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Microbiota-gut-brain axis and toll-like receptors in Alzheimer's disease

Caixiu Lin [a,b], Shuai Zhao [a], Yueli Zhu [a], Ziqi Fan [a], Jing Wang [c], Baorong Zhang [a], Yanxing Chen [a,*]

[a] Department of Neurology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China
[b] Department of Neurology, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, China
[c] Department of Geriatric, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial disease which involves both the periphery and central nervous system (CNS). It has been recently recognized that gut microbiota interacts with the gut and brain (microbiota-gut-brain axis), contributing to the pathogenesis of neurodegenerative diseases, such as AD. Dysbiosis of gut microbiota can induce increased intestinal permeability and systemic inflammation, which may lead to the development of AD pathologies and cognitive impairment via the neural, immune, endocrine, and metabolic pathways. Toll-like receptors (TLRs) play an important role in the innate immune system via recognizing microbes-derived pathogens and initiating the inflammatory process. TLRs have also been found in the brain, especially in the microglia, and have been indicated in the development of AD. In this review, we summarized the relationship between microbiota-gut-brain axis and AD, as well as the complex role of TLRs in AD. Intervention of the gut microbiota or modulation of TLRs properly might emerge as promising preventive and therapeutic strategies for AD.

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Contents

1. Introduction

Modern medicine has achieved great victory in life span expanding. As the incidence of numerous geriatric diseases increases with age, the new challenges include alleviating symptoms, reducing the complications of diseases, and delaying the onset with the intervention of risk factors. Dementia is one of these diseases, which exerts heavy burden on both the family and society. The prevalence of dementia increases exponentially with age. The global prevalence of dementia is about 0.7–1.8% in population aged 60–64 years, while in people aged over 90 years the figure is between 28.7% and 63.9% [1]. Alzheimer's disease (AD) is the most common type of dementia accounting for 50–60% of all cases.

* Corresponding author at: Department of Neurology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, 88 Jiefang Rd, Hangzhou 310009, China.
E-mail address: chenyanxing@zju.edu.cn (Y. Chen).

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Extracellular senile plaques and intracellular neurofibrillary tangles are the main pathological hallmarks of the disease. The biomarker framework has been established for clinical diagnosis, including amyloid β-42 (Aβ42) level, total tau and phosphorylated tau (p-Tau) level in cerebrospinal fluid (CSF), positron emission tomography (PET) amyloid imaging, Fluorodeoxyglucose (FDG) uptake on PET and structural magnetic resonance imaging (MRI) [2,3].

Although great efforts have been made targeting the two pathological hallmarks, there still remains no disease modifying treatment for AD. To date, therapeutic strategies targeting against Aβ, including active vaccines, passive immunization, as well as β- and γ-secretases inhibitors, were fraught with failure and confusing results [4–10]. Clinical trials focused on tau protein immunotherapy are still pending [11]. Several hypotheses other than amyloid cascade hypothesis have been raised [12–16]. It is considered that the imbalance between Aβ production and clearance leads to Aβ accumulation and subsequently neuronal dysfunction. Accumulating studies have implicated that neuroinflammation might participate in the clearance of Aβ and even promote the pathological process of AD [17–19]. Epidemiological and observational studies indicate that non-steroidal anti-inflammatory drugs (NSAIDs) users had a lower risk of developing dementia [20–22]. Genome-wide association studies (GWAS) revealed that some genes involved in immune response were associated with AD risk [23]. Acute systemic inflammation events including various infections, surgical interventions, myocardial infarction and so on, could boost neuroinflammation and exaggerate cognitive decline [24]. In animal experiments, it has also been shown that systemic inflammation could accelerate AD-like pathological changes [25]. All these evidences indicate a close relationship between AD and inflammation. It is assumed that infection event could exaggerate neuroinflammation, promote Aβ production, and then resulting in exacerbation of cognitive impairment.

In recent years, a large number of studies revealed that dysbiosis or the localized intestinal infection may trigger systemic immune response, resulting in exacerbated inflammatory response in AD brain [26]. Dysbiosis refers to microbial imbalance on or inside the body. Intestinal microbiota can bidirectionally interplay with the central nervous system (CNS) through neural, immune, endocrine, and metabolic signals, which is regarded as the microbiota-gut-brain axis [27]. There were over 150,000 microbial genomes reconstructed from global, body-wide metagenomes [28]. The human gastrointestinal (GI) tract is the largest reservoir and harbors approximately 10^{14} microorganisms. Gut microbiota not only has metabolic and trophic functions, but also promotes host defense and immune homeostasis [29,30]. As numerous lymphoid tissues locate in the intestinal mucosa, GI tract incessantly monitors the pathogens and the dynamic microenvironment of the gut. The alteration of gut microbiota may lead to intestinal infection or inflammatory bowel disease, and prime the immune response [31,32].

Toll-like receptors (TLRs), the crucial sentinels, are the first line of defenders, participating in recognizing molecules broadly shared by pathogens and the activated immune system. TLRs are involved in commensal colonization, maintenance of the homeostasis, and integrity of the intestinal barrier [33]. Apart from an assortment of gut bacteria and their excreta, Aβ is also a ligand of TLRs, which, under certain conditions, can initiate the inflammatory process in the gut and the brain, leading to the development of neurodegenerative diseases, including AD. Here, we review the current knowledge concerning the relationship between TLRs and microbiota-gut-brain axis in AD, and discuss the potential mechanisms underlying the role of TLRs in AD (Fig. 1).

2. Toll-like receptors signaling

TLRs are a family of transmembrane pattern recognition receptors. TLRs initiate the downstream signaling transduction upon recognition of damage- and pathogen-associated molecular patterns (DAMPs and PAMPs). To date, 11 human and 13 mouse TLRs have been identified. The TLRs can roughly be classified into two groups referring to their space distribution. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are expressed on the plasma membrane, which recognize microbial products such as lipids, lipoproteins, and proteins, whereas TLR3, TLR7, TLR8, and TLR9 are localized in cytoplasmic compartments, which can be activated by nucleic acid species [34].

TLRs are composed of three major domains, a leucine-rich repeat (LRR) ligand-binding domain, a single membrane spanning helix, and a signaling Toll-interleukin-1 receptor (TIR) domain. Upon recognizing PAMPs or DAMPs, TLRs undergo conformational changes following dimerization to recruit the downstream signaling adaptors, which triggers the activation of specific transcription factors and the subsequent innate immune responses. A total of four adaptor proteins have been identified, including myeloid differentiation primary response protein 88 (MyD88), TIR domain–containing adaptor molecule (TIRAP, also known as MyD88-adaptor-like protein, MAL), TRIF-related adaptor molecule (TRAM, also known as TIR-domain-containing molecule 2, TICAM2), TIR domain–containing adaptor protein inducing interferon-β (TRIF, also known as TIR-domain-containing molecule 1, TICAM1) [35]. MyD88 is a universal adaptor protein for all TLR-mediated signaling pathways except for TLR3 [36]. MyD88-dependent pathway can induce the activation of nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), leading to the expression of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6. Both TLR3 and TLR4 are capable of recruiting TRIF, resulting in the production of type-I interferon (IFN). TRAM is specifically necessary for TRIF-dependent signaling pathway through TLR4, but not TLR3 [37]. Besides, TLR7, TLR8, and TLR9 can also induce the production of type-I IFN through the MyD88-dependent pathway [36].

The activation of TLRs can be regulated by sialic acid-binding immunoglobulin superfamily lectin receptors (Siglecs), which are known to inhibit the immune response. Extensive Siglec-TLR interactions negatively regulate the activation of TLRs [38]. Disruption of their interactions can result in the activation of TLRs and the immune responses [38]. Siglec-3 (CD33) has been shown to regulate the presentation of LPS to TLR4, leading to down-regulation of TLR4-mediated signaling [39]. Besides, triggering receptors expressed on myeloid cells-1 (TREM-1) also acts synergistically with receptors for PAMPs, including TLRs. TREM-1 amplifies the TLR-mediated immune response to microbial products, resulting in a dramatic upregulation of pro-inflammatory cytokines secretion [40].

3. TLRs in Alzheimer's disease

Broad expression of TLRs have been found in human CNS. TLR1-9 encoding mRNA were detected in primary cultures of microglia from postmortem human brain. Astrocytes and oligodendrocytes were also found to express TLR2 and TLR3, and to some extent, TLR1 and TLR4 [41]. Besides, certain TLRs were also found in neurons [42,43].

Microglia are the resident macrophages and primary immune cells in the CNS, responsible for the elimination of invading pathogens and injured neurons. As early as in the 1990s, microglia had been revealed can be activated by Aβ [44,45]. TLRs are also the endogenous binding sites for Aβ. It has been revealed that CD14,

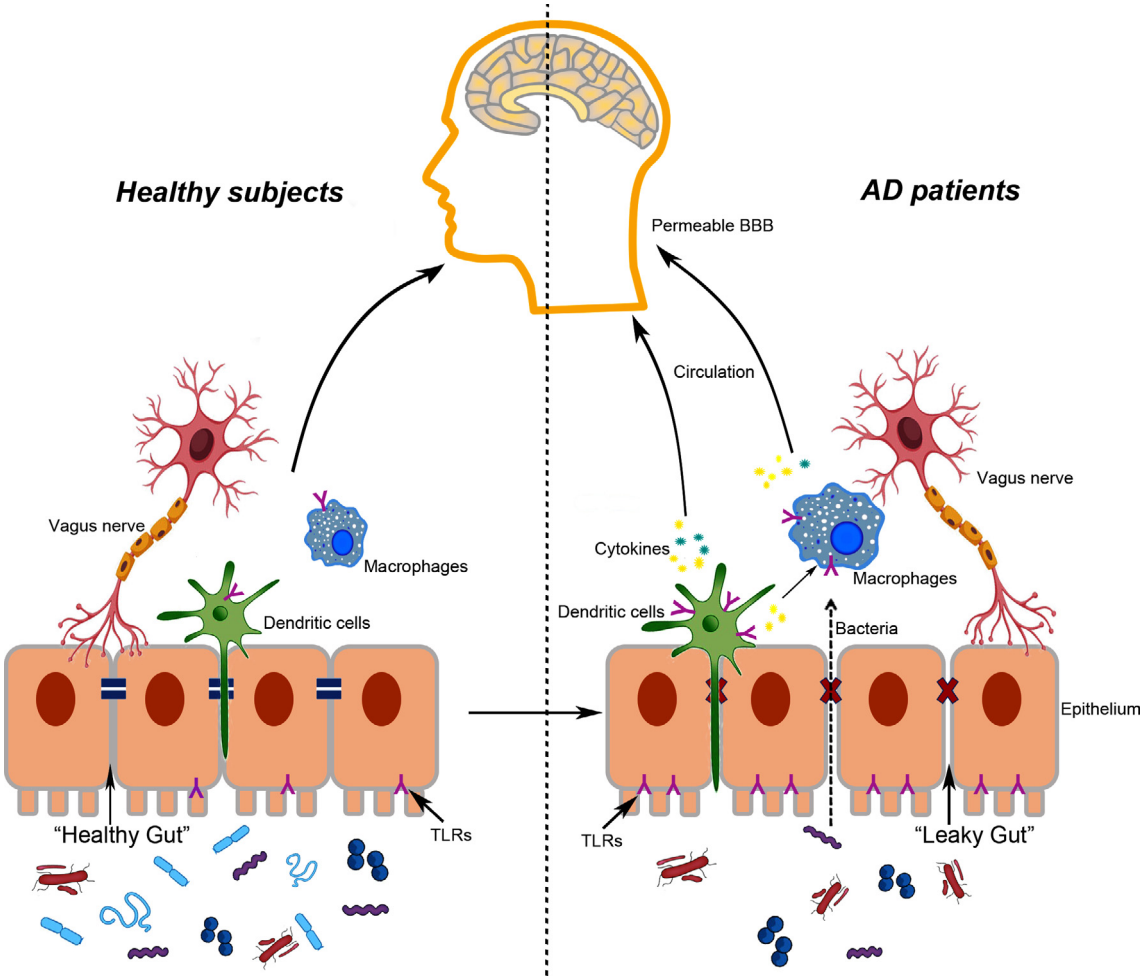

Fig. 1. Potential implications of TLRs and gut-brain-axis for AD. In healthy subjects, the gut epithelium is guaranteed by tight junctions between the cells. TLRs are expressed on macrophages, dendritic cells (DCs), and intestinal epithelial cells, serving as sentinels to monitor the pathogens in gut. Vagus nerve appears to modulate communication between the gut and the brain. The whole microenvironment maintains in homeostasis. During aging, the tight junction of intestinal and BBB become permeable. In AD patients, the diversity of gut microbiota decreased, while the population of pro-inflammation bacteria increased. Bacteria and their excretions could cross the leaky gut and then activate the TLRs in epithelium, IECs and macrophages, leading to production of pro-inflammation cytokines. These cytokines make their way through circulation or vague nerves to the brain, enlarge the neuroinflammatory responses, and promote neurodegeneration in CNS.

TLR2, and TLR4 are required for the activation of microglia by Aβ [46]. Overexpression of TLR2, TLR4, and CD14 (the lipopolysaccharide (LPS) receptor) have been found in the brains of both AD patients and AD mouse models [47,48]. AD mouse models, mostly generated by over-expression of mutated human PS1, APP and/or tau, are valuable tools to investigate the mechanisms of AD. These transgenic mouse models generally develop amyloid plaques and/or neurofibrillary tangles in the brain, resembling the hallmarks of AD. Triple-transgenic AD (3xTg-AD) mouse, APP/presenilin 1 (APP/PS1) mouse, and tau transgenetic mouse are the commonly used AD mouse models to investigate the role of TLRs in AD [49].

The role of TLR2 in AD is controversial. TLR2 can recognize Aβ42, triggering the release of pro-inflammatory cytokines, including TNF-α, interleukin-6 (IL-6), and interleukin 1-β (IL-1β), which are detrimental to the CNS, promoting the pathogenesis of AD [50,51]. Inhibiting TLR2 by anti-TLR2 antibody could attenuate Aβ-induced pro-inflammatory cytokines release and amyloid accumulation, leading to improved performance in spatial learning in AD mouse models [52,53]. Additionally, TLR2 deficiency enhanced phagocytosis and clearance of Aβ in cultured microglia [54]. All these evidences indicate that inhibition of TLR2, which participates in Aβ deposition and Aβ-induced neuroinflammation, might be beneficial for AD. However, Richard et al. reported that TLR2 knockout APP/PS1 mice had lower amyloid burden, but higher toxic Aβ1-42 species and more heavily cognitive damage [55]. This was supported by another study, which demonstrated a markedly increased uptake of Aβ42 by microglia via the activation of TLR2 [56]. These discrepancies might be due to different animal models used, as well as their representing disease stages. Thus, the role of TLR2 in AD still needs further investigation.

Similar to TLR2, TLR4 seems to play a dual role in the pathogenesis and progression of AD as well. On one side, microglial TLR4 mediates Aβ-induced neurotoxicity [48]. Cytokines, including IL-1β, IL-10, IL-17, and TNF-α, were upregulated in a TLR4-dependent way in AD mice [57]. On the other side, TLR4-mutant AD mice had less microglial activation, and as a result, more Aβ accumulation and severer cognitive deficits than TLR4 wild type AD mice, suggesting that activation of microglia via TLR4 signaling could enhance the clearance of Aβ and preserve cognitive function from Aβ-induced neurotoxicity [58]. Another study found that neuroinflammation could promote neuronal autophagy, and that chronic mild stimulation of TLR4 was associated with a reduction in cerebral p-Tau levels and improved cognitive function of tau-transgenic AD mice [59]. However, activation of microglia by LPS, a TLR4 ligand, was markedly blunted in 12-month-old APP/PS1 mice compared to their 2-month-old counterparts, indicating

TLR4 signaling dysfunction due to chronic exposure of microglia to Aβ deposits [60]. Collectively, these studies suggest that TLR4 signaling is essential for the clearance of Aβ by microglia. However, persistent chronic activation of microglia by Aβ exposure would dampen the TLR4 signaling, leading to further Aβ accumulation and neurodegeneration. Thus, modulating the activation of TLR4 towards facilitating Aβ clearance without activating neuroinflammation should be a promising treatment target for AD.

In addition to TLR2 and TLR4, TLR9 can also be detected in both microglia and neurons. TLR9 polymorphism has been reported to be associated with a decreased risk of AD [61]. Activation of TLR9 signaling could protect neurons from stress [62]. TLR9 knockout mice showed impaired synaptic function [63]. Targeting CpG motifs, which function as TLR9 agonists, can reduce both Aβ and tau pathologies in various AD transgenic mouse models, and rescue their cognitive deficits [64–66]. These studies provide valuable evidence in support of immunomodulation via TLR9 as a potential therapeutic approach for AD.

4. Gut microbiota and aging

Human aging is an intrinsic physiological process with a gradually function decline in the organs, including intestine, brain, and gut microbiota. The normal intestinal barrier is comprised of tight junctions between epithelial cells, mucus, bicarbonate, and antimicrobial peptides secreted from Paneth cells [67].

An integrated and healthy gut wall is essential to protect the host from the attack of pathogenic bacteria. Disorders of the GI tract are prevalent amongst the elderly population. For example, chronic constipation is common in the elderly and reaches an incidence rate of 30–40% among those over 65 years of age [68]. The underlying mechanism is poorly understood, but impaired intestine mobility, intrinsic aging of the cells in the gut, and some extrinsic factors like gut microbiota, may influence the physiological function of the GI tract [69]. Age related loss of enteric neurons by about 38% was found in old man [70]. Animal studies also demonstrated neuronal loss and degenerative changes with age in the enteric nervous system (ENS), which might be associated with a age-related phenotypic shift of macrophages and altered neural response to inflammatory signals, resulting in increased apoptosis and loss of enteric neurons and neural stem cells [71]. Besides, intestinal epithelial stem cells (IESC), which are responsible for the renewal of the intestinal epithelium, have also been shown to experience age-related dysfunction in mice, such as hyper-proliferation and expansion, and increased expression of genes associated with cellular stress, DNA damage and apoptosis [72].

Gut microbiota transmission occurs during the *peri*-partum period from mothers to their infants, which could be affected by several perturbations, including birth by Cesarean section and the use of antibiotics during pregnancy [73,74]. The composition and diversity of the infant microbiota is highly dynamic during the first year of life, and gets to resemble those of adult microbiota by around 3 years old [75]. Thereafter, the gut microbiota generally maintains stable. However, the increasing disappearances of microbiota and its diversity due to decreased vertical transmission from mother to child, decreased horizontal acquisition of commensal microbiota from other humans, and disrupted maintenance of key microbiota taxa in the early life by multiple insults like antibiotics exposure have exerted cumulative effects over generations, particularly the development of immunity in the gut [76–79]. Gut microbiota has been suggested to be associated with the development and organization of ENS, and the formation of gut immune system, although little is known about how the balance between immune response and host health is maintained [76,80]. In terms of aging, age-dependent, microbiome-modulated immunosenescence have been identified. The diversity and configuration of microbiota in the elderly can be affected by factors including residence location, diet, and health status, leading to the incidence of a wide variety of aging-related diseases [81,82].

5. The blood brain barrier during aging

The blood brain barrier (BBB) is a highly selective barrier that acts to separate the circulating blood from the brain in the CNS. It is composed of the continuous capillary endothelium connected by the tight junctions, astrocytic end feet, and basal membrane. The physical function of BBB is selectively impermeable to the microscopic objects (e.g., bacteria), large or hydrophilic elements diffusing into the CSF [83]. However, BBB dysfunction and leakage, associated with tight junction impairment and pericytes loss, are common during aging [84,85]. BBB needs much more mitochondrial volumes than tissues from non-BBB area to maintain its unique structure and the corresponding function [86]. However, this high mitochondrial content makes it vulnerable to accumulated oxidative stress and damage during aging, such as reactive oxygen species (ROS). The compromised BBB allows pathogens to get into the brain, leading to neuronal damages.

Cross-talks exist between the gut and brain, though the exact mechanisms have not been fully elucidated. The impact of gut microbiota on gut-brain axis is proposed to involve neural, immune, neuroendocrine, and metabolic systems [87]. The vagus nerve is the longest cranial nerve in the body and has afferent (sensory) and motor (efferent) nerves. Neurochemical and behavioral changes induced by bacteria exposure to the gut were not found in vagotomized mice, suggesting the vagus as a modulatory communication pathway between the gut and brain [88]. The spread of certain pathologies between the gut and brain have also been identified in animal studies via the vagus nerve [89]. Certain live bacteria may be beneficial to the establishment of BBB defense [90]. Germ-free mice showed significantly increased permeability of BBB, as well as lowered levels of endothelial tight junction proteins [91]. On the other hand, overresponse of the immune system due to gut dysbiosis can result in increased intestinal permeability, gut-vascular barrier (GVB) disruption, and systemic inflammation, which may further lead to the impairment of BBB integrity and neuroinflammation [92]. The existence of GVB was firstly identified by Spadoni et al, who demonstrated the disruption of GVB by pathogenic bacteria, leading to a systemic immune response [67]. Additionally, metabolic products produced by microbiota, such as short-chain fatty acids (SCFAs), can be sensed by vagus nerve. They can modulate the function of cholinergic neurons of the gut and neuronal activity in the brain after crossing the BBB, resulting in behavioral and cognitive changes.

Environmental and dietary influences, including chronic bacterial or viral infections can progressively alter BBB permeability and thereby facilitate cerebral colonization by opportunistic pathogens as we age. Given the existence of the gut wall, gut microbiota, immune system, and BBB dysfunction during aging, the microbiota-gut-brain axis may play an important role in age related neurodegenerative diseases such as AD.

6. The gut microbiota in Alzheimer's disease

Early in 1989, Aβ protein deposits were detected in the intestine [93]. Amyloid-β protein precursor (AβPP) from which Aβ is derived, and total tau, are also expressed in the enteric neurons, making it plausible that AD pathophysiology could involve the ENS [94,95]. However, this concept still needs further verification due to contro-

versial reports which showed similar amount of Aβ and tau pathologies between AD patients and elderly controls [96,97].

Animal studies have shown a direct effect of gut microbiota on AD pathologies. Intestinal inflammation induced by gut microbiota perturbation has been identified contributing to the pathogenesis and progression of AD. The local gut inflammation induced by infection significantly enhanced microglia activation and neuroinflammatory response in 3xTg-AD mice [26]. A lower level of pro-inflammatory cytokine IL-17 was found in gut-associated lymphoid tissue (GALT) cells of aged AD mice compared to their controls, suggesting that the surveillance to gut microbiota and immune barrier were impaired in AD [98]. Altered gut microbiota composition in the fecal samples from AD patients and AD mouse models have been reported [99–101]. The altered microbial composition could influence the levels of Aβ42, amyloid deposition, and pro-inflammatory cytokines in the brain [99]. A recent report revealed different genera abundance of fecal microbiota between AD patients and cognitively normal controls (increased in AD: *Dorea, Lactobacillus, Streptococcus, Bifidobacterium, Blautia*, and *Escherichia;* decreased in AD: *Alistipes, Bacteroides, Parabacteroides, Sutterella, and Paraprevotella*) [102]. A significantly negative relationship between amyloid burden and relative abundance of *Lactobacillus* in AD feces was observed [102]. In another study, cognitively impaired patients with brain amyloidosis showed lower abundance of the anti-inflammatory *E. rectale* and higher abundance of pro-inflammatory *Escherichia/Shigella* in their fecal samples compared to healthy controls or amyloid negative controls. Besides, amyloidosis-positive patients had increased serum levels of the pro-inflammatory cytokines, including IL-6, CXCL2, NLRP3 and IL-1β, and lower serum levels of anti-inflammatory cytokine IL-10 [103]. These findings support that there is an association between gut-microbiota-related inflammation and brain amyloidosis in AD.

Although great efforts have been made focusing on the role of gut-brain-axis in AD, the relationship between antibiotic treatment and the development of AD in humans has not been identified. Animal studies have demonstrated that antibiotic-induced perturbations in gut microbiota could influence neuroinflammation and amyloidosis in the brain. Antibiotic treatment over 6 months induced distinct alterations in microbial diversity in APP/PS1 mice, alongside alterations in peripheral inflammatory cytokines and chemokines, which coincided with attenuated Aβ plaque deposition and neuroinflammatory responses [104]. The same group also found that 1 week postnatal antibiotic treatment of APP/PS1 mice resulted in altered gut microbial diversity and reduced Aβ deposition at 6.5 months of age [105]. The underlying mechanism has not been elucidated. However, these findings indicate the close relationship between altered host innate immunity and amyloidosis in AD.

7. Microbiota-gut-brain axis and TLRs: potential implications for AD

It is well known that TLRs are expressed on numerous cell types in gut, including macrophages, dendritic cells (DCs), T lymphocytes, and intestinal epithelial cells (IECs). Intestinal epithelial cells are located on the front line of a microbial-rich environment, therefore, TLRs act as the essential mediators between microbiota and the host.

A broad spectrum of compounds are excreted by GI microbiota, including bacterial amyloids and LPS. The alterations of gut microbiota composition might induce perturbation of bacterial amyloids and LPS. Both of them can directly activate TLRs. Bacterial amyloids have been detected in both gram-negative and gram-positive bacteria, like *Proteobacteria, Bacteriodetes, Chloroflexi, Actinobacteria, and Firmicutes* [106,107]. There are a variety of bacterial amyloids which contribute to numerously different functions [108]. It has been known that bacterial amyloids are involved in biofilms formation and host defense [109]. However, bacterial amyloids also function as toxins, triggering apoptosis in some human cell lines [110]. The existence of vast quantities of amyloids imply that human physiology may be potentially exposed to a tremendous systemic amyloid burden. It is remarkable that amyloids produced by human microorganisms are biologically similar to CNS amyloids, such as CsgA, Aβ42 [111]. When bacteria invade the intestinal mucosa, following interaction with a receptor complex of TLR1/TLR2, bacterial amyloids can initiate a robust release of inflammation cytokines, including IL-17 and IL-22 [112].

Higher bacterial LPS level was found in AD brains than that of the controls [113]. The mean LPS levels varied from 2 to 26 folds increases in brain samples from AD over age-matched controls, depending on the brain area and the severity of the disease [114]. Infusion of bacterial LPS into the fourth ventricle of rat brains reproduced AD-like pathological alterations and cognitive impairment, which did not recover with time [115]. Administration of LPS peripherally led to prolonged elevation of Aβ and cognitive deficits [116]. Besides, AD mice exhibited enhanced expression of microglial LPS receptor, CD14, the blockade of which reduced excessive microglial activation and toxicity [117]. Additionally, an in vitro study demonstrated that LPS could potentiate Aβ fibrillogenesis [118]. These results suggest that bacterial infection events are potential catalyst to promote the progression of AD. On the other hand, Aβ has also been reported to be an innate immune protein, which protects the brain from invading pathogens by entrapping and neuralizing them within the β-amyloid [119]. Aβ has been shown to exert antimicrobial activity in vitro [119]. The antimicrobial activity was significantly higher in brain homogenates from AD than in samples from age matched controls, which can be ablated with the treatment of anti-Aβ antibodies [119]. However, chronic sustained activation of this protective antimicrobial pathway leads to excessive Aβ deposition and tangle formation, and subsequently neurodegeneration and dementia [120].

Under physiological conditions, despite constant exposure to microbial-derived TLR ligands, IEC is in a state of hyporesponsiveness with low expression of TLRs. If the intestine is infected by pathogenic bacteria or when inflammatory bowel disease occurs, TLRs are upregulated in an inflammation-dependent way in IECs and macrophages [121,122]. As a result, tremendous pro-inflammation cytokines and chemokines are released into the blood. Altered gut microbiota profile has been found associated with elevated levels of plasma LPS, inflammatory cytokines (IL-6, IL-8, IL-12, and TNF), and activated T-cells [123]. Gut infection could also enhance systemic pro-inflammatory response, characterized by the production of pro-inflammatory cytokines and chemokines such as TNF-α, IL-6, CCL5, and CXCL-1, which was associated with increased activation of microglia in 3xTg-AD mouse brain [124]. Once the pro-inflammatory cytokines are released by TLRs, they make their way to the brain by crossing the BBB via both diffusion and cytokine transporters, especially during aging when the GI epithelial barrier and BBB become significantly more restructured and permeable. In the brain, these cytokines act on receptors expressed by neurons and glial cells, particularly microglia, altering their activation status and physiology [125]. A recently published work by Wang et al. observed accumulation of Aβ in the brains of 5xFAD mice, which is accompanied by shifts in gut microbial population [126]. Besides, as activated M1 microglia increased in the brain, so did the number and pattern of peripheral pro-inflammatory T helper 1 (Th1) cells, indicating that gut dysbiosis alters peripheral inflammation, promoting the activation of microglia and amyloidosis, and eventually cognitive

impairment. A drug named GV-971, remodeled the gut microbiota, could reduce Th1 cell proliferation in the blood and harness neuroinflammation and cognitive impairment, which has also been demonstrated effective in a phase 3 clinical trial [126].

8. The potential therapeutic targets

To date, there is no disease modifying therapy available for AD. Therefore, novel insights into AD pathologies are imperative to discover new therapeutic strategies. The existing interactions between TLRs and the gut-microbiota-brain axis in AD might provide opportunity for intervention. Emerging studies are focusing on regulating inflammatory response in the gut and brain to delay the progression of AD.

Administration of probiotics appears to be a novel and safe method to preserve a healthy intestinal microbiota and intestinal barrier, reducing the initiation of pro-inflammatory responses and propagation of neuroinflammation in neurodegeneration diseases [125]. Cell surface macromolecules in probiotics, such as peptidoglycan, cell wall teichoic, and lipoteichoic acid (LTA), exopolysaccharides, surface layer associated proteins (SLAPS), and fibronectin binding proteins are able to interact directly with the intestinal epithelium, mucus, and TLRs of the GI mucosa [127–132]. It has been found that LPS-induced neuroinflammation and memory impairment could be attenuated by consumption of probiotics [133]. In animal experiments, treatment of probiotics, including *Bifidobacterium* and *Lactobacillus,* could ameliorate cognitive impairment, decrease the size and number of amyloid plaques, and reduce the immune response and neuroinflammation [134,135]. Clinical trials also demonstrated that probiotics administration could significantly increase the mini-mental state examination score of the AD patients [136,137]. Antibiotic treatment and fecal microbiota transplantation are potential options, but still need further investigation. As mentioned above, TLRs might be the possible therapeutic targets for AD. Although the role of TLR2 in AD brain is still controversial, studies have demonstrated the association of TLR2 signaling with the activation of microglia and the clearance of Aβ. Further investigations are needed to better characterize the TLR2 signaling, which would shed light on how to target TLR2 as a therapy for AD. Activation of TLR4 signaling have been found to promote microglia-mediated Aβ clearance. Besides, TLR4 activation could also be probably beneficial due to its autophagy effect. However, LPS-induced TLR4 signaling activation was dampened in AD mice during aging, suggesting TLR4 signaling might become tolerant to persist Aβ exposure in the brain [60]. Chronic and systemic administration of Monophosphoryl lipid A (MPL, a non-pyrogenic TLR4 agonist), through enhancing phagocytic capacity without inducing immune tolerance of innate immune cells, can attenuate the cerebral Aβ load [138]. What's more, TLR9 could be another possible therapeutic target. Intraperitoneal injection of TLR9 agonist significantly reduced Aβ and tau pathologies, as well as levels of toxic oligomers in AD mouse models [64,66,139]. TLR9 stimulation also effectively ameliorated the cognitive deficits of these mice. These beneficial effects might result from increased phagocytic activity and upregulation of anti-inflammatory cytokines [66]. Given the complexity of the roles of TLRs in AD, a more profound understanding of the TLR signaling pathway and their association with AD pathologies are essential for the development of effective treatments.

9. Summary and outlook

In conclusion, we summarized the role of microbiota-gut-brain axis and TLRs in the pathogenesis of AD. It can be assumed that when gut dysbiosis occurs, microbial amyloids, LPS and other small compounds segregated can disrupt the gut wall and increase its permeability, which further undermine the BBB via blood or ENS pathway. TLRs can be activated by microbial amyloids or LPS in the gut, leading to the release of pro-inflammatory and/or anti-inflammatory cytokines, which results in the imbalance of the immune system, contributing to the progression of AD pathologies and cognitive decline. Attempts to restore the gut microbiota to a composition that found in healthy adults may slow down the progression of AD. However, interventions directly targeting TLRs still have a long way to go before more extensive studies carried out to elucidate the TLR signaling pathway and its impact on the immune system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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