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Inflammation in Alzheimer's Disease: Lessons learned from microglia-depletion models

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Abstract

Microglia are the primary immune cell of the brain and function to protect the central nervous system (CNS) from injury and invading pathogens. In the homeostatic brain, microglia serve to support neuronal health through synaptic pruning, promoting normal brain connectivity and development, and through release of neurotrophic factors, providing support for CNS integrity. However, recent evidence indicates that the homeostatic functioning of these cells is lost in neurodegenerative disease, including Alzheimer's disease (AD), ultimately contributing to a chronic neuroinflammatory environment in the brain. Importantly, the development of compounds and genetic models to ablate the microglial compartment has emerged as effective tools to further our understanding of microglial function in AD. Use of these models has identified roles of microglia in several pathological facets of AD, including tau propagation, synaptic stripping, neuronal loss, and cognitive decline. Although culminating evidence utilizing these microglial ablation models reports an absence of CNS-endogenous and peripheral myeloid cell involvement in A β phagocytosis, recent data indicates that targeting microglia-evoked neuroinflammation in AD may be essential for potential therapeutics. Therefore, identifying altered signaling pathways in the microglia-devoid brain may assist with the development of effective inflammation-based therapies in AD.

Keywords

Microglia; Colony-stimulating factor 1 receptor; Inflammation; Alzheimer's disease; Amyloid; Tau; Spines

I. Introduction

Microglia are the brain's resident immune cells, comprising approximately 5–12% of all cells found in the brain. They function as the brain's first line of defense to protect the CNS

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Conflict of Interest

The authors E.E.S and K.N.G have no conflicting financial interests.

from injury and invading pathogens. Originally presumed to be “resting,” microglia in the healthy adult brain are highly dynamic, surveying the entire brain parenchyma every 24 hours (Nimmerjahn et al., 2005). In this “surveying” state, microglia exhibit a ramified morphology and serve to support neuronal function and health via physical and biochemical interactions. Upon detection of an insult, microglia respond by becoming activated. This process may involve the migration to and proliferation of these cells at the site of the insult, as well as dramatic transformation into an amoeboid morphology, depending on the type and extent of the insult. Activated microglia produce and secrete several proinflammatory mediators, including tumor necrosis factor- α (TNF α), interleukin (IL)-6, and nitric oxide (NO), all of which can confer neurotoxicity (Akiyama et al., 2000). Neuroimmune regulatory proteins (NiReg) modulate the microglia-mediated immune response to resolve the inflammatory process (Hoarau et al., 2011), which then promotes tissue repair through the secretion of several neurotrophic factors, including insulin-like growth factor 1 (IGF-1), brain-derived neurotrophic factor (BDNF), transforming-growth factor- β (TGF- β), and nerve growth factor (NGF) (Polazzi and Monti, 2010). In acute inflammatory events, the pro-inflammatory response resolves and microglia continue their surveillance of the brain parenchyma. However, in neurodegenerative disease, the equilibrium between microglial surveillance and activation is disturbed, creating a feedforward loop that results in a chronic neuroinflammatory state, promoting inflammation and tissue atrophy. Although astrocytes also participate in and propagate the neuroinflammatory environment in AD, for the purposes of this review, the focus will remain on microglia-mediated inflammation in AD pathogenesis. Here, we briefly review the various implications of microglia and other myeloid cells in neurodegeneration and discuss current methods that allow for investigations into the biology of microglia in AD.

2. Homeostatic microglial functions

2.1. Phagocytosis

One of the more extensively studied functions of microglia in the brain is their role in clearance via phagocytosis, by which these cells both protect the brain from invading pathogens as well as remove cellular debris from the neural environment. Aside from the clearance of cellular debris, microglia may also phagocytose viable neurons in a process known as “phagoptosis,” which specifically targets senescent or damaged cells (Brown and Neher, 2014). For this reason, proper degradation of internalized components by microglia is essential for normal CNS function. Consequently, dysregulated or abnormal degradation of material can result in the intracellular accumulation of toxic molecules, including reactive oxygen species (Underhill and Goodridge, 2012). Importantly, studies have shown that microglia are involved in the phagocytosis of supranumerous apoptotic neuroblasts in the subgranular zone of young mice, implicating their involvement in neurogenesis (Sierra et al., 2010).

2.2 Synaptic sculpting and cognition

In addition to phagocytosis, microglia are also involved in the removal of synapses from neuronal cell bodies via synaptic stripping. This phenomenon was first observed in a model of facial nerve injury in rats (Blinzinger and Kreutzberg, 1968), in which microglial

localization to the site of injury and interaction with facial motor neurons resulted in the removal of synaptic contacts. In the developing brain, microglia form brief, repetitive contacts with synapses, eliminating any weak or unnecessary synaptic structures, a process which is modulated by sensory experiences (Tremblay et al., 2010). Recently, it was shown that knockout of the microglial purigenic receptor P2Y₁₂, which mediates process motility during injury response, also disrupts plasticity in the visual system (Sipe et al., 2016). While the exact mechanism behind microglia-mediated synaptic elimination (whether it be by phagocytosis or the secretion of various factors) has yet to be elucidated, it is clear that the interaction between microglia and synapses is crucial for activity-dependent plasticity in the developing brain. Accumulating evidence points to neuron-microglia crosstalk as an essential mechanism for proper synapse and network maintenance. One pathway implicated in this crosstalk involves the fractalkine receptor (CX₃CR1) expressed on microglia and its ligand CX₃CL1, released by neurons. For example, knockout of CX₃CR1 during development produces deficits in synaptic pruning, characterized by an excess of dendritic spines and immature synapses, resulting in weakened synaptic transmission and decreased functional brain connectivity (Paolicelli et al., 2011). Behaviorally, loss of CX₃CR1 in juvenile mice manifests in impaired social interactions reminiscent of autism spectrum disorder and other neuropsychiatric disorders (Zhan et al., 2014). Furthermore, disruption of signaling between complement 3 (C3), which is localized to synaptically-enriched regions, and its receptor, complement receptor 3 (CR3), in the mouse retinogeniculate system impairs microglial phagocytosis of synaptic inputs, leading to sustained deficits in brain wiring (Schafer et al., 2012). Collectively, these studies underscore the role of microglia as regulators of the synaptic landscape in the developing brain, implicating neuron-microglia crosstalk as a crucial process for proper brain development. In addition to complement and fractalkine signaling, paired immunoglobulin-like receptor B (PirB) is also involved in the regulation of synaptic plasticity. In the CNS, PirB is expressed by both neurons and glia and binds several ligands, including major histocompatibility complex-I (MHC-I) (Syken et al., 2006), which is believed to direct cellular recognition by immune cells. In adult mice, disruption of PirB signaling in the visual cortex increased dendritic spine density and induced the formation of functional synapses, as evidenced by increases in miniature excitatory postsynaptic currents (Bochner et al., 2014). Whether microglia are mediating the PirB-induced synaptic changes remains unresolved, although this cell type is a likely candidate.

While the role of microglia in synaptic sculpting during development is well-described, it remains unclear whether microglia contribute to the synaptic landscape in adulthood. We recently reported that the absence of microglia in the healthy adult mouse brain increases total dendritic spine density and intensity of immunolabeling for the synaptic surrogates PSD95 and synaptophysin (Rice et al., 2015), indicating that microglia continue to regulate the synaptic landscape in adulthood. Collectively, these studies point to microglia as critical mediators of synaptic sculpting in development and adulthood, providing an important role in shaping and modulating neuronal circuitry to maintain normal brain connectivity.

As microglia are heavily implicated in shaping the synaptic landscape of the brain during the incorporation of new memories and experiences into the neural network, the CNS immune system is also thought to be involved in cognitive function. Genetic ablation of microglia

using CX3CR1^{CreER} mice to drive diphtheria toxin receptor expression in these cells found that mice devoid of microglia, following administration of diphtheria toxin, exhibited impaired performance on cue-based fear condition and novel object recognition tasks, as well as impaired dendritic spine remodeling (Parkhurst et al., 2013). However, it is important to note that since behavioral analysis occurred no later than postnatal day 34, an age at which microglia actively refine synapses and eliminate weak and unnecessary neuronal connections, it is not surprising that microglia-deficient mice exhibit memory-related deficits. Additionally, death of microglia with this genetic approach may induce widespread inflammatory processes from surviving cells (Bruttger et al., 2015), including the production of pro-inflammatory cytokines, which can impair cognitive functioning (Bellinger et al., 1993; Dugan et al., 2009; Terrando et al., 2010). In contrast to this, the administration of the CSF1R inhibitor, PLX3397, to eliminate microglia in adult mice revealed no behavioral or cognitive impairments, indicating that microglia are not necessary in these tasks in adulthood (Elmore et al., 2014). Providing an additional explanation for the differences between these studies, one report indicated impairments in spatial memory with one week of PLX3397 treatment, which subsided with longer treatments (Torres et al., 2015). The transient impairment in cognitive function is presumably due to behavioral assessment occurring at a time point in which microglia are still in the process of dying. Importantly, multiple groups have utilized PLX3397, as well as the CSF1R-specific inhibitor PLX5622 to eliminate microglia (De et al., 2014; Valdearcos et al., 2014; Asai et al., 2015; Klein et al., 2015; Schreiner et al., 2015), without inducing adverse effects on cognitive function in adult mice (Dagher et al., 2015; Rice et al., 2015; Spangenberg et al., 2016).

3. AD pathophysiology

In contrast to the beneficial roles of microglia in the homeostatic brain, the activation of these cells is heavily implicated in the progression of several neurodegenerative diseases, including AD. AD is a progressive neurodegenerative disease characterized by the extracellular deposition of A β -associated plaques and intracellular tau-associated neurofibrillary tangles. It is also one of the most common forms of dementia, affecting roughly 10% of the population aged 65, and up to 50% of people aged 85 and over (Hebert et al., 2003), with the number of cases expected to triple by 2050 (Hebert et al., 2013). Therefore, understanding the mechanistic changes in the human brain leading to pathophysiology of the disease is essential to creating effective therapies. According to the amyloid cascade hypothesis, the triggering factor for the disease is the accumulation of amyloid aggregates composed of A β peptides (Hardy and Selkoe, 2002). The presence of aggregated A β initiates downstream effects, including sustained chronic neuroinflammation, tau hyperphosphorylation, and a loss of synapses and neurons that ultimately lead to considerable brain atrophy and cognitive decline. In human AD, evidence indicates that these amyloid deposits may present decades before the cognitive deficits associated with this disorder are evident (Mintun et al., 2006). Following amyloid deposition, the presence of intraneuronal neurofibrillary tangles comprised of hyperphosphorylated tau is observed in AD, and importantly, tau pathology more closely correlates with cognitive status, and neurodegeneration, than amyloid pathology (Nelson et al., 2009). Another critical facet of AD is chronic neuroinflammation, which is characterized by astro- and microgliosis

(Akiyama et al., 2000). Together, these neuropathological alterations exert toxic effects on the brain, leading to brain degeneration. Even in the mild cognitive impairment (MCI) phase of the disease, which is the intermediate stage between the expected cognitive decline associated with normal aging and dementia, there appears to be substantial accumulation of amyloid and tau, as well as neuronal loss (Morris and Price, 2001). These findings highlight the importance of treating the disease at the earliest stages in order to effectively control and limit the progression of AD.

4. Microglial contribution to AD pathogenesis

4.1. Microglial dysfunction and inflammation in AD

Over one hundred years ago, Alois Alzheimer first identified the presence of plaque-associated microglia in post-mortem brains (Alzheimer et al., 1995) and since then, activated microglia have been considered a key feature of AD pathology. In the AD brain, the number and size of microglia directly increase proportionally to the size of the plaques (Wegiel et al., 2001) and proliferate in the vicinity of plaques leading to the accumulation of these cells at amyloid deposits (Frautschy et al., 1998; Bornemann et al., 2001). Plaque-associated microglia have been shown to encircle plaques and it is postulated that the close proximity of microglia regulates plaque dynamics in AD mice (Condello et al., 2015). Recently, the emergence of inflammation-associated positron emission tomography (PET) imaging radioligands have allowed researchers to track inflammatory processes in humans with various diseases, including AD. Prospective studies utilizing PET ligands in AD patients found that microglial activation occurs well before clinical symptoms of cognitive decline present (Yasuno et al., 2012; Hamelin et al., 2016), highlighting the potential for immune-specific PET ligands as tools to monitor the inflammatory progression in a clinical setting for AD.

The hypothesis of compromised microglial function as a contributor to AD pathogenesis gained momentum following the publication of recent genome wide association (GWAS) studies. These studies identified several single nucleotide polymorphisms (SNPs) that convey risk of developing AD, with many of these SNPs associated with or related to microglial function (Malik et al., 2015) including *TREM2*, *CD33*, *CR1*, *CLU*, *CD2AP*, *EPHA1*, *ABCA7*, and *INPP5D* (Lambert et al., 2009; Hollingworth et al., 2011; Naj et al., 2011; Guerreiro et al., 2013; Lambert et al., 2013), indicating that microglia play a critical role in the development of AD. The generation of knockout mice for various immune-associated GWAS genes have allowed for *in vivo* investigations into the contribution of these genes in AD pathogenesis. Inactivation of *CD33* in APP/PS1 mice reduced A β accumulation and plaque burden (Griciuc et al., 2013), whereas *ABCA7* deficiency in APP/PS1 and TgCRND8 mice exacerbated A β load (Satoh et al., 2015; Sakae et al., 2016), highlighting the importance of these genes in regulating A β pathology. In contrast to *CD33* and *ABCA7*, attempts to define the exact function of *TREM2* in AD have been less clear. In APP/PS1 mice, removal of *TREM2* mitigated A β accumulation (Wang et al., 2015), whereas in 5xfAD mice, knockout of *TREM2* worsened A β pathology (Jay et al., 2015). In both studies, however, removal of *TREM2* signaling prevented the association of myeloid cells – whether it be microglia or monocytes – with plaques. A subsequent parabiosis study using

both AD models found no indication of infiltrated monocytes surrounding A β plaques (Wang et al., 2016), providing evidence that endogenous microglia are the source of plaque-associated myeloid cells. Importantly, loss of one (Yuan et al., 2016) or two copies of TREM2 (Wang et al., 2016) impaired the ability of microglia to compact amyloid into dense deposits, thereby increasing damage to local neuritic structures. Collectively, these studies indicate that TREM2 signaling regulates the microglial response to A β in AD, although whether this alteration in microglial function is beneficial or detrimental requires further experimentation. Furthermore, the role of inflammation in AD progression, as well as facilitating A β deposition, neuronal loss, and cognitive deficits is well described (see Fig. 1). Exposing microglia to A β induces the production and release of pro-inflammatory cytokines, including IL-1 β , IL-6, TNF α , TGF- β , as well as chemokines, such as macrophage inflammatory proteins (MIP)-1a, 1b, -2, and chemokine (C-C Motif) ligand 2 (CCL2) (Akiyama et al., 2000) in an attempt to facilitate the clearance of A β . However, microglia in AD are ineffective A β phagocytes, as evidenced by the continued presence of plaques surrounded by activated microglia in the AD brain, resulting in an enduring chronic neuroinflammatory environment for the duration of the disease. Furthermore, the sustained release of neurotoxic factors likely leads to neurodegeneration and many of these neurotoxic factors are evidenced to be microglia-derived, including TNF- α , NO, IL-1 β , and ROS (Lull and Block, 2010). Knockout of NLRP3 – a critical component in the inflammasome pathway and major contributor to neuroinflammatory insult in the CNS – in APP/PS1 mice mitigated inflammatory signaling, decreased deposition of A β , restored synaptic plasticity, and improved cognitive function as assessed by Morris water maze (Heneka et al., 2013). Importantly, the formation and secretion of the NLRP3 inflammasome is restricted to the microglial compartment in the mouse brain (Gustin et al., 2015), which together with the NLRP3 knockout data indicate that dysfunctional regulation of microglial NLRP3 signaling may underlie AD pathogenesis.

In addition to the general functions of these cells in AD, the timing of neuroinflammation in the progression of AD is also crucial to our understanding of the disease – recent evidence indicates that inflammatory changes occur before the appearance of amyloid plaques (Heneka et al., 2005; Ferretti et al., 2012; Wright et al., 2013). One study exploring early immune activation in AD revealed that inflammation alone can drive AD pathogenesis. Specifically, systemic immune stimulation in prenatal mice with a viral mimetic (i.e., double stranded RNA) followed by immune challenge later in adulthood, was sufficient to induce the development of sporadic-like AD, characterized by deposition of A β and tau proteins, impairments in working memory, as well as chronic microglial activation (Krstic et al., 2012). Interestingly, this study provides evidence for immune activation early in life as a sufficient factor to trigger the onset of AD. In addition to chronic microglial activation, the senescence of this cell type that normally ensures neuroprotection may underlie AD pathogenesis (Streit, 2002). Analysis of postmortem AD brains showed elevated expression of the microglial dystrophic marker, ferretin, as well as thinning and fragmentation of microglial processes (Lopes et al., 2008). Subsequent studies found that tau-positive neuronal structures were associated with dystrophic rather than chronically activated microglia, suggesting that microglial senescence, which results in diminished

neuroprotection, may contribute to the onset of AD through neurofibrillary degeneration (Streit et al., 2009).

Although gray matter is predominantly affected in AD, reports indicate that white matter from AD patients also displays considerable damage (Rose et al., 2000). It is proposed that axonal degeneration may occur in early AD (Terry, 1998), although the contribution of microglia in white matter damage remains to be clarified. Assessment of cerebrospinal fluid (CSF) markers and regional microstructure from asymptomatic adults found that the neuroinflammatory markers were associated with markers of axonal damage and altered microstructure on diffusion tensor imaging (DTI) (Melah et al., 2016), suggesting that neuroinflammation worsens white matter damage in preclinical AD. However, PET imaging with the second generation TPSO ligand DPA-714 in early stage and prodromal AD patients showed that patients with increased ligand binding declined slower, suggesting that microglial activation may be protective in the early stages of the disease (Hamelin et al., 2016). Collectively, these studies highlight the importance of inflammation-targeted therapeutic approaches for treating this disease, but caution that timing may be critical.

4.2. Microglial regulation of A β dynamics

Traditionally, it was believed that microglia respond to the presence of A β deposits by clearing them from the brain via phagocytosis of A β -fibrils (Pan et al., 2011). *In vitro* studies have shown clear evidence that microglia are able to internalize and degrade A β aggregates (Paresce et al., 1997; Li et al., 2000; Koenigsnecht-Talboo and Landreth, 2005; Hellwig et al., 2015). However, there was no clear consensus *in vivo* as to whether microglia possess the capacity to clear A β , as some studies show internalization of A β to the lysosome of microglia (Bolmont et al., 2008), whereas others do not observe A β plaque clearance by these cells (Stalder et al., 2001; Meyer-Luehmann et al., 2008). In order to directly assess the roles of microglia in A β clearance and plaque dynamics, several methods for *in vivo* microglial ablation have been developed, which allows researchers to probe the roles of AD-associated microglia, and how they contribute to pathophysiology. For example, clodronate liposomes induce apoptosis in phagocytizing macrophages (Claassen et al., 1990). Using this method in P0 5xfAD mouse organotypic hippocampal slice cultures, microglia are depleted leading to the rapid accumulation of A β deposits, showing that microglia in development and/or in slice cultures actively clear A β (Hellwig et al., 2015). However, upon replenishment with juvenile microglia, A β clearance is restored resulting in fewer A β deposits. Notably, replenishment with adult 5xfAD microglia does not reduce plaque deposits, indicating that the phagocytic ability of microglia is quickly lost with age, somewhere between 1 and 6 months of age. In CD11b-HSVTK (TK) mice, the thymidine kinase of herpes simplex virus is expressed under the CD11b promoter. Thymidine kinase converts ganciclovir into cytotoxic kinases, leading to cell death. In the brain, microglia exclusively express CD11b, and are therefore inducibly ablated with this method, with current studies maintaining ablation for up to four weeks. Researchers crossed TK and AD mice to assess alterations in plaque dynamics with the elimination of microglia and found no changes in plaque load, A β levels, or dystrophic neuritic structures near plaques, in either young or aged animals (Grathwohl et al., 2009). As the absence of microglia had no impact

on A β dynamics, this suggests that aged microglia may not be actively phagocytosing and clearing plaques in the AD brain.

In addition to toxin models, microglia can also be eliminated via inhibition of the CSF1R. Knocking out the CSF1R (Ginhoux et al., 2010) or either of its ligands, CSF1 (Wegiel et al., 1998) and IL34 (Wang et al., 2012), results in robust decreases in microglial number, emphasizing the importance of this signaling pathway in myeloid cell development. Our lab discovered that pharmacological inhibition of the CSF1R in adult mice led to the rapid elimination of microglia from the entire CNS. With seven days of treatment, >95% of microglia can be eliminated with the drug PLX3397, and microglia remain eliminated for as long as treatment is continued (Elmore et al., 2014). This provides a non-invasive approach to study microglial dynamics in any mouse model. Importantly, treatment with PLX3397 induces microglial cell death, as opposed to a downregulation of microglial-related genes (Spangenberg et al., 2016). CSF1R inhibitors can be formulated in chow and consumed by mice *ad-libitum*, while also maintaining blood-brain barrier (BBB) integrity. Thus, this provides a non-invasive context in which to study microglial dynamics in the brain, in both health and disease. Consistent with the prior data (Grathwohl et al., 2009), elimination of microglia from either aged or pre-pathological 5xfAD mice had no effects on A β accumulation or deposition (Spangenberg et al., 2016), confirming that microglia are not serving to remove amyloid from the brain. Notably, we found evidence of heterogeneity of myeloid cell populations associated with plaques, as ~50% of plaque-associated myeloid cells resistant to elimination with CSF1R inhibitor treatment. These surviving cells may be infiltrated monocytes, which are not dependent on CSF1R signaling for their survival (Jay et al., 2015), or may alternatively upregulate TREM2, which can act as a survival signal in place of CSF1R (Wang et al., 2015).

In accordance with these findings, recent evidence suggests that plaque-associated microglia *in vivo* are in a suppressed phagocytic state due to the overproduction of IL-10 (Chakrabarty et al., 2015), prostaglandin E2 (PGE2) (Johansson et al., 2015), and arginase-1 (Kan et al., 2015). Indeed, subsequent exposure of microglia to certain stimuli, including LPS (DiCarlo et al., 2001), IL-1 β (Shaftel et al., 2007), IL-33 (Fu et al., 2016), as well as the retinoid X receptor agonist bexarotene (Cramer et al., 2012) and ultrasound (Leinenga and Götz, 2015) are sufficient to induce microglial phagocytosis of A β . Together, these studies indicate that microglia are fully capable of A β phagocytosis, given a favorable environment in which to do so. Additionally, in a transgenic model of A β arrest, switching off the APP transgene halted the progression of A β pathology, but did not induce the breakdown or clearance of plaques (Jankowsky et al., 2005), providing further evidence that microglia are not regulating A β dynamics in the AD brain. Although microglial association with plaques is pervasive in different murine models of AD, as well as AD patients, the function of this association was previously unknown. Using *in vivo* methods to examine the role of microglia in regulating plaque dynamics revealed that microglia constitute a barrier surrounding amyloid deposits, serving to limit their outward expansion. Moreover, plaque-associated microglia limited the toxic effects of A β 42 hotspots on nearby neurons (Condello et al., 2015). As smaller (and presumably newer) plaques were most restricted in size, these data provide evidence for a neuroprotective role of the microglial barrier early in the disease to shield neurons from toxic species of A β , and perhaps with age, this method of plaque

restriction loses its effectiveness. Recently, it was reported that TREM2-deficient (Wang et al., 2016) and β -haploinsufficient (Yuan et al., 2016) mice display greater plaque diffusion and damage to nearby neuronal structures by modulating the microglial response, indicating that TREM2 signaling is crucial for the compaction of amyloid plaques, as well as limiting their toxicity to nearby neurons. Although these studies may appear to contradict data published by our lab, it is important to note that thorough A β plaque analysis in microglia-devoid and -intact animals was performed only in aged 5xfAD mice, whereby the ability of microglia to compact amyloid into dense plaques is presumably lost. Moreover, and perhaps more importantly, microglia-association with plaques and their ability to restrict plaque growth is perturbed throughout the entire lifetime of TREM2-deficient mice. With PLX3397-treatment, plaque-associated microglia are reduced only during treatment with the CSF1R inhibitor; therefore, earlier and longer treatment paradigms may allow for the detection of alterations in plaque compaction with the absence of microglia in AD mice, and may be informative as to how aging modulates microglial function to facilitate AD pathologies.

Collectively, numerous studies implicate microglial reactivity in AD pathogenesis, but the current consensus is that these cells are not key regulators of A β /plaque levels *in vivo*. Thus, further experimentation is needed to determine what effect these cells have in the disease and how GWAS identified myeloid gene changes influence the risk for AD.

5. Impacts of chronically reactive microglia in the AD brain

5.1 Cognitive dysfunction mediated by synaptic and neuronal loss

Modulation of microglial function in AD has been repeatedly shown to improve cognitive function (i.e. (Parachikova et al., 2010; Yamanaka et al., 2012)), indicating that chronically reactive microglia are promoting the cognitive decline that occurs in the disease. In accordance with this, chronically eliminating microglia in 5xfAD mice improved hippocampal dependent-memory, as assessed by contextual fear conditioning (Spangenberg et al., 2016). Notably, synapse degeneration is the best pathological correlate of cognitive decline in AD (Scheff et al., 2006), raising the question of whether microglia continue to prune synapses in adulthood and if this process goes askew in AD, resulting in the pathological stripping of synapses in the brain and, potentially, impairments in cognitive function. We recently reported a 35% increase in number of synapse-bearing dendritic spines in healthy, adult mice devoid of microglia compared to microglia-intact mice (Rice et al., 2015), suggesting that these cells continue to sculpt the synaptic landscape into adulthood. Subsequent examination into the role of microglia in mediating pathological synaptic stripping in 5xfAD mice revealed a significant loss of total dendritic spine density, and in 5xfAD microglia-devoid mice dendritic spine analysis showed a significant increase in total dendritic spine densities, particularly in mushroom and thin spines (Spangenberg et al., 2016). These data indicate that microglia are stripping dendritic spines in the AD brain, and mechanistically, microglial phagocytosis of synaptic material in the AD brain was recently reported as being mediated by the complement system (Hong et al., 2016). As synapse degeneration is the best pathological correlate of cognitive deficits in AD, preventing the loss of dendritic spines or allowing lost spines to regenerate through modulating microglial function may be an important area to pursue for AD therapeutics.

Importantly, the 5xfAD mouse model is the only model of AD to date that exhibits profound neuronal loss, specifically in the subiculum and layer V cortex (Eimer and Vassar, 2013), permitting inquiries into the role of chronically activated microglia in promoting the loss of neurons. We reported a 25% loss of subiculum neurons in these mice, and the neuronal loss was entirely prevented with the elimination of microglia (Spangenberg et al., 2016). Moreover, studies investigating the neurotoxic roles of microglia have revealed that knockout of CX3CR1, which is myeloid/microglia-expressed, prevents neuronal loss in 3xTg-AD mice (Fuhrmann et al., 2010), again suggesting that these cells are critically involved in the loss of neurons that occurs in AD. CX3CR1-deficient mice did not show any differences in soluble or insoluble A β levels, indicating that this signaling pathway is either not affected or not involved in the phagocytosis of A β , while also uncoupling A β /plaque pathology from neuronal loss. Collectively, these studies indicate that chronically activated microglia in the AD brain are major contributors to pathological synaptic stripping and neuronal loss, both of which likely underlie deficits in cognitive function.

5.2 Intervention in AD with CSF1R inhibitors that modulate microglial function

As prolonged treatment with high-doses of CSF1R inhibitors may not be a viable option for the treatment of AD, we have conducted studies to explore the effects of lower, clinically-relevant doses of CSF1R inhibitors on AD pathologies (Dagher et al., 2015). Importantly, lower doses modulate microglial function without eliminating all microglia from the brain. We identified a dose of PLX5622 that had minimal effects on microglial numbers in the brain (up to a 30% sustained overall reduction). This dose in 3xTg-AD mice, an AD transgenic model, administered just prior to the initial plaque forming period for 6 or 12 weeks, improved hippocampal dependent memory. Investigations into possible mechanisms underlying this improvement in cognition revealed no changes in amyloid pathology, but a complete prevention of the association of myeloid cells/microglia with plaques. These findings have been confirmed with a different CSF1R inhibitor, GW2580, in APP/PS1 mice, which reduced the number of plaque-associated microglia, normalized behavioral impairments, and recovered dendritic spine density (Olmos-Alonso et al., 2016). Collectively, modulating CSF1R signaling appears to affect both chemotaxis and proliferation, thus preventing the myeloid cells/microglia from migrating to and reacting to the plaques. Again, preventing microglia from associating with plaques did not affect the number of plaques or A β levels, supporting the argument that these cells do not clear plaque derived A β from the brain. Curiously, CSF1R inhibition results in a phenotype that recapitulates that seen in TREM2-deficient mice – a lack of plaque-associated myeloid cells (Jay et al., 2015; Wang et al., 2015; Wang et al., 2016), further suggesting a relationship between CSF1R and TREM2.

6. Contribution of peripheral myeloid cells in A β clearance

In certain murine models, such as repeated social defeat and irradiation conditioning in AD, peripheral myeloid cells are capable of crossing the BBB to mediate differential effects in the brain (Mildner et al., 2007; Wohleb et al., 2013). It is theorized that peripherally-derived myeloid cells possess a greater capacity to phagocytose amyloid than endogenous microglia, as reducing their association with plaques (Jay et al., 2015) or restricting the infiltration of

myeloid cells into the brain (Mildner et al., 2011) promotes AD-like pathology, although the latter found reductions in cerebrovascular A β load while parenchymal A β levels remained unchanged. In line with this, increasing the recruitment of myeloid cells in the AD brain, through the specific overexpression of TGF β in peripheral populations, enhances the removal of A β deposits (Town et al., 2008). Therefore, understanding the roles of peripheral myeloid cells in A β clearance is crucial, as these cells may provide some therapeutic benefit to AD. To that end, researchers depleted microglia from APPPS1/TK mice and allowed for repopulation to occur following the cessation of ganciclovir treatment. Analysis of CNS cells evidenced a substantial portion of these cells to be peripherally-derived myeloid cells, thereby allowing researchers to address the role of these cells in A β clearance. Assessment of the monocyte-repopulated brain revealed that the number of A β deposits was unchanged (Prokop et al., 2015). In an analogous study published at the same time, a different research group used the same APPPS1/TK mice, as well as APP23/TK mice, to repopulate the brain with peripheral myeloid cells and also found that amyloid burden in the brain was unchanged in both APP transgenic models (Varvel et al., 2015). In contrast to the previous study, repopulation was allowed to occur for up to six months in these mice, and even long-term exposure of amyloid to peripheral myeloid cells did not impact amyloid dynamics. Together, these studies indicate that the origin of myeloid cells does not necessarily govern their functionality in AD, and rather, the brain environment dictates the behavior of myeloid cells in the AD brain, as the repopulated peripheral myeloid cells inherited a similar phenotype to that of endogenous microglia. As it is generally believed that BBB breakdown in AD patients and animal models accelerates disease onset, and may also provide a route for potential therapies into the brain, researchers sought to assess BBB integrity in patients with AD and mouse models of AD. Analysis of BBB antibody permeability showed no differences between transgenic (e.g., hTau P301L, ApoEKO and ApoEKI, PS2-APP, etc.) and wildtype mice (Bien-Ly et al., 2015), and in humans, no differences in brain farct percentage or volume were observed between AD patients and controls. This indicates that in AD, BBB disruption is not overtly observed, diminishing the likelihood of infiltration of peripheral myeloid cells in AD. Altogether, these data suggest that peripheral myeloid populations are not largely contributing to the maintenance of amyloid pathology, even in circumstances in which these cells can cross the BBB.

7. Microglial maintenance of tau pathology

In the healthy brain, tau proteins are abundant in the CNS and function to stabilize microtubules in axons. However, in a disease state, such as AD, the binding of tau proteins to microtubules is interrupted, leading to high levels of free tau which is ultimately converted to aggregated and fibrillized tau (Kuret et al., 2005). Microglial activation is implicated as a driver of this pathological change, as it was shown to precede the accumulation of neurofibrillary tangles in a tauopathy mouse model (PS301S) (Yoshiyama et al., 2007). In a study investigating the relationship between microglia-induced inflammation and neurofibrillary tangles, researchers found that a deficiency in CX3CR1 in a humanized tau (hTau) mouse model altered microglial activation and increased levels of phosphorylated tau protein, resulting in behavioral impairments (Bhaskar et al., 2010). Importantly, this provides clear evidence that microglial activation plays a direct role in

accelerating tau pathology. Further studies in CX3CR1-deficient hTau mice found that microglial activation precedes the spreading of tau pathology and is correlated with the propagation of tau protein from the CA1 to subiculum (Maphis et al., 2015). The transfer of microglia from CX3CR1-deficient hTau mice into non-transgenic recipient mice found that reactive microglia are sufficient to induce tau hyperphosphorylation (Maphis et al., 2015), providing perhaps the most compelling evidence for microglial activation in driving tau pathology. Interestingly, tau protein has been identified in exosomes from CSF samples of AD patients (Saman et al., 2012) and is elevated in AD (Fiandaca et al., 2015), suggesting that secretion of phosphorylated tau via exosomes may play a role in tau-associated neurodegeneration. As microglia have secretory properties, the focus shifted towards these cells in mediating the spreading of tau in the brain. Moreover, in human brains, the nonsynaptic spread of tau pathology is often observed, and the mechanism by which this happened remained elusive. In an AAV-based, rapid tau propagation model, it was demonstrated that the elimination of microglia, using the CSF1R inhibitor PLX3397 as described earlier, halted the propagation of tau. Furthermore, inhibiting exosome synthesis in primary microglia revealed a reduction in transmission of tau from microglia to neurons in culture (Asai et al., 2015), indicating a necessity for microglia in the propagation of tau protein. However, the method of microglial phagocytosis of tau from neurons remains undetermined, although the authors propose that it may occur a phagoptosis-related mechanism. Altogether, these studies point to the activation of microglia as a driver of tau deposition and implicate these cells in spreading pathological tau protein in the brain. Therefore, microglia-targeted therapies may prove beneficial to suppress tau pathologies in AD, and other tau-related neurodegenerative disorders.

8. Anti-inflammatories to alleviate AD

Increasing evidence indicates that inflammation is a major element in the promotion of progressive CNS damage. Approaches to mitigate microglia-mediated neuroinflammation in neurodegenerative disease have long been developed, with PPAR γ agonists, statins, flavonoids, COX2 inhibitors, minocycline, and glatiramer acetate showing positive effects on mouse models of AD or patients enrolled in clinical trials (Zipp and Aktas, 2006). Delivery of an IL-1 receptor antagonist via injection of neural precursor cells in AD mice rescued spatial and contextual learning impairments and restored neurogenesis deficits, suggesting that blocking pro-inflammatory signaling may ameliorate cognitive outcomes in AD (Ben-Menachem-Zidon et al., 2014). Additionally, the administration of bexarotene, an RXR agonist which exerts anti-inflammatory effects, to APP/PS1 mice revealed stark reductions in A β levels and plaque burden, as well as restoration of learning and memory function, as assessed by contextual fear conditioning (Cramer et al., 2012). This compound proceeded to clinical trials, in which four weeks of treatment of bexarotene did not reduce brain amyloid in AD patients, nor did it improve cognitive function (Cummings et al., 2016). However, in AD patients with the ApoE4 allele, bexarotene treatment slightly lowered amyloid levels, hinting that the drug may help clear amyloid in an APOE isoform-dependent manner. Moreover, findings from epidemiological studies indicate that chronic intake of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced incidence of AD (Zandi et al., 2002), and additionally, the administration of certain NSAIDs to AD

mouse models reduced A β plaque load (Prokop et al., 2013). Although treatment with NSAIDs has failed to slow cognitive decline in patients with mild to moderate AD (de Jong et al., 2008; Green et al., 2009) and has failed to prevent AD in prospective clinical trials of aged subjects (Group et al., 2007), there may still be value in pursuing this as a preventative AD therapeutic if intervention occurs well before disease onset.

9. Acute inflammation mediated by microglia

Although it is accepted that chronic neuroinflammation is detrimental to the brain, acute inflammation may serve an important function for tissue repair and angiogenesis (Varin and Gordon, 2009). Therefore, the timing of microglial modulation is critical, as these cells can have both harmful and beneficial effects in the brain during inflammatory events. In a genetic hippocampal lesion model, we sought to determine the effects of eliminating microglia during different phases of a neuronal lesion. Importantly, this model has relevance to TBI, stroke, hippocampal sclerosis, and AD, as these diseases feature extensive neuronal loss and chronic microglial reactivity. Notably, the elimination of microglia for 4 weeks immediately following the lesion promoted functional recovery, whilst the elimination of microglia during and following the lesion promoted further neuronal loss, suggesting that microglia are beneficial during the acute phase of an injury (Rice et al., 2015). Importantly, these findings highlight the diverse roles of microglia in the brain during different phases of an insult. In line with this, microglia have been reported to serve beneficial functions in acute damage to the BBB via a localized laser lesion (Nimmerjahn et al., 2005). Importantly, the severity of the lesion dictated the number of microglia responding to the injury, and the accumulation of microglial processes in the lesion vicinity served to shield the injured area, demonstrating a neuroprotective role of microglia that is dependent on injury severity. Collectively, these studies emphasize the complexity of microglia biology, and that careful consideration needs to be given when developing/employing microglia-targeted interventions to the phase and severity of the injury or disease.

10. Conclusions – Net impact of microglia in AD

Together, much remains to be determined about the role of microglia in AD. In development, these cells serve to shape neuronal connectivity through refinement of extraneuronal synapses to establish a functional network, and importantly, microglia continue to sculpt the synaptic landscape throughout adulthood. However, the ways in which their function changes in AD and to what extent requires further experimentation. Whether microglia are exerting neuroprotective or neurodegenerative effects in the AD brain largely depends on the severity/stage of the disease, with some evidence indicating beneficial roles of microglial activation in early AD (Condello et al., 2015; Hamelin et al., 2016). Moreover, whether these cells are phagocytosing amyloid in the human AD brain remains uncertain. Is A β phagocytosis a microglial function that goes awry with age or are these cells simply not involved in the clearance of amyloid? If microglia are phagocytosing A β , do these cells contribute to the spreading of amyloid throughout the brain in an exosome-related manner, as has been observed with tau protein? Current studies implicate the spreading of amyloid to involve exosome trafficking (Nath et al., 2012), but whether microglia are propagating this spread is unclear. Furthermore, teasing out the effects of peripheral versus endogenous

myeloid cells in the regulation of amyloid requires additional study. To date, distinguishing myeloid cell populations (resident versus infiltrating) in the brain is challenging due to shared expression of marker proteins, and so, identification of unique cell signatures will be necessary to determine if and/or which population of myeloid cells are involved in amyloid phagocytosis. Here, we argue that microglia are not involved in the phagocytosis of amyloid in the AD brain, unless provided with sufficient stimulation, and thus, identifying signaling molecules/pathways that revert microglia back into a phagocytic phenotype may prove beneficial in AD.

Several studies assessing cognitive function of AD patients with mini mental status scores found that the number of activated microglia is highly correlated with cognitive decline (Cagnin et al., 2001; Versijpt et al., 2003; Edison et al., 2008). Possibly, microglia become problematic in AD and other neurodegenerative diseases due to excessive proliferation, and therefore, the supranumerous microglia may be contributing to the pathophysiology of the disease. In this way, possible therapies that target the excess number of microglia could prove beneficial for patients with AD. Regardless of what effects these cells are exerting in the AD brain, one point is clear – therapeutic intervention at late stages of AD has little hope of being effective, as lost neurons are incapable of regeneration, and rather, successful therapies likely require intervention during preclinical stages of AD. Collectively, microglia appear to become dysfunctional in AD, whereby these cells exert detrimental effects in the brain, and thus, developing ways to harness and mitigate microglia-mediated effects in the brain may provide the key to creating effective therapies for AD.

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Highlights

- Genetic and pharmacological manipulations of CSF1R signaling allow for investigations into microglial function in AD
- Chronically activated microglia promote tau pathology, synaptic stripping, neuronal loss, and cognitive decline in AD, but do not regulate A β dynamics
- Peripheral myeloid cell populations are not largely involved in the maintenance of amyloid pathology
- Targeting microglia-mediated effects may highlight important therapeutic avenues for AD

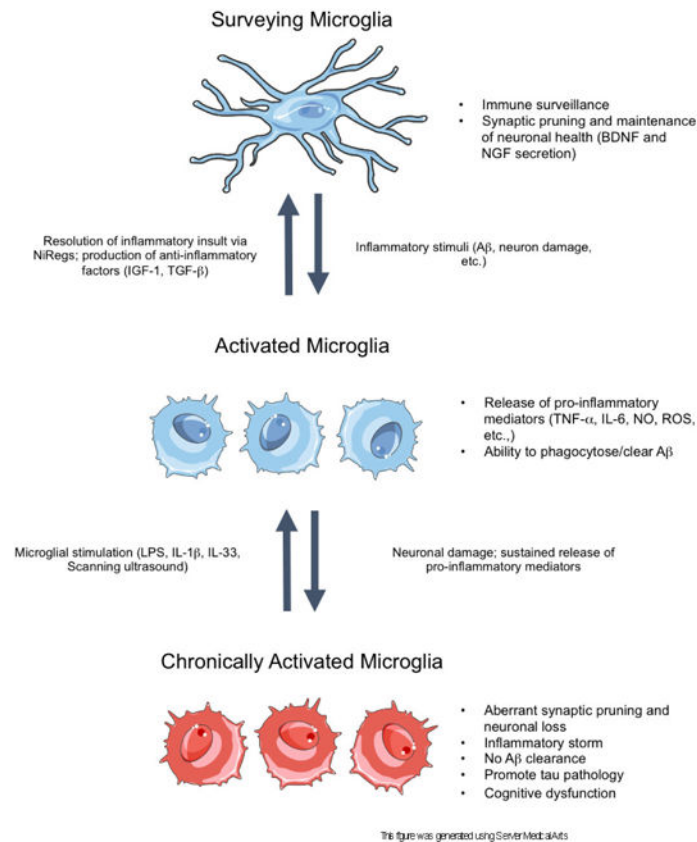


Figure 1.

A schematic of acute and chronic microglial activation in the context of Alzheimer's disease. In the surveillance state, microglia sample the brain parenchyma to detect anomalous materials, while concomitantly secreting anti-inflammatory factors and pruning unnecessary or weak synapses to support neuronal health. Disturbances in brain homeostasis, such as detection of A β , result in the activation of microglia. In the activated state, microglia release pro-inflammatory mediators to recruit other cells to the insult and to phagocytose the detected insult. When the insult is cleared, the inflammatory response resolves via NiReg (Neuroimmune regulatory proteins) activity, and microglia secrete anti-inflammatory factors to promote tissue repair and growth. However, in neurodegenerative diseases, the sustained release of pro-inflammatory mediators, as well as increasing neuronal death, drives the cells into a state of chronic activation. In this state, the homeostatic functioning of microglia is lost, resulting in an accumulation of aggregated proteins, the stripping of synapses from neurons, as well as further neuronal loss. Importantly, in this reactive state, microglia can be stimulated with certain proteins or methods to induce microglial phagocytosis of A β , thereby restoring the phagocytic ability of microglia.