Toll-Like Receptor Signaling in Alzheimer’s Disease Progression

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Abstract
Evidence continues to underpin the role of the innate immune system in pathologies associated with neuroinflammation. Neuroinflammation is the complex innate immune response of neural tissue to control infection, and Toll-like receptors (TLRs), a major family of pattern recognition receptors (PRRs) that mediate innate immunity, have emerged as players in Alzheimer’s disease (AD). Upon ligation with their ligand, TLRs induce signaling involving recruitment of various adaptors and signaling molecules that culminate in the activation of genes including interferons and cytokines. TLR expression has been identified on resident central nervous system cells including microglia and neurons, with an altered expression profile for this receptor determined in microglia from AD patients. Furthermore, TLR activation on microglia is associated with amyloid-β (Aβ) clearance from the brain, suggesting that modulation of TLR signaling may be a therapeutic strategy for plaque removal. This review will highlight evidence linking the TLR system with the progression of AD, assessing TLR involvement in events associated with AD in cellular systems, transgenic murine models of AD and in humans.

Keywords: Alzheimer’s disease; Innate immunity; Neuroinflammation; Toll-like receptors; Microglia

AD Neuropathology
Alzheimer’s Disease (AD) is a chronic neurodegenerative condition associated with progressive cognitive decline and estimated to afflict 10% of people over 65 years of age and 25% of people over 80 years of age. Aging is the single biggest risk factor for developing AD, with a direct correlation between age and AD incidence [1]. The disease can be divided into two categories, early-onset AD (accounting for ~2% of all cases and developing between the ages of 30-60 years) and late-onset AD (the common form of the disease) [2]. Memory loss is the most frequent symptom with a definitive diagnosis of AD requiring not only the presence of severe dementia but post-mortem confirmation of histopathological hallmarks [3]. A small proportion of AD cases have a genetic basis (~10%), due to an inherited autosomal dominant gene mutation (familial AD), the majority of which are genes encoding amyloid precursor protein (APP) or presenilin (PS) components of γ-secretase. However, the majority of cases are sporadic, with unknown aetiology. Regional neuronal degeneration, synaptic loss, presence of neurofibrillary tangles and senile plaques are hallmarks of the disease [4]. Senile plaques are extracellular lesions composed of a core of β-amyloid (Aβ) aggregates, surrounded by dilated neurites, activated microglia and reactive astrocytes [5]. Aβ is a ~4-kDa peptide derived from the processing of the transmembrane APP by either the α- or β-secretase (BACE), and subsequently the γ-secretase. The excessive accumulation of this peptide in hippocampal, cortical and amygdalar regions of the brain has been proposed as a pivotal event in aberrant neuronal cell death associated with the disease [2,4,6]. Hyperphosphorylation of the microtubule-associated protein, tau, resulting in the formation of neurofibrillary tangles is the second pathological hallmark of the disease, although not specific to AD [7]. Tangles impair interneuronal communication and tau has been implicated as a mediator of Aβ-induced neurotoxicity.

AD: Therapeutic Avenues
Effective treatments for this disease are lacking as only palliative approaches are currently available. There are four major categories of drugs used in AD treatment: cholinergic treatment, anti-glutamatergic treatment, vitamins and anti-oxidants, and nonsteroidal anti-inflammatory drugs (NSAIDs) [8]. Acetylcholinesterase inhibitors (AChEIs; donepezil, rivastigmine) are the mainstays of AD treatment, increasing the availability of acetylcholine and thereby facilitating cholinergic neurotransmission, with significant benefits on cognition, neuropsychiatric measures and global measures related to dementia reported. AChEIs are considered safe compounds, with side effects limited to gastrointestinal symptoms; however agreement on the duration of treatment is lacking [9]. A second approach to AD treatment involves blockade of glutamatergic neurotransmission by use of the uncompetitive N-methyl-D-aspartate (NMDA) antagonist memantine, which subsequently blunts excitotoxicity due to intracellular calcium abundance. Symptomatic, rather than disease-modifying, benefits are reported in AD with memantine use, and like AChEIs, minimal adverse effects have been reported [9]. Clinical evidence has demonstrated that an anti-oxidant strategy with vitamin E may be an effective therapy for AD, limiting oxidative stress and free radical accumulation in the AD brain [10]. Indeed, a double-blind randomized multicentre trial reported no improvement in cognition, with a higher incidence of falls and syncope reported. Although not recommended as AD treatments, retrospective studies have demonstrated that NSAIDs may be protective against AD development. However, NSAIDs trials in patients with established AD have reported limited benefits [10]. Current AD therapies are based on neurotransmitter replacement or modulation, which although providing symptomatic benefit, limits disease progression minimally, highlighting the need for neuroprotective, anti-inflammatory and anti-amyloid therapies.

AD: Neuroinflammatory Profile
The role of neuroinflammation and microgliosis in AD is attracting considerable attention as recent evidence suggests that both factors...
are early events in the pathogenesis of the disease [11]. Indeed, much evidence has indicated the presence of pro-inflammatory mediators (complement, proteases, cytokines) in the brain and cerebrospinal fluid of AD patients [12]. Although the exact role of microglia in AD is unclear, microglia are reactive in the brain of AD patients and APP mice, secreting both neurotrophic and neurotoxic agents [13]. Indeed, evidence suggests that microglia, or blood-derived monocytes, may be involved in Aβ clearance in a transgenic mouse model of AD (discussed below), suggesting that immune cells shield mice from AD [14]. Furthermore, although there is still little evidence supporting blood-brain barrier (BBB) disruption in AD patients, morphological evidence suggests abnormalities in brain microvasculature in AD [15], which may have important implications both in terms of central nervous system (CNS) infiltration of leukocytes and plasma-derived Aβ in the disease. The interaction of fibrillar forms of Aβ with microglia triggers tyrosine kinase-based cascades involving the Src family kinases and Syk kinase that culminate in the generation of reactive oxygen species (ROS), cytokines, prostaglandins and phagocytosis [16]. Microglia assemble a cell surface complex consisting of the B class scavenger receptor CD36, the α5β1-integrin, the integrin-associated protein/CD47 and the class A scavenger receptor (SRA), that bind Aβ and transduce intracellular signaling in the glial cell [17].

**Innate Immune System**

The immune system is divided into two branches, the innate and adaptive immune systems, with innate immunity acting as the first line of defense against pathogens. The cells of the innate immune system recognize highly conserved structures referred to as pathogen-associated molecular patterns (PAMPs), expressed by large groups of microorganisms [18]. This system is orchestrated by a number of cells including mast cells, dendritic cells (DCs), neutrophils, natural killer (NK) cells, γδ T cells, glial cells and macrophages, and these cells act as crucial initiators and effectors of innate immune responses [19]. Innate immunity is tightly regulated by a complex mechanism involving pattern recognition receptors (PRRs) that recognize PAMPs, orchestrating transcriptional expression of inflammatory mediators that coordinate the elimination of pathogens and infected cells. Signaling PRRs include the family of Toll-like receptors (TLRs), along with retinoic acid-inducible gene I (RIG-I)–like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs) [14]. If left unchecked, or not tightly regulated, dysregulation of this system can lead to conditions such as sepsis, asthma, and autoimmunity [20].

TLRs mediate innate immune immunity by recognising PAMPs and endogenous damage-associated molecular patterns (DAMPs) that are released by injured tissue [21]. To date, 12 functional TLRs have been discovered in mice and 10 in humans, with TLR1–TLR9 conserved in both species. TLRs are type-I transmembrane glycoproteins composed of an extracellular leucine-rich repeat (LRR) motif required for PAMP recognition, a transmembrane domain, and a cytoplasmic Toll/interleukin IL-1 receptor (TIR) domain [22,23]. The TIR domain has similarity to the type-I IL-1 receptor (IL-1R) [24], consisting of a conserved region of approximately 200 amino acids [25]. Despite complex effector mechanisms involved in the immune system, TLRs are capable of distinguishing between pathogens to produce specific signaling cascades, thus inducing gene transcription and controlling immune processes. This specificity is dependent on the TLR adaptor proteins that are used [26]. To date, five adaptor proteins have been identified: myeloid differentiation factor 88 (MyD88), MyD88-adaptor like (Mal), TIR-domain-containing adaptor protein inducing IFN-β (TRIF), TRIF-related adaptor molecule (TRAM), and sterile a and armadillo-motif containing protein (SARM). Once TLRs are ligated to their associated ligand, they form homodimers, heterodimers or oligomers, which is crucial for intracellular signaling to occur. This is believed to cause a conformational change in the TIR domains, thus allowing adaptor recruitment. With the exception of TLR3, all TLRs recruit the adaptor MyD88. TLR3 induces MyD88-independent signaling to regulate NF-κB via the TRIF adaptor protein. The adaptor proteins (with the exception of SARM), actively engage downstream signaling involving transcription factors such as nuclear factor (NF)-κB, and induction of genes encoding interferons (IFNs), cytokines and chemokines [23].

**TLRs and Neuroinflammation**

Peripheral systemic infections are associated with cognitive decline in many neurological conditions, including patients with AD, and such decline can outlast the infection [27]. While global TLR expression pattern studies have characterized TLRs on an array of immune cells (DCs, T cells, macrophages) and their role in orchestrating a comprehensive programme to remove invading pathogens [28], it has become increasingly clear that TLRs have a distinct role in immune surveillance and inflammatory responses in the CNS. Indeed, TLR expression profiles on resident CNS cells continue to be elucidated, with complements of receptors expressed on microglia (TLRs 1-9) [29,30], astrocytes (TLRs 1-5, 9) [23,30,31], and neurons (TLRs 2-5, 8, 9, 11-12) [31,32]. TLR expression has also been determined on glial cells in human post-mortem CNS tissue derived from patients suffering from AD [33], and knockout studies targeting TLRs and associated adaptors (discussed below) continue to elucidate the complex role of TLRs, and TLR signaling proteins, in neuroinflammatory conditions such as AD.

**TLRs and AD**

Overall expression

Interestingly innate immune receptor expression increases both in normal aging and in AD brain. Using real-time PCR, Li et al. [33] demonstrated that the expression of TLR1, TLR2, TLR4, TLR5, TLR7 and CD14 (a co-receptor for TLR2 and TLR4) was upregulated in the whole hemisphere in 17-month-old, compared to 3-month-old, mice. This group published a follow-up study conducting a systematic assessment of innate immune receptor expression in three neurodegenerative models, including a transgenic mouse AD model (APP TgCRND8) [34]. Transcription levels of TLR2, TLR7 and CD14 were higher in the cortex of APP TgCRND8 transgenic mice compared to the levels seen in aged-matched control mice, indicating that a marked elevation in innate immune receptors occurs in this AD model [34]. These findings are supported elsewhere indicating a strong upregulation of TLR2, TLR4, TLR5, TLR7 and TLR9 mRNAs in plaque material, compared to plaque-free tissue, in an APP23 transgenic mouse model [35], while an age-related induction of TLR2, TLR4, TLR7 an TLR9 transcripts was identified in the hemispheres of APP/PS1K1 transgenic mice [36]. In AD human brain sections, TLR2 and CD14 have been co-localized with microglial cells in post-mortem studies [34], while AD patients show enhanced CD14 expression in parenchymal microglia of the frontal cortex and hippocampus [37] and an association of TLR4 with Aβ plaque deposition in the entorhinal cortex [38]. Recent evidence also indicates that monocytes derived from elderly individuals display defective TLR signaling, particularly TLR1 and TLR2, with further decreases in relative TLR1 and TLR4 expression determined on peripheral blood monocytes from older subjects [39,40], while increased expression of TLR2 and TLR4 has
been reported in peripheral blood mononuclear cells (PBMCs) from patients with late-onset AD [41].

**CD14**

CD14, a glycosylphosphatidylinositol (GPI)-anchored myeloid glycoprotein, acts as a co-receptor for TLR2 and TLR4 and is responsible for the uptake of Gram-negative bacterial lipopolysaccharide (LPS) via macrophagocytosis [42]. Although polymorphism in CD14 has not been demonstrated as a risk factor in AD in some groups [43], others have demonstrated that subjects having a specific combination of polymorphisms in CD14 and Liver X receptor (LXR) β have a reduction in the risk of developing AD [44]. In addition, much evidence from animal studies associates CD14 with AD progression. Indeed, in vitro studies in microglia from CD14 knockout mice indicates that fibrillar Aβ requires this co-receptor for downstream inflammatory signaling, indicating that CD14 may act as part of the Aβ receptor complex [45]. Since CD14 is required for uptake of a variety of pathogens, Liu et al. [46] conducted in vitro experiments investigating the involvement of CD14 in Aβ internalization and demonstrated that CD14 has a direct effect on Aβ phagocytosis in primary murine microglia. Furthermore, transgenic mice (APPswe/PSEN1dE9) lacking CD14 exhibit reduced cortical plaque burden and microgliosis, with enhanced tumour necrosis factor (TNF)-α and interferon-γ (IFN-γ) expression at 7 months of age [46], indicating that this receptor has a profound impact on neuroinflammation in the model.

**MyD88**

All TLRs, with the exception of TLR3, recruit the MyD88 adaptor. In vitro evidence indicates that antisense knockdown of MyD88 inhibits Aβ-induced expression of inducible nitric oxide synthase (iNOS) and TNF in primary mouse microglial cells and BV-2 cells [47], and this is supported by unpublished evidence in macrophages from my laboratory. In mouse AD models, partial deletion of MyD88 exaggerates cognitive deficits in APPswe/PSE1dE9 mice, increasing brain soluble oligomeric Aβ while reducing brain interleukin-1 beta (IL-1β) levels in the forebrain, indicating the profound effects of this adaptor in the mouse model [48]. In support of this, APPswe/PSE1dE9 mice lacking MyD88 display improved spatial memory in the water maze irrespective of the transgene [49]. In addition, decreased fibrillar Aβ deposits in cerebrum have been determined in this mouse AD model deficient for MyD88 [49]. Recent intricate experiments by Michaud et al. [50] have further clarified the role of this adaptor in AD, indicating that MyD88-deficient microglia and monocytes have a reduced capacity to clear Aβ oligomers which contributes to the progression of the behavioural deficits in the mouse APPswe/PSE1 transgenic model.

**TLR2**

A large body of evidence associates TLR2 with AD neuropathology. Importantly, polymorphisms in the TLR2 gene have linked TLR2 with AD incidence. Indeed the risk of developing late-onset AD has been linked with -196 to -174 del polymorphism [51] and microsatellite polymorphisms in intron II [52] in the TLR2 gene in a Han Chinese population. TLR2 co-localizes with microglial cells in the AD cortex [34] and clear evidence demonstrates that Aβ binds microglial TLR2 to induce cellular activation in primary mouse cultures and BV-2 microglial cells [47]. Furthermore, the Aβ peptide receptor, formyl peptide receptor-like 2 (FPR2), is upregulated by cooperation between TLR2 and the intracellular NOD2 receptor in the murine microglial cell line N2 [53]. Studies in microglia from TLR2 knockout mice indicates that fibrillar Aβ requires this receptor for inflammatory signaling, suggesting that TLR2, like CD14, is an integral component of the Aβ receptor complex [45]. In addition TLR2/-/- mice are protected from cognitive impairment following Aβ immunization (subcutaneous administration of 100 μg/animal human Aβ peptide) [54], while lentivirus/TLR2 treatment improved spatial and contextual memory in APP/TLR2 knockout mice [55]. Evidence also indicates that upregulating this receptor may be a therapeutic option in AD by regulating the phagocytic activity of microglia. Indeed, Chen et al. demonstrated that TLR2 activation is associated with Aβ uptake via FPR2 in N2 microglial cells [53].

**TLR4**

Like TLR2, polymorphisms in the TLR4 gene are associated with the incidence of AD. Susceptibility to late-onset AD has been associated with TLR4 Asp299Gly polymorphism [56] while TLR4/11367 population [52]. A recent investigation has also concluded that strong association exists between TLR4 polymorphisms (rs1927907 and HAPI GACGG) and late-onset AD risk, especially among ApoE e4 carriers [57]. TLR4 mediates binding of fibrillar Aβ to microglial cells, regulating phagocytosis (in BV-2 microglial cells) [58] and ROS production (in primary murine microglia) [45]. In primary murine cortical neurons, TLR4 has been demonstrated to mediate Aβ-induced apoptosis via jun N-terminal kinase (JNK)- and caspase-3-dependent mechanisms [59]. Song et al. [60] demonstrated that TLR4 mutant TgAPPswe/PS1dE9 transgenic mice have the proclivity to maintain cognitive function in the water maze via TLR4-mediated Aβ clearance by microglia, while data from the same group indicates that TLR4 directly upregulates cortical pro-inflammatory cytokines in the same model [61]. Recent intricate data from the Rivest group indicate that systemic administration of a TLR4 agonist at nonpyrogenic concentrations reduces cortical Aβ load and improves spatial memory in the T water maze in APPswe/PS1 mice [62]. Interestingly, repeated intraperitoneal administration of the TLR4 agonist LPS to mice has been suggested as an AD model. Indeed, this treatment regimen has been demonstrated to enhance Aβ concentration in the hippocampus and cortex of mice, promoting glial activation and inducing cognitive impairment [63]. In support of this, central administration of LPS to the frontal cortex and hippocampus of transgenic mice carrying parental tau mutations (rTg4510) enhanced tau phosphorylation and microglial activation in this model [64]. In contrast, intracranial administration of LPS promotes microglial activation and reduces hippocampal Aβ pathology in Tg2576 APP transgenic mice [65], indicating that CNS inflammation has diverse effects on tau and amyloid pathology.

**TLR9**

TLR9 functions to specifically bind DNA that contain unmethylated cytosine-guanosine (CpG) sequences, which are commonly found in the genomes of prokaryotes (bacteria) and viruses and under-represented in those of eukaryotes. In relation to AD, TLR9 signaling has emerged as a probable mechanism of reducing plaque burden. Indeed, TLR9 stimulation in the mouse Tg2576 AD model reduces hippocampal and cortical plaque burden and is associated with a reduction in cognitive deficits associated with working memory [66]. This is in line with evidence that TLR9 stimulation prevents Aβ-induced cell death in primary mouse cortical neurons in vitro, and reduces Aβ-induced impairment of recognition memory in wild type mice in vivo [67]. Furthermore, BV-2 microglia treated with the TLR9 ligand CpG oligodeoxynucleotide enhances uptake of Aβ in vitro [58]. Interestingly, TLR9-induced NO and TNF production in primary murine microglia
is antagonized by Aβ treatment indicating that signaling cross-talk occurs between both the TLR9 and Aβ receptor pathways [68].

Conclusion

Evidence discussed herein highlights the complex role of the TLR system in AD progression. This system plays a multi-functional role in the disease, and clearly further research is needed to decipher distinct approaches with real therapeutic value. Indeed effective treatments for AD are lacking as only palliative approaches are currently available. TLR-targeted antibodies, small molecules and nucleic acid-based therapies are at various stages of clinical development for a wide range of conditions in the fields of oncology, infectious and immune disease (reviewed in [69]). To date, few TLR-based therapies have passed all clinical stages, with the TLR7 agonist Imiquimod approved to treat skin disorders such as basal cell carcinoma and warts. In relation to inflammatory disorders, humanized anti-TLR2 monoclonal antibody therapy (OPN305) is in clinical trials for myocardial ischemia/ reperfusion injury, while a TLR4 targeted antibody (NI0101) is in preclinical development for several indications including rheumatoid arthritis. Hence, a large body of research continues to demonstrate the involvement of TLRs, their signaling adaptors and intermediates in AD pathogenesis, which adds weight to the on-going clinical trials assessing the role of TLR agonists and antagonists in an array of neuroinflammatory conditions.

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References


