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Chapter 8. TLRs in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is characterized by the formation of insoluble deposits of β -amyloid ($A\beta$) within the parenchyma of the brain. These deposits are associated with a robust microglial-mediated inflammatory response. Recent work has demonstrated that Toll like receptors (TLRs) participate in this inflammatory response. This chapter reviews the mechanisms whereby TLRs contribute to induction of a microglial inflammatory response to promote AD pathogenesis. Specifically the involvement of CD14 and the TLRs in microglial activation is delineated. The TLR-mediated microglial response has both beneficial roles in stimulating phagocytosis as well as detrimental roles in the $A\beta$ -stimulated release of neurotoxic products.

8.1 Alzheimer's Disease

Alzheimer's disease (AD) was first described by Dr. Alois Alzheimer in 1907. He described a 51 year-old woman named August D. exhibiting progressive memory loss, cognitive decline, changes in behavior, and loss of language function, which comprise the cardinal symptoms of the disease. AD is the most common cause of dementia in the elderly. Currently, there are 5 million cases in the United States, with over 15 million cases worldwide. The number of AD cases continues to rise as the average life expectancy increases, reaching perhaps 16 million Americans by 2050. Average disease progression occurs over the course of 8 years, culminating in death of the patient. It has been argued that if a treatment could delay symptom onset by 5 years, the number of individuals with AD could ultimately be reduced by nearly 50%. This would relieve both economic and medical burdens, as currently the national total for direct and indirect costs of AD are over \$148 billion annually (from www.Alz.org).

The AD brain is characterized by extensive extracellular deposits of β -amyloid ($A\beta$) that condenses to form senile plaques. In addition to amyloid deposits, the AD brain exhibits intracellular neurofibrillary tangles composed of the hyperphosphorylated microtubule-associated protein tau and extensive neuronal atrophy within the hippocampus, and the entorhinal and the temporoparietal cortices. The disease is also characterized by a robust inflammatory response with increased levels of inflammatory cytokines, chemokines, immune cell surface proteins, acute phase proteins, complement proteins, and oxidative damage within the brain.

Age is the single biggest risk factor for AD; there is a positive correlation between increased age and the incidence of AD (Gorelick 2004). Although most cases are sporadic, a small number of the total cases are inherited and are linked to autosomal dominant mutations in genes whose products participate in APP processing and $A\beta$ production (Findeis 2007). People with these mutations have disease onset prior to age 65, whereas individuals affected after the age of 65 have late-onset AD. A number of susceptibility genes have been identified that influence the risk of late onset AD as well (Findeis 2007). Moreover,

vascular, environmental, and social factors, in addition to life habits and medication have been shown to be associated with increased or decreased AD risk (Fratiglioni et al. 2007).

The most widely held hypothesis for AD pathogenesis is known as the “amyloid hypothesis.” It posits that a gradual and chronic imbalance in the production and clearance of A β leads to an increase in its steady state levels within the brain over the course of decades, thus resulting in the complex molecular and cellular changes within the brain that typify AD (Selkoe 2000). A β is produced by the sequential proteolytic processing of the amyloid precursor protein (APP) by either the α - or β -secretase (BACE), and subsequently the γ -secretase. The pathogenic processing is initiated by the cleavage of APP by BACE, releasing APPs- β , leaving the C99 fragment in the membrane. C99 is then cleaved by γ -secretase to produce the A β peptide, with the two major forms being 40 and 42 residues in length. In a normal individual, the majority of the A β produced is the 40 amino acid species, whereas 5–15% of the total A β pool is made up of A β ₄₂ (Younkin 1998). Familial APP mutations increase the relative production of the more toxic and more amyloidogenic species, A β ₄₂, which is important in development of AD (Crouch et al. 2008). In the non-pathogenic APP processing pathway, APP is cleaved by the α -secretase to produce APPs- α and the C83 fragment. C83 is then cleaved by the γ -secretase to produce the non-amyloidogenic p3 fragment.

8.2 Inflammation and Alzheimer's disease

Extensive research has demonstrated the involvement of inflammation in AD. There is an extensive literature documenting that the brains and cerebrospinal fluid of AD patients contain a variety of pro-inflammatory mediators, including complement, cytokines and chemokines, acute phase proteins, proteases, as well as reactive oxygen and nitrogen species that are responsible for the oxidative damage in the AD brain (reviewed in (Akiyama et al. 2000)). In addition, a subset of epidemiological studies have shown chronic use of some non-steroidal anti-inflammatory drugs (NSAIDs) drastically reduce the risk, onset, severity, and progression of AD (Rogers et al. 1993; Rich et al. 1995; McGeer et al. 1996; Stewart et al. 1997; in't Veld et al. 2000).

The principal immune effector cells of the brain are microglia. A number of hypotheses exist for the origin of microglia, currently, the consensus is that they are derived from mesodermal/mesenchymal sources in the periphery and infiltrate the brain during development (reviewed in (Chan et al. 2007)). Recently, it has been suggested that peripherally-derived monocytes and macrophages can traffic into and populate the brain, and subsequently perform microglial-related functions (Simard and Rivest 2004). However, this phenomenon has now been shown to be an artifact of the experimental manipulation (reviewed in (Ransohoff 2007)).

Under normal, non-pathogenic conditions, microglia have a small soma and highly ramified morphology, and exhibit a “resting” morphology, but are far from dormant. Two recent studies have demonstrated the dynamic nature of microglia in immune surveillance of the brain (Davalos et al. 2005; Nimmerjahn et al. 2005). Using the microglial-specific *Cx3cr1* locus to drive enhanced green fluorescent protein (eGFP) expression, 2-photon microscopy revealed that microglia are evenly spaced throughout the brain at approximately 6 cells per cubic millimeter. These cells contain highly mobile processes that continuously extend and retract, resulting in inspection of the entire brain parenchyma every few hours. Neighboring microglial cells alternate in scanning overlapping regions, ensuring thorough examination of the brain parenchyma while avoiding contact. These dynamic and finely tuned surveillance mechanisms enable stationary microglia to scan their environment without disrupting the neuronal network of the brain parenchyma (Hanisch and Kettenmann 2007). Upon

recognition of an insult or foreign material, microglia shift their activation state from a surveillance mode to a reactive mode where they act as immune effector cells.

Microglial activation status is determined by their immediate environment (Goerdts and Orfanos 1999; Gordon 2003). The response of microglia, like other tissue macrophages, to cytokines and a pro-inflammatory environment (Th1 response) results in a classical activation. This activation status is characterized by up-regulation of a variety of cell surface receptors, pro-inflammatory molecules, nitric oxide, and prostaglandins (Mantovani et al. 2002). In contrast, microglia exposed to the anti-inflammatory cytokines interleukin 4 (IL-4), IL-10, IL-13, and transforming growth factor β (TGF- β), reflective of a Th2 type response become alternatively activated. This alternate activation state demonstrates a greater capacity for phagocytosis and does not produce nitric oxide (Goerdts and Orfanos 1999). A pro-inflammatory environment reduces the phagocytic capacity of microglia (Koenigsnecht-Talboo and Landreth 2005; Zelcer et al. 2007). In these studies, LPS was used to induce a pro-inflammatory environment as it stimulates cytokine gene expression via NF- κ B. The suppression of phagocytosis by pro-inflammatory cytokines is reversible by NSAIDs, anti-inflammatory cytokines, and liver $\times$ receptor agonists.

Microglia surrounding A β plaques show an activated phenotype and extend processes which envelop the A β plaque (Bornemann et al. 2001; Bolmont et al. 2008). Compared to those found in the non-demented brain, these microglia express higher levels of a number of cell surface proteins, including the major histocompatibility complex II (MHC II) cell surface glycoprotein (Luber-Narod and Rogers 1988; Rogers et al. 1988; Haga et al. 1989; McGeer et al. 1989; Styren et al. 1990). In addition, a variety of other cell surface proteins are up-regulated on activated microglia neighboring A β plaques. Microglia surrounding plaques have also been shown to proliferate, contributing to their accumulation at the plaque periphery (Bornemann et al. 2001; Stalder et al. 2001; Bolmont et al. 2008). Some aspects of the microglial inflammatory response represent positive influences with respect to AD pathogenesis, such as phagocytosis which may participate in the removal of A β from the brain. However, decades of direct and bystander damage from a chronic microglial-mediated inflammatory response mechanism likely exacerbates disease pathogenesis. Importantly, the levels of pro-inflammatory cytokines are dependent on the magnitude of plaque burden in the AD brain (Patel et al., 2005). It has been suggested that the inflammatory response facilitates the production and deposition of A β (Akiyama et al. 2000; Patel et al. 2005).

8.3 Innate immunity in Alzheimer's disease

The innate immune system was first described over one hundred years ago by Dr. Elie Metchnikoff. This system mediates the detection of constitutive and conserved products of microbial metabolism and mobilizes a robust immune response resulting in death or disposal of the invading pathogen (reviewed in (Medzhitov 2001)). Although bacterial products exhibit variation in their chemical structures, they are typically found in the context of a common molecular pattern called pathogen-associated molecular patterns (PAMPs). These PAMPs are recognized by a variety of pattern recognition receptors (PRRs). As PRRs are encoded in the germ line, there is no requirement for previous exposure, so the response is rapid; thus, the innate immune system is the first line of defense in host defense.

Toll-like receptors (TLRs) are a family of PRRs characterized by an extracellular leucine-rich repeat domain and an intracellular Toll/IL-1 receptor (TIR) domain (Kielian 2006). In mammals there are at least ten TLRs; despite a high degree of structural similarity, each receptor has a distinct function in innate immune recognition. Although each TLR has some degree of ligand specificity, this specificity is extended through dimerization of the TLRs.

Some TLRs employ additional co-receptors that assist in pathogen recognition, such as CD14 for TLR4 (Kielian 2006).

The binding of ligand to TLRs initiates intracellular signal transduction cascades. The TLR-stimulated signaling cascades act to up-regulate the expression of pro-inflammatory cytokines and chemokines, nitric oxide synthase and other anti-microbial peptides that directly destroy microbial pathogens (reviewed in (Han and Ulevitch 2005)). TLRs possess an intracellular Toll/IL-1 receptor (TIR) domain that serves as a scaffold to generate intracellular signaling cascades that induce changes for mechanisms of host defense. Following recognition of ligand, TLRs recruit specific combinations of adaptor proteins that include myeloid differentiation primary response gene 88 (MyD88), TIR-containing adaptor protein/MyD88-adaptor like (TIRAP/MAL), TIR-containing adaptor inducing interferon- β (IFN- β)/TIR-domain-containing adaptor molecule 1 (TRIF/TICAM1) and TIR-domain-containing adaptor molecule/TRIF-related adaptor molecule 2 (TRAM/TICAM2) (Kawai and Akira 2007). TLR signaling is divided into MyD88-dependent and TRIF-dependent pathways. The MyD88-dependent pathway involves the IL-1 receptor-associated kinase (IRAK) family members IRAK4 and IRAK1, which interact with tumor necrosis factor receptor-associated factor 6 (TRAF6) to promote ubiquitination for recruitment of transforming growth factor- β -activated kinase-1 (TAK1) and TAK1 binding proteins (TABs) (Chen et al. 2006a; Adhikari et al. 2007; Kawai and Akira 2007). These molecules then activate pathways involving the IKK complex and the mitogen-activated protein kinase (MAPK) pathway for NF- κ B-dependent pro-inflammatory gene expression (Wang et al. 2001; Sanjo et al. 2003; Shim et al. 2005). In contrast, TRIF-dependent signaling leads to NF- κ B activation through TRAF6 which activates TAK1 in a manner similar to that of the MyD88-dependent pathway via receptor-interacting protein-1 (RIP1) (Yamamoto et al. 2002; Sato et al. 2003; Jiang et al. 2004; Meylan et al. 2004; Cusson-Hermance et al. 2005). The TRIF-dependent pathway also activates the IKK-related kinases TRAF family member-associated NF- κ B activator binding kinase-1 (TBK1) and IKKi through TRAF3 (Pomerantz and Baltimore 1999; Nomura et al. 2000; Kawai and Akira 2007). These kinases phosphorylate the transcription factors interferon regulatory factor 3 (IRF3) and IRF7, activating them for IFN- β induction (Fitzgerald et al. 2003; Sharma et al. 2003).

8.3.1 Increased TLR expression

It has been shown that there are changes in neuro-inflammation associated with aging (Letiembre et al. 2007a). It has been argued that a pro-inflammatory status originates as an adaptive mechanism of aging that builds up over time, representing a biologic background favoring disease susceptibility. The addition of various risk factors, genetic or environmental, results in the development of overt age-related diseases having an inflammatory pathogenesis such as AD (Franceschi et al. 2000). Age-related changes in the regulation of innate immunity may be of clinical significance for development of mild cognitive deficits or for predisposition to neurodegenerative diseases in the elderly (Letiembre et al. 2007a).

An unexplained feature of normal aging is an increase in innate immune receptor expression in the brains of aging mice (Letiembre et al. 2007a). Importantly, there is also increased expression of these receptors in brains of AD patients (Liu et al. 2005; Letiembre et al. 2007b; Walter et al. 2007) and in animal models of the disease (Fassbender et al. 2004; Letiembre et al. 2007b; Walter et al. 2007). High levels of CD14 were found in the microglia of the cortex and hippocampus of the APP23 mouse model of AD (Fassbender et al. 2004). AD patients showed increased CD14 expression in parenchymal microglia of the frontal and occipital cortex, hippocampus, and around the senile plaques. Some perivascular cells of the brain exhibited CD14 immunoreactivity as well (Liu et al. 2005). The AD mouse model TgCRND8 was reported to have higher TLR4 mRNA expression as compared to age

matched controls, and brains of AD patients exhibited pronounced TLR4 expression by immunofluorescence that was associated with A β plaque deposition in the entorhinal cortex (Walter et al. 2007). Infusion of A β ₄₂ into the hippocampus induced TLR2 expression (Richard et al. 2008). A screen of innate immune receptors in the TgCRND8 animal also revealed an up-regulation of TLR2 and TLR7 expression (Letiembre et al. 2007b). CD14 expression was localized around the A β plaques present in the cortex. However, TLR2 exhibited a more complex pattern of expression; it was absent from the cortex, but was found surrounding A β plaques in the amygdala, where CD14 was largely undetectable. In human AD brains, both TLR2 and CD14-positive microglia were found associated with A β plaques, and a greater number of CD14-positive cells are found surrounding diffuse plaques compared to dense-core plaques (Letiembre et al. 2007b). These studies suggest that increased expression of CD14 and TLRs is associated with increased inflammation associated with aging, and when combined with various AD risk factors, this inflammatory environment results in exacerbation of disease progression through an inflammatory response.

Interestingly, a recent study of a TLR4 polymorphism that exhibits a blunted TLR signaling response is associated with a 2.7 fold reduction in risk of late onset AD (Minoretti et al. 2006). An adenine to guanine substitution in TLR4 causes the replacement of an aspartic acid residue by a glycine at amino acid 299. This affects the structure of the extracellular domain of TLR4 and results in attenuated efficacy of LPS signaling and a reduced capacity to elicit inflammation (Arbour et al. 2000). Work by Minoretti et al. demonstrated that the frequency of the 299Gly allele was significantly higher in the non-demented age matched controls than in the AD cases, suggesting that TLR signaling may play a role in AD pathogenesis (Minoretti et al. 2006). Another study subjects having a specific combination of polymorphisms in CD14 and LXR β had a 6-fold reduction in the risk of developing AD (Rodriguez-Rodriguez et al. 2008). The polymorphism in the promoter region of CD14 decreases CD14 expression on circulating monocytes and in plasma. This coupled with a polymorphism in LXR β intron 5 is thought to lower the inflammatory response in the brain, which in turn, decreases the risk of AD (Rodriguez-Rodriguez et al. 2008).

8.3.2 TLRs in microglial activation

Only recently have TLRs been implicated in microglial activation in AD, and how microglial TLRs function in the inflammatory response in AD is now under active investigation. TLR ligands were typically thought to be “exogenous”, in that they were of microbial origin. However, recent evidence suggests the presence of “endogenous” TLR ligands. Interestingly, the majority of endogenous TLR ligands interact with TLR4 or TLR2 and 4 and not with other members of the TLR family (reviewed in (Tsan and Gao 2007)). The diversity of TLR ligands has been explained by a hydrophobicity model (Seong and Matzinger 2004), where hydrophobic domains in any molecule can become molecular patterns recognized by TLRs. Because A β forms hydrophobic aggregates in AD plaques, it was investigated whether CD14 could mediate A β -induced neuroinflammation (Fassbender et al. 2004; Liu et al. 2005). Using a variety of techniques, CD14 was shown to bind A β ₄₂. The interaction between CD14 and fibrillar A β ₄₂ was 20 times greater than that between CD14 and non-fibrillar A β ₄₂, indicating the importance of the fibrillar structure of A β ₄₂ in binding CD14. Although the affinity of the A β ₄₂-CD14 interaction is approximately 50 times lower than that of LPS and CD14, it is thought that because AD brains contain high concentrations of A β ₄₂ over a period of decades, it is likely that even at this submaximal affinity, this interaction is sufficient to maintain chronic neuroinflammation (Fassbender et al. 2004). Fassbender and colleagues concluded that CD14 along with “accessory receptors” may bind highly hydrophobic A β aggregates in a manner similar to that of exogenous PAMPs in a “structural mimicry” mechanism (Fassbender et al. 2004). Inhibition of CD14

function, either through the use of a function blocking antibody or the use of CD14-deficient microglia, demonstrated CD14 was required for A β /IFN γ -induced release of nitrite, IL-6, and TNF- α in both murine microglia and in human peripheral blood monocytes. This response was associated with NF κ B nuclear translocation and activation (Fassbender et al. 2004).

These studies led to the question as to whether TLRs participated in A β -induced microglial activation. Fibrillar A β engages TLRs to stimulate “host defense” mechanisms, resulting in pro-inflammatory activation of microglia. Inhibition of TLR4 in human monocytes or murine microglia, through the use of function blocking antibodies, resulted in reduced nitrite, TNF- α , and IL-6 production following aggregated, fibrillar A β ₄₂ exposure (Walter et al. 2007; Udan et al. 2008). Non-fibrillar forms of A β ₄₂ were unable to stimulate IL-8 secretion in HEK293 cells expressing CD14, TLR4, and MD2, suggesting again, that the aggregated conformation of A β is a structural prerequisite for cellular activation by innate immune receptors (Walter et al. 2007). Microglia without functioning TLR2 were unable to increase expression of TNF- α , IL-1 β , IL-6, the integrin markers CD11b, CD11c, and CD68, in addition to inducible NO synthase following fibrillar A β ₄₂ (Jana et al. 2008; Udan et al. 2008). TLR2 expression in HEK293 cells resulted in A β -induced IL-8 production, and was enhanced after CD14 co-expression (Walter et al. 2007). Moreover, animal models of AD have shown a requirement for TLRs in microglial activation and inflammatory gene expression. A mouse model of AD lacking functional TLR4 had increased levels of the microglial marker CD11b and the reactive astrocyte marker GFAP (Jin et al. 2008). In addition, AD mice deficient TLR2 were shown to have increased TGF- β mRNA than their TLR2^{+/+} littermates (Richard et al. 2008).

A microglial receptor complex comprised of the $\alpha_6\beta_1$ integrin, the integrin associated protein CD47, and CD36 has been described for fibrillar A β (Bamberger et al. 2003). This receptor complex is required for A β -induced cytokine production, reactive oxygen species production and a phagocytic response (Bamberger et al. 2003; Koenigsnecht and Landreth 2004). Microglia lacking CD14, TLR4, or TLR2 are unable to produce reactive oxygen species or a phagocytic response following stimulation with fibrillar A β (Reed-Geaghan and Landreth 2007). Wilkinson et al. demonstrated that following engagement of this receptor complex by fibrillar A β , Lyn and Syk kinases associate with Vav, a Rac guanine nucleotide exchange factor, phosphorylating, and thereby activating it (Wilkinson et al. 2006). Vav activation permitted Rac participation in NADPH oxidase complex formation for reactive oxygen species production and for participation in actin cytoskeletal re-arrangement for phagocytosis. Microglia deficient for CD14, TLR4, or TLR2 were unable to stimulate signaling through this Src-Vav-Rac cascade (Reed-Geaghan and Landreth 2007). The MAPK p38 becomes activated following fibrillar A β exposure (McDonald et al. 1998), and has been shown to function in TLR-dependent endosome maturation (Blander and Medzhitov 2004) as well as in phosphorylation of the NADPH oxidase subunit p47^{phox} for reactive oxygen species production (El Benna et al. 1996). Following treatment with fibrillar A β , microglia from CD14, TLR4, or TLR2-null animals were unable to activate p38 (Reed-Geaghan and Landreth 2007). These studies demonstrate the involvement of CD14 and the TLRs in microglial recognition of fibrillar A β and subsequent signal transduction cascades for microglial activation.

Persistent activation of microglia results in their migration to the activating stimulus. Human G protein-coupled formyl peptide receptor like 1 (FPRL1) and its mouse homolog murine formyl peptide receptor 2 (mFPR2) mediate the chemotactic activity and uptake of A β ₄₂ by monocytic cells, and thus may be involved in recruiting microglia to amyloid plaques (Tiffany et al. 2001; Yazawa et al. 2001; Cui et al. 2002; Ying et al. 2004; Iribarren et al. 2005b). The TLR ligands LPS, CpG, and PGN up-regulate mFPR2 mRNA in both a

microglial cell line and in primary murine microglia, accompanied by an increased chemotactic response to A β and increased endocytosis of A β (Iribarren et al. 2005a; Chen et al. 2006b). Activation of the p38 and ERK1/2 MAP kinases as well as activation of NF- κ B by TLR ligands is required for the induction of mFPR2 mRNA and the chemotactic response (Iribarren et al. 2005a; Chen et al. 2006b). Here the activation of TLR4 may promote a microglial response in AD where the mFPR2 agonist A β is elevated. Thus, TLRs on the surface of microglia act as sensors for pro-inflammatory signals and orchestrate the host response in the brain (Iribarren et al. 2005a).

While all previous studies point to TLR ligands enhancing the activation of microglia by A β , a recent study by Lotz et al. suggests that not all TLR agonists enhance the stimulatory effect of A β on innate immunity (Lotz et al. 2005). In these studies, simultaneous treatment of primary mouse microglia or murine peritoneal macrophages with LPS or the TLR2 ligand Pam3Cys together with A β_{40} had an additive effect on nitric oxide and TNF- α release compared to LPS or Pam3Cys alone. However, simultaneous treatment of microglia or macrophages with the TLR9 ligand single-stranded unmethylated CpG-DNA and A β_{40} , while having little effect at low doses, significantly decreased NO release at higher concentrations. Interestingly, low doses of CpG with A β_{40} reduced TNF- α release. Treatment of microglia with A β_{40} resulted in microglial activation characterized by cell rounding, loss of ramification, and formation of cytoplasmic vacuoles. A β was detected both at the surface and within the cell, but did not colocalize with CpG. The authors propose that the distinct responses of microglia to individual TLR agonists is the result of receptor localization and the difference in signaling cascades (Lotz et al. 2005).

8.3.3 TLRs in the death of neurons

Neuronal loss is a prominent element of AD pathology. Combs et al. demonstrated neuronal apoptosis was induced by the addition of conditioned media from A β -stimulated monocytes (Combs et al. 2001). TLRs have been shown to have a role in neuronal apoptosis as well. Hippocampal neurons exposed to conditioned media from microglia treated with aggregated A β_{42} resulted in neuronal death. However, media from CD14- or TLR4-knockout microglia treated with A β_{42} was unable to kill neurons (Fassbender et al. 2004; Walter et al. 2007). These data suggest that CD14 and TLR4 function in the production of neuro-toxic molecules.

Another mechanism whereby TLRs function in neuronal death is through the recognition of A β -damaged neurons. Bate et al. have shown microglia kill A β_{42} -damaged neurons through a contact-dependent mechanism (Bate et al. 2004). The addition of microglia to neurons treated with sub-lethal doses of A β_{42} resulted in the death of neurons in a manner inversely related to both the concentration of A β and the number of microglial cells added (Bate et al. 2006). Involvement of CD14 in this response was demonstrated by attenuated killing of A β -treated neurons following pretreatment of microglia with an antibody to CD14, as well as the inability of microglia from CD14^{-/-} mice to kill A β -treated neurons (Bate et al. 2004). Moreover, neurons treated with A β_{42} possess higher levels of CD14-reactive molecules on their cell surface, as they bound higher amounts of a CD14-IgG chimera than did untreated neurons (Bate et al. 2006). Significantly, the addition of this CD14-IgG chimera to A β -treated neurons prevented microglial killing of neurons without altering the direct effects of A β (Bate et al. 2004). The authors hypothesized that neurons which have survived low (i.e. non-toxic) concentrations of A β undergo phenotypic changes which lead to them being recognized and killed by microglial cells via CD14. This model may mimic some of the earliest neuronal changes, such as loss of synapses and axon terminal degeneration, observed during disease progression (Bate et al. 2006).

8.3.4 TLRs and the clearance of A β from the brain

The imbalance between A β production and clearance influences AD pathogenesis. In vitro, treatment of microglia with the TLR4 ligand LPS or the TLR9 ligand CpG oligodeoxynucleotide stimulates uptake of A β (Tahara et al. 2006). Fiala et al. examined the difference in A β uptake and TLR expression (Fiala et al. 2007) and found that macrophages from control subjects usually showed extremely efficient phagocytosis of A β and rapid intracellular transport of A β . In contrast, macrophages from AD patients minimally ingested A β , and did not transport A β into endosomes and lysosomes. Control macrophages stimulated with A β increased expression of TLRs, but AD macrophages down-regulated their TLR ratios. The authors hypothesized that the lower expression levels of TLRs on AD macrophages may be indicative of more global innate immune defects beyond A β phagocytosis, where the innate and adaptive immune systems are in various states of dissonance (Fiala et al. 2007).

Because CD14 has been shown to be required for uptake of a variety of pathogens, the involvement of CD14 in A β internalization has been studied (Liu et al. 2005). Confocal analysis of wild-type microglia showed CD14 and fibrillar A β ₄₂ colocalized at the cell surface, and after 30 minutes, this complex was internalized and colocalized with the lysosomal marker LAMP2. CD14^{-/-} microglia phagocytosed less fibrillar A β ₄₂ than did their wild-type counterparts, though this was not due to general impairment of the phagocytic machinery. These data clearly demonstrate the requirement for CD14 in microglial phagocytosis of A β .

The importance of TLR4 and TLR2 in A β uptake has also been assessed in animal models of AD. The APP^{swe}/PSEN1^{dE9} mouse model of Alzheimer's disease (Jankowsky et al. 2004) has as part of its background the C3H/HeJ strain. These animals contain a co-dominant destructive point mutation in the TLR4 gene, resulting in the failure of TLR4 activation by LPS (Poltorak et al. 1998). Tahara et al. took advantage of this intrinsic mutation to evaluate the role of TLR4 in amyloidogenesis in vivo (Tahara et al. 2006). As compared to their wild-type littermates, APP^{swe}/PSEN^{dE9} mice with inactive TLR4 showed increased cortical and hippocampal A β load, without changing steady state APP or presenilin1 levels. These data strongly argue that the change in A β load was due to a change in microglial-mediated A β clearance that is reliant upon TLR4 function. APP^{swe}/PSEN^{dE9} mice have also been mated to TLR2^{-/-} mice, and the resulting transgenic TLR2^{-/-} mice show delayed A β deposition through 6 months of age, but had comparable deposition by 9 months of age, compared to their TLR2^{+/+} littermates (Richard et al. 2008). The different results in A β deposition between TLR4 and TLR2 deficient animals could be explained by the age of the animals assessed. Jin et al. used mice that were aged at least 14 months, while Richard et al. examined their animals no later than 9 months of age. The APP^{swe}/PSEN^{dE9} mouse has decreased expression of various A β -binding receptors and degrading enzymes at 8 months of age in addition to increased pro-inflammatory gene expression (Hickman et al. 2008). It is hypothesized that while microglia clear A β in early stages of AD, as the disease progresses, genes involved in A β clearance are down-regulated due to increased pro-inflammatory gene expression, contributing to A β accumulation (Hickman et al. 2008). Thus, CD14, TLR4, and TLR2 function in the recognition and binding of A β for its internalization and clearance from the brain parenchyma.

8.4 Conclusions

The AD brain contains many potential inflammatory stimuli, including A β and damaged neurons. Due to the presence of these stimuli, there is a discrete, localized inflammatory reaction. Over the course of decades, this chronic inflammation produces direct and indirect damage within the brain. Amyloid deposition begins 10–20 years before the appearance of

clinical dementia, allowing inflammatory processes to proceed for years unchecked. A small number of studies prospectively investigated the relationship between systemic markers of inflammation and the risk of cognitive decline (reviewed in (Dziedzic 2006)). Individuals with high levels of the inflammatory markers C-reactive protein, IL-6, and α 1-antichymotrypsin were associated with increased dementia. Work has shown that, to a degree, the microglial inflammatory response has beneficial effects. Microglia migrate to plaques for phagocytosis and degradation of A β . They up-regulate a variety of factors that aid in clearance of A β , such as complement, integrins, and Fc receptors (reviewed in (Blasko and Grubeck-Loebenstein 2003)). However, while microglial activation is suppressed by electrically active neurons (Neumann and Wekerle 1998), in AD brain, neurons continuously exposed to A β lose the ability to suppress immune responses, so that a chronic inflammatory response exists, thereby contributing to disease pathology.

Work discussed above illustrates the role of CD14 and TLRs in this process. CD14 has the ability to recognize and bind A β aggregates, and along with TLRs mediate microglial activation and clearance of A β from the brain (Fassbender et al. 2004; Liu et al. 2005; Tahara et al. 2006; Walter et al. 2007; Udan et al. 2008). In addition to CD14 and TLRs functioning in microglial activation, CD14 functions in the recognition and clearance of A β -damaged neurons (Bate et al. 2004; Bate et al. 2006). Thus, the innate immune system is truly a double-edged sword. It has been proposed that at low A β concentrations corresponding to those observed in the brain of early/middle stage AD, CD14 and TLRs may activate microglia promoting phagocytic clearance of A β , whereas at higher A β concentrations corresponding to those at late stage AD, microglial activation through CD14 and the TLRs results in production of neurotoxins as well, thereby damaging surrounding neurons (Liu et al. 2005; Walter et al. 2007) and killing these damaged neurons. The recognition of the involvement of TLRs and their co-receptors in AD pathogenesis suggests that they may be an appropriate target for therapeutic intervention within the disease progression.

Glossary

Aβ	β -amyloid
AD	Alzheimer's disease
APP	amyloid precursor protein
BACE	β -secretase
FPRL1	formyl peptide receptor like 1
IFN	interferon
IL	interleukin
IRAK	IL-1 receptor associated kinase
IRF	interferon regulatory factor
MAPK	mitogen activated protein kinase
MHC II	major histocompatibility complex II
mFPR2	murine formyl peptide receptor 2
MyD88	myeloid differentiation primary response gene 88
NSAIDs	non-steroidal anti-inflammatory drugs
PRRs	pattern recognition receptors

PAMPs	pathogen-associated molecular patterns
RIP1	receptor-interacting protein 1
TABs	TAK1 binding proteins
TAK	TGF- β -activated kinase
TBK 1	TRAF family member-associated NF- κ B activator binding kinase-1
TGF-β	transforming growth factor- β
Th2	T helper 2
TIR	Toll/IL-1 receptor
TIRAP/MAL	TIR-containing adaptor protein/MyD88-adaptor like
TNF-α	tumor necrosis factor α
TLRs	toll like receptors
TRAF	TNF-associated factor
TRAM/TICAM2	TIR-domain-containing adaptor molecule/TRIF-related adaptor molecule 2
TRIF/TICAM 1	TIR-containing adaptor inducing IFN- β /TIR-domain containing adaptor molecule 1

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