633.

100. Sparks Stein P, Steffen MJ, Smith C, et al. Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease. Alzheimers Dement 2012;8:196-203.

101. Noble JM, Scarmeas N, Celenti RS, et al. Serum IgG antibody levels to periodontal microbiota are associated with incident Alzheimer disease. PLoS One 2014;9:e114959.

102. Akbari E, Asemi Z, Daneshvar Kakhaki R, et al. Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial. Front Aging Neurosci 2016;8:256. eCollection 2016.

103. Carter C. Alzheimer's disease: APP, gamma secretase, APOE, CLU, CR1, PICALM, ABCA7, BIN1, CD2AP, CD33, EPHA1, and MS4A2, and their relationships with herpes simplex, *C. Pneumoniae*, Other suspect pathogens, and the immune system. Int J Alzheimers Dis 2011;2011:501862.

104. Fani L, Wolters FJ, Ikram MK, et al. *Helicobacter pylori* and the risk of dementia: a population-based study. Alzheimers Dement 2018;14:1377-1382.

105. Tremlett H, Bauer KC, Appel-Cresswell S, Finlay BB, Waubant E. The gut microbiome in human neurological disease: a review. Ann Neurol 2017;81:369-382.

106. Lacy BE, Mearin F, Chang L, et al. Bowel disorders. Gastroenterology 2016;150:1393-1407.

107. Chen CH, Lin CL, Kao CH. Irritable bowel syndrome is associated with an increased risk of dementia: a nationwide population-based study. PLoS One 2016;11:e0144589.

108. Mancuso C, Santangelo R. Alzheimer's disease and gut microbiota modifications: the long way between preclinical studies and clinical evidence. Pharmacol Res 2018;129:329-336.

109. Plaza-Díaz J, Ruiz-Ojeda FJ, Vilchez-Padial LM, Gil A. Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. Nutrients 2017;9:E555.

110. Divyashri G, Krishna G, Muralidhara, Prapulla SG. Probiotic attributes, antioxidant, anti-inflammatory and neuromodulatory effects of *Enterococcus faecium* CFR 3003: *in vitro* and *in vivo* evidence. J Med Microbiol 2015;64:1527-1540.

111. Musa NH, Mani V, Lim SM, Vidyadaran S, Abdul Majeed AB, Ramasamy K. *Lactobacilli-fermented* cow's milk attenuated lipopolysaccharide-induced neuroinflammation and memory impairment *in vitro* and *in vivo*. J Dairy Res 2017;84:488-495.

112. Fasano A, Bove F, Gabrielli M, et al. The role of small intestinal bacterial overgrowth in Parkinson's disease. Mov Disord 2013;28:1241-1249.

113. Evrensel A, Ceylan ME. Fecal microbiota transplantation and its usage in neuropsychiatric disorders. Clin Psychopharmacol Neurosci 2016;14:231-237.

114. Perez-Pardo P, Kliest T, Dodiya HB, et al. The gut-brain axis in Parkinson's disease: possibilities for food-based therapies. Eur J Pharmacol 2017;817:86-95.

115. Szablewski L. Human gut microbiota in health and Alzheimer's disease. J Alzheimers Dis 2018;62:549-560.

116. Pistollato F, Iglesias RC, Ruiz R, et al. Nutritional patterns associated with the maintenance of neurocognitive functions and the risk of dementia and Alzheimer's disease: a focus on human studies. Pharmacol Res 2018;131:32-43.

117. Itzhaki RF, Lathe R, Balin BJ, et al. Microbes and Alzheimer's disease. J Alzheimers Dis 2016;51:979-984.

118. Pistollato F, Ohayon EL, Lam A, et al. Alzheimer disease research in the 21st century: past and current failures, new perspectives and funding priorities. Oncotarget 2016;7:38999-39016.