Review Article

The Innate Immune System in Alzheimer's Disease

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Alzheimer's disease (AD) is the leading cause for dementia in the world. It is characterized by two biochemically distinct types of protein aggregates: amyloid β (Aβ) peptide in the forms of parenchymal amyloid plaques and congophilic amyloid angiopathy (CAA) and aggregated tau protein in the form of intraneuronal neurofibrillary tangles (NFT). Several risk factors have been discovered that are associated with AD. The most well-known genetic risk factor for late-onset AD is apolipoprotein E4 (ApoE4) (Potter and Wisniewski (2012), and Verghese et al. (2011)). Recently, it has been reported by two groups independently that a rare functional variant (R47H) of TREM2 is associated with the late-onset risk of AD. TREM2 is expressed on myeloid cells including microglia, macrophages, and dendritic cells, as well as osteoclasts. Microglia are a major part of the innate immune system in the CNS and are also involved in stimulating adaptive immunity. Microglia express several Toll-like receptors (TLRs) and are the resident macrophages of the central nervous system (CNS). In this review, we will focus on the recent advances regarding the role of TREM2, as well as the effects of TLRs 4 and 9 on AD.

1. Introduction

Alzheimer's disease is the most common cause of dementia globally [1]. AD is characterized by the presence of amyloid β (Aβ) deposits in the forms of parenchymal amyloid plaques and congophilic amyloid angiopathy (CAA) as well as aggregated tau protein in the form of neurofibrillary tangles (NFT). These lesions are associated with neuronal loss and synaptic damage, which produce the cognitive dysfunction which characterizes AD. Mutations in three genes have been shown to cause early-onset AD (EOAD): the amyloid precursor protein (APP), Presenilin 1 (PS1), and Presenilin 2 (PS2) [2, 3]. Mutations associated with these genes affect <1% of all AD patients and are not informative regarding the causes of the much more common late-onset AD (LOAD) [2, 3]. Inheritance of the apolipoprotein E (ApoE4) allele is the major

genetic risk factor that is associated with late onset AD [4, 5]. Several environmental factors are known as risk factors for LOAD including, aging, head trauma, type 2 diabetes, hypertension, hypercholesterolemia, and vascular pathology [6].

Recently, two independent groups of investigators have identified a rare variant in the gene encoding the triggering receptor expressed on myeloid cells 2 protein (*TREM2*), which is predicted to result in a R47H substitution that causes an ~3-fold increase in the susceptibility to LOAD. Although the odds ratio of Trem2 R47H is comparable to the presence of a single copy of ApoE4, this variant has a population frequency of only ~0.3% [7–9]. TREM2 is located on chromosome 6p21.1 and encodes five exons. It is a transmembrane glycoprotein, made up of an extracellular immunoglobulin-like domain, a transmembrane domain, and a cytoplasmic tail, that couples with DAP12 for its signaling function [10].

TREM2 is expressed in microglia, macrophages, osteoclasts, and dendritic cells. TREM2 was initially identified as a phagocytic receptor of bacteria [11]. It recognizes anionic lipopolysaccharides (LPS) on the surface of bacteria, via signaling through DAP12, triggering their phagocytosis. Other pattern recognition receptors which have been shown to play a critical part in macrophage/microglial function and have a role in AD-related pathology are the Toll-like receptors (TLRs) [12–14]. In this review, we will focus on the potential roles of TLRs and TREM2 in AD.

2. TREM2 in AD

The *TREM2* gene encodes 5 exons that code for a 693 pb DNA which translated into 230 amino-acids called TREM2 [15–17]. In the normal brain, TREM2 is highly expressed in white matter, hippocampus, and neocortex, while low levels are found in the cerebellum of the human brain [18]. This distribution of TREM2 expression is consistent with the distribution of AD-related pathology. In AD animal models, it has been reported that TREM2 is upregulated in amyloid plaque-associated microglia, such as in aged APP23 mice [19], a result which was confirmed by another group [20]. In AD model TgCRND8 mice TREM2 mRNA was increased, correlating with the rise in $A\beta$ levels [7].

TREM2 is a phagocytic receptor of bacteria [11] and forms a receptor signaling complex with the TYRO protein tyrosine kinase binding protein (TYROBP, also called DAP12), that triggers phagocytosis and the release of reactive oxygen species [21]. TREM-2 is defined as an innate immune receptor expressed on the cell surface of microglia, macrophages, osteoclasts, and immature dendritic cells [22]. Microglia play a key role in the immune response in the central nervous system (CNS) and are the resident innate immune cells responsible for the early control of infections. TREM2 is known to have anti-inflammatory properties; it suppresses inflammatory responses by repression of cytokine production and secretion [23]. TREM2 reduces macrophage activation and inhibits cytokine production in response to both TLR2 and TLR4 ligands zymosan and LPS [24, 25]. Conversely reduction of *TREM2* expression by either RNA interference or by targeted gene deletion amplified inflammatory cytokine responses by macrophages following stimulation of multiple different TLRs including TLR2, 4, and 9 [26]. Hence, it has been speculated that TREM2 has a protective role in AD pathogenesis; its anti-inflammatory properties could reduce inflammation-induced innocent bystander neuronal damage [8, 16, 17]. In addition to the anti-inflammatory roles of TREM2, it is also known to effect phagocytosis of damaged/apoptotic cells. TREM2 interacts with endogenous ligands on neurons, leading to the direct removal of damaged cells [27]. In various models of multiple sclerosis increased microglial expression of TREM2 is associated with increased phagocytosis and a promotion of a M2-like activation state of microglia, which is thought to have protective effects [28, 29]. The removal of damaged or apoptotic neurons mediated via TREM2 could promote tissue repair in response to AD-related pathology. This TREM2 mediated phagocytic activity also has been linked to an enhanced ability of microglia to

clear $A\beta$ and amyloid plaques in vitro and in AD model APP23 Tg mice [20]. Microglia are well known to have the potential to acquire a broad array of cytotoxic and cytoprotective functional states; TREM2 appears to be important in the regulation of this balance in relation to AD pathology.

In 2013, Jonsson et al. performed whole genome sequencing on 2261 Icelandic individuals and found that a rare mutation (rs75932628-T, frequency of 0.63%), predicted to result in a TREM2 R47H substitution, was associated with an increased risk of AD (odds ratio 2.92). Subsequently, this association was replicated in cohorts from the USA, Germany, The Netherlands, and Norway [9]. Concurrently, Guerreiro et al. confirmed the link between LOAD and the R47H variant by meta-analysis of three imputed data sets of genomewide association studies (EADI, GERAD and ANM) [7]. They also found six additional variants (Q33X, Y38C, T66M, D87D, R98W, and H157Y) that were present in affected cases and not in controls, which could be related to AD pathology. Three of these variants (Q33X, Y38C, and T66M) had been previously reported in the homozygous state to be associated with a frontotemporal dementia-like syndrome (without AD-related plaques and NFT) [30]. The critical role of TREM2 for neuronal health is highlighted by patients with autosomal recessive disorder with near complete loss of TREM2 function called polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL or Nasu Hakola disease) [31–33]. Affected individuals have a progressive inflammatory neurodegenerative disorder with formation of multiple bone cysts. They typically present in the second decade of life with psychiatric symptoms and/or bone fractures, which is followed by a worsening dementia, leading to death in the 4th or 5th decades of life. The TREM2 mutation associated with this disorder (Q33X) has been identified, as discussed previously, in the heterozygous state with an increased risk of LOAD [7]. Patients with a partial loss of function of Colony-stimulating factor 1 receptor (CSF1R), which like TREM2 is a microglial receptor that signals via DAP12, have a corticobasal syndrome called hereditary diffuse leukoencephalopathy with spheroids [34]. Neither the latter disorder nor PLOSL is associated with amyloid plaques or NFT pathology; however, there is increased microgliosis along with neurodegeneration, highlighting the likely importance of the balance between microglial phagocytic and inflammatory pathways in AD.

3. Relationship between Tau and Trem2

Neurofibrillary tangles (NFT) are one of the pathological hallmarks of AD, which are formed by the intracellular, neuronal accumulation of abnormally aggregated and hyperphosphorulated tau protein. NFT deposition correlates better with the degree of dementia, compared to the amyloid plaque burden [35]. A number of studies have shown that increases of hyperphosphorylated tau protein (ptau) in CSF correlate with neuronal loss and is predictive of cognitive decline in AD [36–40]. A recent large GWAS study has shown that the Trem2 R47H variant has a strong association with both elevated CSF tau and ptau levels [41].

4. Toll-Like Receptors Structure

Toll was first identified as a receptor expressed by insects and was found to be essential for establishing dorsal-ventral orientation during embryonic development in Drosophila melanogaster [42] and for being important for defense against microbial infection [43, 44]. To date, 11 members of TLR family have been identified in humans and 13 in mice, which trigger both innate and adaptive immune responses [45–47]. Each TLR has at least one known binding ligand and/or adaptors except TLR10 [48, 49]. *TLR11, 12*, and *1* that are not present in the human genome [50]. TLR 4 was the first mammalian homolog of Toll identified as a pattern recognition receptor required for adaptive immunity [43]. The TLRs are important for regulating microglial responses to Aβ. Fibrillar Aβ triggers microglia inflammatory cytokine production via TLR4-TLR6 heterodimers, whose assembly is regulated by CD36 [51]. Treatment of microglia with plaque material produces marked upregulation of TLR2, TLR4, TLR5, TLR7, and TLR9 mRNA [52].

5. Roles of TLR4 and TLR9

In addition to amyloid plaques and neurofibrillary tangles that characterized AD, inflammation is observed with the progression of the disease, which is linked to production of cytokines by activated microglia. Microglia can also play a neuroprotective role by clearing Aβ via increased phagocytosis and proteolytic degradation [53–55]. Microglia can activate both innate and adaptive immune response as well express several Toll-like receptor (TLRs). TLRs play a key role in the innate immune system. Innate immunity is the first line of defense against invading microbes [56]. When a pathogen invades the body, it typically possesses a pathogen-associated molecular pattern, better known as PAMP. These PAMPs are sensed by pattern-recognition receptors (PRRs) and one specific group of PRRs is the Toll-like receptors (TLRs) [57]. TLRs are critical for eliciting an innate immune response to invading pathogens and are also important for triggering the adaptive immune responses [58, 59]. TLR engagement on antigen-presenting cells (APCs) induces cytokine release and costimulatory molecule expression that primes cells for subsequent activation and expansion of antigen-specific T cells [58–61]. TLRs also recognize a variety of danger-associated molecular patterns, called DAMPs. TLRs are expressed in all glial cells including microglia, astrocytes, oligodendrocytes, and a limited repertoire in neurons.

TLR4 is the most actively investigated and characterized in relation to AD pathology. It recognizes microbial motifs of LPS (lipopolysaccharides) [62, 63]. The gene encoding *TLR4* in humans has 4 exons and is located on chromosome 9q32-q33, with 4 exons. TLR4 is mostly expressed in lymphocytes, monocytes, macrophages, and splenocytes [64, 65]. TLR4 has been found expressed in many other cells including epithelial [66], endothelial cells [67], and cancer cells [68, 69]. TLR4 expression on cerebral vascular endothelium increases following subarachnoid hemorrhage. TLR4 deficiency has been shown to be protective against ischemic damage and enhance neuronal survival in stroke mouse models [70].

TLR9 is a protein encoded by the *TLR9* gene which has been designated as CD289. TLR9 was originally identified as a receptor that could differentially recognize bacterial DNA versus mammalian DNA, based on the high frequency of hypomethylated CpG motifs in nonmammalian DNA [71, 72]. TLR9 recognizes intracellular pathogen-derived nonmethylated CpG motifs of bacterial and viral DNA. TLR9 is expressed by several cells including dendritic cells, B lymphocytes, monocytes, and natural killer cells. TLR9 is expressed in the cytoplasm within the endoplasmic reticulum, as well as on other intracellular vesicles but not on the plasma membrane [73].

6. TLRs, TREM2, and Alzheimer's Disease

Numerous studies have shown that TLRs have a crucial role in immune surveillance and inflammatory responses in the central nervous system (CNS) [12]. TLRs expression was identified on glial cells in human postmortem AD [74]. TLRs are important in AD, specifically those expressed on microglia (TLRs 1–9) [75, 76]. It has been shown that TREM2 is coupled with the immunoreceptor tyrosine-based activation motif (ITAM) sequence containing signaling adapter, DAP12, which negatively regulates TLR responses in both macrophages and dendritic cells [26, 77]. Hence, it likely TREM2 can regulate phagocytosis and/or inflammatory responses mediated via TLRs in response to AD pathology. It has been reported that TLRs on the surface of microglia cells bind Aβ, which triggers downstream intracellular signaling cascades [78, 79]. In AD patients high expression of CD14 (coreceptor for TLR4) was observed in parenchymal microglia of the frontal and occipital neocortex, hippocampus, and around senile plaques. Immunoreactivity with CD14 was detected in some perivascular areas [80]. In AD brains, high expression of TLR2 and CD14 was detected in microglia associated with amyloid plaques [74]. In AD Tg models, such as APP23, high levels of CD14 were observed in the microglia detected in the cortex and hippocampus [81]. Increased TLR4 mRNA was reported in another AD Tg model TgCRND8 [82]. In addition, TLR4-deficient mice displayed increased diffuse Aβ and fibrillar Aβ deposits compared with control mice [78], suggesting that TLR4 signaling is involved in Aβ clearance [83]. Microglia deficient in TLR2, TLR4, or the coreceptor CD14 are not activated by Aβ and do not show a phagocytic response [84]. Transgenic AD mice lacking TLR4 have markedly elevated levels of diffuse and fibrillar Aβ. Furthermore, stimulation of microglial cells with TLR2-, TLR4-, or TLR9-specific agonists accelerates Aβ clearance both in vitro and in vivo [85]. It has been reported that TLR9-mediated pathways regulate inflammatory mediator expression in astrocytes. Neurons are thought to express intracellular TLRs, including TLR9, suggesting a role for TLRs during both physiological and pathological conditions [86]. The intracerebroventricular administration of CpG in AD model Tg2576 mice has been shown to ameliorate cognitive impairments [87]. Our group has shown that the administration of the TLR9 agonist CpG oligonucleotides (ODN) containing unmethylated CpG sequences to Tg2576 mice induced a reduction of cortical and vascular Aβ levels

without apparent toxicity and improved cognitive function [88]. We had previously shown that CpG stimulation is beneficial for the generation of an immune response to prion disease [89]. Several CpG DNA drugs have demonstrated good safety profiles in humans and have been tested in numerous clinical trials as antitumor, antimicrobial agents, and adjuvants in vaccines [90, 91]. Recently, we have shown that TLR9 stimulation with CpG in 3xTg AD mice with both amyloid plaque and NFT pathology greatly reduces both of these pathologies in association with cognitive benefits [92]. These results indicate that stimulation of the innate immune system through TLR9 with CpG ODN is an effective and safe method to reduce the amyloid burden and also tau-related pathology in AD model mice.

7. Conclusion

Studies conducted in the early 1990s have suggested the potentially critical role of microglia for both the formation and clearance of amyloid lesions [93–95]. Numerous studies on the relationship of TLRs to AD have shown that modification of these signaling pathways can have profound effects on AD-related pathology, through modification of the inflammatory state of microglia/macrophages. TLRs may either have a positive or negative impact on cellular responses during AD. Studies have shown that appropriate stimulation of TLR9 can ameliorate both Aβ and tau-related pathology. The recent finding by two large consortiums that a rare variant of *TREM2*, a gene which regulates phagocytosis and the activation state of microglia/macrophages, is linked to LOAD has further highlighted the important role of innate immunity in AD. This finding adds to prior data linking other genes that are associated with microglia function and a low increased risk of LOAD, such as CR1, CD33, and MS4A4A/MS4A6A [96]. These studies indicate that modification of microglial function in AD is an important therapeutic target.

Acknowledgments

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