Published in final edited form as:
*CNS Neurol Disord Drug Targets*. 2010 April ; 9(2): 156–167.

# Microglia and Inflammation in Alzheimer's Disease

**Shweta Mandrekar** and **Gary E. Landreth***
Alzheimer's Research Laboratory, Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106, USA

## Abstract

One hundred and fifty years have elapsed since the original discovery of the microglial cell by Virchow. While this cell type has been well studied, the role of microglia in the pathology of many central nervous system diseases still remains enigmatic. It is widely accepted that microglial-mediated inflammation contributes to the progression of Alzheimer's disease (AD); however, the precise mechanisms through which these cells contribute to AD-related inflammation remains to be elucidated. In the AD brain, microglial cells are found in close association with amyloid β (Aβ) deposits. Histological examination of AD brains as well as cell culture studies have shown that the interaction of microglia with fibrillar Aβ leads to their phenotypic activation. The conversion of these cells into a classically 'activated' phenotype results in production of chemokines, neurotoxic cytokines and reactive oxygen and nitrogen species that are deleterious to the CNS. However, microglia also exert a neuroprotective role through their ability to phagocytose Aβ particles and clear soluble forms of Aβ. These cells have been documented to play integral roles in tissue repair and inflammation, and in recent years it has been appreciated that this cell type is capable of facilitating a more complex response to pathogens by changing their activation status. A variety of new findings indicate that their role in the central nervous system is far more complex than previously appreciated. In this review we discuss the role of microglia in the normal brain and their phenotypic heterogeneity and how this may play a role in AD-related pathophysiology. We touch on what is known about their ability to recognize and clear Aβ peptides as well as more controversial topics, including various activation states of microglia and the ability of peripheral macrophages or monocytes to infiltrate the brain.

### Keywords

Alternative activation; Alzheimer's disease; amyloid-β; classical activation; inflammation; microglia

## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss and dementia. The key pathological hallmarks of AD include the deposition of beta amyloid (Aβ) plaques within the brain parenchyma and neurofibrillary tangles. Analysis of inherited forms of the disease has demonstrated the centrality of Aβ homeostasis in AD pathogenesis [1]. Aβ is generated by the sequential proteolytic processing of the amyloid precursor protein (APP), resulting in the formation of peptides 40 to 42 amino acids in length. These soluble peptides spontaneously aggregate to form Aβ oligomers and fibrils



*Address correspondence to this author at the Alzheimer Research Laboratory, Department of Neurosciences, School of Medicine, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106, USA; Tel: 216 368-6101; Fax: 216 368-4650; gel2@case.edu.

that are subsequently deposited within the brain to form both diffuse and dense core amyloid plaques.

Microglial cells, the primary immune effector cells of the brain, play an integral role in maintaining brain homeostasis and protecting the brain from infections and insults. In the AD brain, microglial cells are observed to be phenotypically activated and form intimate associations with amyloid deposits, extending their processes into the plaque core [2, 3]. Microglia are able to recognize and mount an immune response to Aβ peptides as well as migrate to areas of amyloid deposition, however, despite their ability to physically interact with these deposits they are unable to clear plaques from the brain. The role of microglia in the uptake and phagocytosis of amyloid in the brain was first noted in the early 90's by Wisniewski and Frackowiak who utilized electron microscopy to visualize amyloid internalization [4, 5]. They postulated that resident microglial cells associated with plaques in the AD brain played a role in the formation of the amyloid fibrils in the brain, but not its phagocytosis [4]. However, in rare cases in which AD patients suffered from stroke they were able to visualize macrophages that had internalized and trafficked amyloid deposits into lysosomal compartments [5]. This is the first study that hypothesized that peripherally-derived myeloid cells were more competent than endogenous microglia in the clearance of amyloid in the brain, an idea that is currently being re-examined in the field and will be discussed further in this review.

The deposition of amyloid in the parenchyma of the brain is associated with a robust inflammatory response [6]. It is thought that this chronic pro-inflammatory milieu produced by "activated" microglia is neurotoxic and facilitates neurodegeneration. It is now understood that the inflammatory response in AD is multifaceted with the role of microglia in disease progression being both complex and quite controversial. The paradox over whether they play a neuroprotective role in the AD brain or facilitate and contribute to the neurotoxicity observed in AD is a puzzle that is currently under active investigation.

## MICROGLIAL DYNAMICS IN A HEALTHY BRAIN

Microglia are the brain's tissue macrophage and thus the principal representative of the innate immune system in the central nervous system (CNS). It is widely accepted that microglia originate from peripherally-derived mesodermal progenitors, and invade the CNS during embryonic and fetal development, prior to formation of the blood brain barrier [7, 8]. They serve as the first line of defense in the brain and act to protect the CNS from injury and invading pathogens. They also play an important role in normal tissue maintenance by taking up and disposing cellular debris in the brain paranchyma.

In the mature CNS, microglia only account for 5% of the total glial population in the cerebral cortex [9, 10]. Despite their small number they play fundamental roles in maintaining brain homeostasis. In the healthy adult brain, microglia have a low turnover rate and comprise a stable population of cells [11]. Microglia are evenly dispersed at a density of approximately 6 microglia per cubic millimeter [12]. These cells maintain a ramified morphology and express a variety of cell surface markers typical of cells of this lineage, including CD11b, the lectin binding molecule F4/80, the calcium binding protein, Iba1 and MHC II [13, 14].

Originally it was thought that the CNS was populated by a functionally homogeneous population of microglia. However, recent studies have shown that microglia in the brain represent a more phenotypically diverse group of cells that readily react to cues from the extracellular environment by altering their activation status and their expression of phenotypic markers. These cells, like other tissue macrophages, respond to their local environment [15]. Thus, a microglial cell located in one area of the brain may be distinct

from that in another, owing to differences in the surrounding environment and the exposure to a different range of extracellular cues.

In recent years, ideas regarding the role of microglia in the healthy and diseased human brain have evolved dramatically. Conventionally, it was thought that microglia in normal healthy brain were “quiescent” and “resting,” however, it is now recognized that these cells are highly active and very dynamic. The emergence of two-photon microscopy has allowed for a more intimate view of living microglia in their native environment of the brain. Nimmerjahn *et al.* [16] have described microglial dynamics in a healthy brain. They utilized a transgenic mouse model expressing green fluorescent protein from the fractalkine locus and visualized microglial activity *in vivo.* They found that microglial cells continuously sample the extracellular environment by extending and retracting their processes with limited movement of their cell bodies. Furthermore, during this scanning process, microglia were shown to make repeated contact with astrocytes, neurons and the cerebral vasculature [12]. It was estimated that microglia survey the entire brain once every few hours [16]. Additionally, these cells were capable of readily detecting CNS injury and quickly migrating to sites of damage within the brain. In a study by Davalos *et al.* [12], microglia were shown to extend their processes to sites of laser-induced damage within a minute after injury. Following injury, neighboring microglial cells then migrated to the damaged site within 30 minutes of the insult and retracted processes that were not oriented towards the site of damage. It was concluded that astrocytic release of ATP and P2Y12 receptors were necessary for the chemotactic migration of microglial cells to the injury site [12, 17]. Significantly, resting microglia also made direct contact with neuronal synapses. These contacts were short lived, lasting no more than 5 minutes, were activity dependent and occurred on an hourly basis. After ischemic injury, microglia made more frequent and prolonged contact with these neuronal synapses (lasting approximately 1 hour) and resulted in the disappearance of presynaptic boutons [18]. Taken together, these studies suggest that microglia play a rather active and dynamic role in maintaining homeostasis in a healthy brain and can detect and respond efficiently to CNS injury.

## PLAQUE-ASSOCIATED MICROGLIAL DYNAMICS IN ALZHEIMER’S DISEASE

In the AD brain most reactive microglia are associated with dense-core plaques; however, a few are found in the vicinity of diffuse Aβ deposits [19–22]. Additionally, the number of microglia and their size directly increases in proportion to plaque dimension [23–25]. Microglia in the immediate vicinity of plaques also proliferate, allowing for the accumulation of these cells at the periphery of amyloid deposits [26, 27]. Plaque-associated microglia extend their processes and encompass Aβ deposits and through this association can regulate plaque dynamics in transgenic AD mouse models [2, 3]. This association of microglia with amyloid plaques in the brain is consistent in both transgenic animal models of the disease as well as human cases of AD [28, 29].

A recent study, Yan *et al.* [30] visualized plaque growth in an APP/pensenilin 1 (PS1) AD mouse model using serial *in vivo* multiphoton microscopy. Using this method they were able to visualize the growth of individual plaques over a period of time, and demonstrated that plaque growth was more extensive in younger transgenic animals when compared to older ones. Additionally, they found that smaller plaques grew at a greater rate in comparison to larger plaques. The authors also utilized an inhibitor of γ secretase, and found that treatment of transgenic animals with this compound halted the growth of pre-existing plaques, inhibited the appearance of new plaques, and decreased extracelluar levels of Aβ by 20–25% [30].

Using two-photon microscopy Meyer-Luehmann *et al.* [31] were able to identify and follow newly formed Aβ plaques in repeated imaging sessions. They reported that senile plaque formation was a rapid process, and could identify newly formed plaques within 24 hours of an imaging session, a finding which differs from those of Yan *et al.* [30] Microglia were recruited to these plaques within 1–2 days of their appearance. Despite the recruitment of microglial cells to the newly formed plaque, clearance of the amyloid deposits was not observed. Additionally, dystrophic neurites, displaying both dentritic and axonal abnormalities, appeared around the newly formed plaque area and coincided with microglial recruitment [31]. However, the contribution of microglia to the neuritic dystrophy was not addressed. Using a similar technique, Bolmont *et al.* [32] followed single plaques over a period of time, and demonstrated that microglia migrate to plaques while maintaining a highly dynamic interface between the plaques and glial cells. They documented that the migration of microglia to plaques occurred at a rate of about 3 microglia per plaque per month. In contrast to the Meyer-Luehmann *et al.* [31], Bolmont *et al.* [32] were able to observe the internalization and delivery of Aβ fragments to the lysosmomes of microglial cells. These newly developed techniques have proven to be critical our understanding of microglial behavior in the normal as well as diseased brain.

While the development of *in vivo* two-photon microscopy has illuminated our understanding of microglial activity in the mouse, recent advances in positron emission tomography-based technologies have now allowed for imaging of microglial activation in the living human brain as well. This is achieved by utilizing a carbon 11-labeled compound that recognize the mitochondrial protein TP-18 (also known as the peripheral benzodiazepine receptor) which is expressed at high levels by phenotypically activated microglial cells [33, 34]. It also expressed at lower levels in astrocytes [35, 36]. These studies have helped confirm that, in accordance with the literature, microglial phenotype changes in the diseased brain. Several groups were able to determine that the number of activated microglia in the cortex of AD patients was significantly correlated with cognitive decline as assessed using the mini mental status exam scores, rather than amyloid deposition [34, 37, 38]. However, a study by Wiley *et al.* [39] yielded contradictory results. The increase in number of activated microglial cells in aged AD mouse models has also been validated using this PET-based technique [33, 40]. Currently, studies using more sensitive TP-18 binding compounds are underway [41]. These studies support the idea that microglial cells are composed of a heterogeneous population that maintains the capacity to shift activation states in response to disease progression.

## MICROGLIA AND INFLAMMATION IN AD

An extensive literature documents that inflammation plays an integral role in progression of AD, facilitating Aβ deposition, neuronal loss and cognitive deficits [42]. The appearance of amyloid plaques in the brain coincides with a dramatic phenotypic activation of the surrounding microglia which display increased immunoreactivity for CD11b, CD68, complement receptor 3 and CD45 [43]. Postmortum brains from AD patients, as well as brains from APP transgenic animals, display increased levels of inflammatory cytokines as well as chemokines including interferon γ (IFNγ) and tumor necrosis factor α (TNFα), interleukin 1β (IL-1β), and interleukin 6 (IL-6) [6, 44–47]. IFNγ and TNFα not only have toxic effects on neurons but have also reduce levels of insulin degrading enzyme, a key Aβ degrading protease. This may be a secondary mechanism through which inflammation could increase amyloid deposition. Furthermore, both TNFα and INFγ have been shown to increase the production of Aβ from APP expressing cortical neurons as well as impair the ability of microglial cells to degrade Aβ [45].

The association of reactive microglia with amyloid deposits has been demonstrated *in vivo* and their ability to secrete a variety of pro-inflammatory cytokines, chemokines, reactive nitrogen and oxygen species as well as acute phase proteins in response to the fibrillar Aβ has been shown *in vitro* [37–40]. There is an extensive literature that documents the Aβ-induced production of a host of proinflammatory molecules and these findings have been recently reviewed [6, 48, 49]. Notably, microglial exposure to fibrillary forms of Aβ *in vitro* provoke the synthesis and secretion of pro-inflammatory cytokines, including IL-1β, IL-6, TNFα, transforming growth factor-β (TGF-β), a peptide growth factor and chemokines, macrophage inflammatory proteins -1α, -1β, -2, and most prominently CCL2/monocyte chemotactic protein 1 [6, 50, 51]. Aβ-stimulated microglia also produce reactive oxygen species (ROS) and reactive nitrogen species. These cells also elaborate a number of other immune mediators including macrophage colony stimulating factor and the complement protein, C1q [52].

The generation of mouse models lacking inflammatory signaling elements has provided insight into the role of inflammation in AD pathogenesis and Aβ clearance *in vivo*. AD transgenic mouse models lacking expression of either the IFNγ receptor type 1 or TNF type 1 receptor display significant decreases in amyloid deposition as well as microglial activation [45, 46]. Deletion of TNF type 1 receptor also alleviated Aβ-associated cognitive deficits [53]. However, we cannot conclude that inhibition of cytokine signaling promoted microglial clearance of Aβ in the animal models since both these studies documented decreases in beta-site APP-cleaving enzyme 1 activity as well. Since the deletion of these receptors is global it is difficult to assess their specific contribution in the inflammatory response and clearance of Aβ in microglia [53, 54].

A study by Shaftel *et al*. [55] took an alternative approach and examined the effects of sustained inflammatory signaling by over-expression of the pro-inflammatory cytokine IL-1β in astrocytes. To the surprise of the authors, sustained IL-1β activation, which was expected to have detrimental effects, resulted in a significant decrease in amyloid deposition in the hippocampus of these mice. They found that IL-1β over-expression led to an increase in the number of activated plaque-associated microglia that were heterogeneous in their phenotype and were positive for either Iba1 or both Iba1 and MHCII and this phenotypic diversity arose from the IL-1β-stimulated recruitment of blood-borne leukocytes into the brain [55]. Similarly, another study conducted by Chakrabarty *et al*. [56], in which the pro-inflammatory cytokine IL-6 was over-expressed in the brain of murine models of AD using an adenoviral vector, found that amyloid deposition was attenuated in these animals. IL-6 expression resulted in a dramatic increase in gliogenesis in these animals that was associated with enhanced microglial phagocytosis of Aβ, without affecting APP expression or processing [56]. From these studies we can conclude that while inflammation has been shown to be detrimental to the brain, some components of this system may be involved in amelioration of AD-related pathology.

## Intrinsic Mechanisms that Regulate Microglial Activation *In Vivo*

Although inflammation plays beneficial roles in pathogen removal, it can also be detrimental to the surrounding tissue and result in bystander damage if the response is not down-regulated. Thus, the CNS possesses intrinsic mechanisms to suppress microglial activation through cell-cell interactions between microglia and neurons as well as microglia and other glial cell types. One such example is the fractalkine receptor (CX3CR1) expressed on microglial cell surface. The ligand of this receptor, fractalkine (CX3CL1), is expressed on neurons and astrocytes. Interaction of the ligand with the receptor during normal microglial surveillance in the brain prevents microglial activation. It is thought that during neurodegeneration, this ligand-receptor interaction is lost, due to loss of neurons, and contributes to the activation of microglial cells [57]. In the Tg2576 mouse model of AD, the

number of fractalkine-positive cells in the cortex and hippocampal regions is half that of the wild-type control animals at 9 months of age [58].

Similarly, interaction between microglia and neurons *via* the CD200 receptor located on neurons and CD200 ligand expressed on microglial cells also inhibits microglial-mediated inflammation. Levels of both CD200 and CD200 receptor are reduced in the hippocampus and cortex of AD patients [59]. *In vitro,* CD200 interaction with its receptor can attenuate Aβ-induced glial activation [60]. The microglial transmembrane protein-tyrosine phosphatase CD45 also plays a similar role. Engagement of this receptor with its ligand, CD22, inhibits the production of pro-inflammatory cytokines by microglia in response to lipopolysaccharide [61]. CD22 is normally secreted by neurons.

Recent studies have shown that microglial cells can interact *via* the microglial triggering receptor expressed by myeloid cells 2 with an unidentified neuronal cell surface ligand to suppress microglial-mediated inflammatory cytokine production [62]. AD mouse models express increased levels of triggering receptor expressed by myeloid cells 2 in plaque-associated microglial cells [63]. Additionally, two other proteins, signal regulatory protein α expressed on myeloid cells, neurons and astroctyes, and CD47 expressed on microglia and neurons, can interact and signal bidirectionally to suppress expression of proinflammatory cytokines [64].

In contrast, the CD40 receptor on microglial cells participates in induction of an inflammatory response. Engagement of this receptor with the CD40 ligand leads to the production of TNFα and IL-1β [65]. Expression of CD40, as well as its ligand is increased around Aβ plaque deposits in the AD brain [66, 67]. Tg2576 animals deficient for CD40 ligand show a marked reduction in Aβ burden, microglial activation and astrogliosis [68]. Thus, while there are numerous intrinsic mechanisms employed to assure tight control of microglial activation, many of these signaling components are compromised in the AD brain.

## PHAGOCYTOSIS OF Aβ BY MICROGLIAL CELLS

One of the enigmatic features of AD is that while microglia are competent phagocytes, they are unable to efficiently clear amyloid deposits within the brain. Microglial cells clear soluble forms of the Aβ peptide by macropinocytosis and are capable of taking up fibrillar Aβ peptides through phagocytosis [69, 70]. Fibrillar forms of Aβ are recognized by a receptor complex, described in detail below, located on the microglial cell surface. Activation of this complex initiates an intracellular signaling cascade that engages the phagocytic machinery of the cell and results in the phagocytosis of fibrillar Aβ. Once internalized both soluble and fibrillar species of Aβ are delivered to the endolysosomal system [32, 71, 72].

While microglial cells possess the ability to take up fibrillar forms of Aβ its seems as though in the AD brain the microglia have a difficult time degrading these dense aggregates and suggests that disease progression may be paralleled by defective microglial clearance mechanisms. Paresce *et al.* [72] have shown *in vitro* that murine microglial cells are capable of phagocytosing Aβ; however, they observed that once internalized, the peptide can remain undegraded for over 72 hours. In another study it was shown that once microglia internalized Aβ they resecreted the undegraded peptide into the culture medium [73].

The conclusion that endogenous microglia are inefficient or unable to effect plaque clearance has been supported by a recent and provocative study by Grathwohl and colleagues [74]. These authors reported that they could selectively ablate endogenous microglia using a CD11b-driven thymindine kinase gene coupled with ganciclovir

administration to the brain of murine models of AD. They found that loss of microglia had no effect on plaque number or size over a 2–4 week period. However, the interpretation of these experiments is confounded by the morbidity and mortality that accompany ganciclovir treatment, the loss of other cell populations besides microglia, most notably pericytes, the loss of the integrity of the vasculature, and perturbation of normal homeostatic mechanisms in the brain. While this study was argued to undermine a role for microglia in AD pathogenesis, an alternate interpretation of these data is that it just reinforces the view that endogenous microglia do not normally act to efficiently remodel or clear amyloid deposits in the AD brain.

Many *in vitro* studies support the idea that an inflammatory environment negatively affects the capacity of microglial cells to engage in phagocytosis and clear fibrillar Aβ deposits [75, 76]. Yamamoto *et al*. [77] showed *in vitro* that wild-type microglia take up and degrade Aβ; however, they lost their capacity to degrade the peptide following inflammatory cytokine stimulation, resulting in the retention of $A\beta_{40}$ and $A\beta_{42}$ within the cells. Microglial cells isolated from APP/GRKO animals (where IFNγ signaling is disrupted), were able to take up Aβ and did not lose their capacity to degrade exogenously supplied Aβ in response to cytokine stimulation [54].

Koenigsknecht-Talboo *et al*. [75] have shown that inflammatory cytokine treatment inhibits the ability of microglial cells to phagocytose Aβ that occurs upon engagement of the fibrillar Aβ receptor complex; however, this treatment did not alter Fc-mediated phagocytosis by microglia. Indeed, in Aβ vaccination models, microglia can be stimulated to remove plaque through the activation of the FcR in the presence of an inflammatory environment [78]. Treatment of microglial cells with ibuprofen, known for its anti-inflammatory actions, rescues impairments in fibrillar Aβ-induced phagocytosis by microglia in response to a pro-inflammatory environment [75]. Treatment of microglial cells with anti-inflammatory agents such as non-steroidal anti-inflammatory drugs, liver X receptor agonists, peroxisome proliferator-activated receptor (PPAR)γ agonists and anti-inflammatory cytokines can reverse any phagocytic deficits incurred in response to inflammation [75–77, 79]. Similarly, a study conducted by Shie *et al*. [80] examined the effects of the E prostanoid receptor subtype 2 on the ability on microglia to mediate the phagocytosis of Aβ. Prostoglandin E2 is a product of cycloxygenases and can bind to a number of E prostanoid receptors. These authors found that E prostanoid receptor subtype 2 knockout microglia were more efficient at clearing Aβ peptides in solution as well as from AD brain sections [80, 81]. Together with the *in vitro* experiments, these studies suggest that inhibition of inflammatory signaling reduces microglial activation, enhancing their ability to mediate Aβ clearance.

In line with these studies, long term use of non-steroidal anti-inflammatory drugs, which inhibit cycloxygenases, have been shown to reduce the risk of AD as well as delay disease progression [82, 83]. Long-term treatment of transgenic AD mouse models with ibuprofen also reduces inflammation, ameliorates cognitive deficits, and decreases amyloid deposition [84–86]. These studies taken together suggest that inflammation may impact the ability of microglial cells in the brain to take up and degrade Aβ. Therapies geared towards ameliorating the inflammatory response in the AD brain may prove to be a beneficial in treating this disease.

## MICROGLIAL AB RECEPTOR COMPLEX

Microglial cells possess a wide variety of cell surface receptors that are capable of recognizing Aβ aggregates and initiating an inflammatory response. Some postulated cell surface Aβ receptors include receptor for advanced glycation endproducts, and the formyl

peptide receptor-like 1 protein [49, 54, 87–89]. However, the exact role of these receptors in the recognition of fibrillar Aβ by microglial cells remains controversial.

We have previously shown that fibrillar forms of Aβ can interact with a multicomponent receptor complex on microglial cells. This complex is composed of CD36 (a B-class scavenger receptor), the α6β1 integrin, the integrin associated protein CD47 and scavenger receptor A [71]. Engagement of this receptor complex with fibrillar Aβ leads to the activation of an intracellular signaling cascade, resulting in the phenotypic activation of microglia through NFκB-mediated gene transcription. Additionally, activation of this receptor complex results in increased phagocytosis, cytokine expression and production of ROS [70, 90, 91].

Many studies support the idea that this receptor complex plays an important role in AD pathogenesis. Microglia isolated from CD36 null animals exhibit decreased levels of cytokine and chemokine secretion, as well as ROS in response to exposure to fibrillar Aβ species [51]. A study by Hickman *et al.* [92] described a 2.5-fold increase in levels of IL-1β and TNFα mRNA in a PS1-APP AD mouse model with a parallel 2–5-fold decrease in mRNA levels of scavenger receptor A, CD36 and receptor for advanced glycation endproducts in comparison to their age-matched wild-type littermates. The fall in receptor levels was postulated to be due to the increase in levels of pro-inflammatory cytokines, as incubation of N9 microglial cells with TNFα decreased the expression of scavenger receptor A and CD36 and reduced Aβ uptake by cultured microglial cells [92].

Additionally, we have recently reported that the innate immune toll-like receptors (TLRs), and the coreceptor CD14, function as integral members of this fibrillar Aβ receptor complex [93]. Stimulation of both TLR2 and TLR4 by fibrillar Aβ was required for microglial activation [93]. TLR2, TLR4 and its coreceptor CD14 are necessary to mediate and activate intracellular signaling in response to fibrillar Aβ. Cells deficient in any of these proteins fail to initiate Src-Vav-Rac signaling, resulting in reduced levels of ROS and impaired phagocytosis in response to fibrillar Aβ stimulation. These cells also failed to induce phophorylation of IκBα after fibrillar Aβ stimulation. The data provide strong evidence that TLR2, TLR4 and CD14 function as additional members of the microglial fibrillar Aβ receptor complex we have previously described [93]. These studies demonstrate the recognition of Aβ at the cell surface occurs through the association of immune receptors into a multi-subunit receptor complex that is critical in engaging the phagocytic machinery of the microglial cell as well as inducing the classical activation of microglial cells.

## The Role of TLRs in Inflammation and Aβ Clearance

The innate immune system plays a key role in the discrimination between self and non-self. Thus, the host defense system has evolved strategies to detect tissue damage and invading viral and microbial pathogens. Microglial cells are capable of initiating an immune response through the engagement of various pattern recognitions receptors (PRRs) expressed on their cell surface, including the TLRs. TLRs are specialized to recognize pathogen-associated molecular patterns that are conserved within a class of microbes. TLRs can also recognize danger-associated molecular patterns which are released after tissue damage. The TLRs comprise a family of 13 structurally similar receptors in mammals and are expressed on a variety of immune cells, including B cells, T cells, macrophages, monocytes and dendritic cells. Recognition of pathogen-associated molecular patterns and danger-associated molecular patterns by TLRs initiates signaling cascades resulting in the production of inflammatory cytokines, proteases and reactive oxygen and nitrogen species, which leads to the rapid and efficient clearance of these agents and the classical activation of microglia [94].

In the brain, TLRs are expressed mainly on microglia; however, recently it has been reported that a subset of TLRs are expressed on neurons and astrocytes [95, 96]. Microglia associated with Aβ deposits in AD brains as well as murine models of AD express elevated levels of TLR 2/4/5/7/9 mRNAs [97]. Similarly, the AD brain, as well as the brains transgenic murine models of AD, exhibit increased protein levels of CD14, TLR2 and TLR4 [98, 99]. TLR4, TLR2 and the coreceptor CD14, are of particular importance to AD due to their ability to recognize Aβ peptides and oligomers [98].

Both TLR2 and TLR4 activation have been linked to inflammation in the AD brain. Treatment of microglia with function blocking antibodies to TLR2 and TLR4 results in decreased production of the inflammatory cytokines, IL-6, TNFα and reactive nitrogen species in response to fibrillar Aβ stimulation. Neutralizing antibodies or genetic deficiencies in CD14 elicit a similar response [98]. Additionally, Walter *et al*. [100] have shown that media from Aβ-treated wild-type microglia have neurotoxic effects on neurons, while media from Aβ-primed TLR4- or CD14-deficient microglia are no longer toxic, suggesting that these receptors are necessary to mediate microglial neurotoxicity [100]. These data strongly suggest that in microglial cells the TLRs are involved in mediating an inflammatory response to Aβ.

The TLRs as well as the co-receptor CD14 have also been implicated in the uptake of Aβ by microglia. The interaction of CD14 with Aβ has been demonstrated through FLIM-based FRET and real time plasmon resonance spectroscopy [99]. CD14 co-localizes with fibrillar Aβ42 at the cell surface and is subsequently internalized and trafficked to the lysosomes. Deletion of CD14 results in decreased Aβ uptake by microglial cells in comparison to wild-type cells. Tahara *et al*. [101] have shown that activation of microglial TLR2 and TLR4 receptors results in increased Aβ phagocytosis. Moreover, both TLR2 and TLR4 are necessary for fibrillar Aβ stimulated phagocytosis [93]. These *in vitro* studies strongly implicate TLR2, TLR4 and CD14 in the phagocytosis of Aβ by microglial cells.

While a number of *in vitro* studies have shown that TLR activation promotes inflammation and may play a role in plaque clearance, *in vivo* studies examining the effect of genetic inactivation of the TLRs are simply confusing. APPswe/PSEN1ΔE9 mice (14 month old) expressing an inactive, mutated TLR4, displayed increased levels of both diffuse and dense core-amyloid plaques in both the hippocampus and cortex [101]. The authors suggest that TLR4 functions in microglia to mediate the clearance of Aβ and thus, the absence of functional TLR4 results in a dramatic change in Aβ load. In another study, peripheral injection of LPS, a TLR4 ligand, promoted microglial activation and ultimately resulted in the clearance of diffuse, but not compact, amyloid deposits [102]. Studies conducted by Herber *et al*. [43] demonstrated a similar result. Together these studies support a role for TLR4 in microglia activation and promotion of plaque removal. However, a study by Sheng *et al*. [103] reported contradictory results; they demonstrated that lipopolysaccharide treatment increased overall Aβ peptide levels.

A different result was reported on examination of TLR2 knockout animals crossed with the APPswe/PSEN1dE9 AD mouse model, and revealed delayed Aβ deposition and enhanced cognitive decline at 3 and 6 months of age [104]. However, at 9 months of age these animals exhibit comparable levels of amyloid deposition to their wild-type littermates. Thus, the role of TLRs in AD-related pathophysiology remains unclear. These differences in Aβ deposition between the TLR2 and TLR4 deficient mouse models may possibly be attributed to the age of analysis.

A recent study has also described the possible role of another toll-like receptor, TLR9, in Aβ clearance. TLR9 is located in endosomal compartments within the cell and recognizes CpG

DNA from viruses and bacteria that have been internalized [105]. Activation of TLR9 by CpG ODNs (unmethylated cytosine-guanosineoligodeoxynucleotides) induces phagocytosis in murine microglial cells *in vitro* [106]. The study by Scholtzova *et al*. [107] stimulated TLR9 by intraperitoneal injection of CpG ODN. These mice displayed a 66% reduction in cortical amyloid burden as well as an 80% reduction in vascular amyloid deposition. Treatment with CpG ODN also rescued behavioral deficits seen in the radial arm maze. Since CpG ODNs are unable to penetrate the blood-brain barrier, it is speculated that the effects of TLR9 activation were on peripheral macrophages which then infiltrated the brain [107].

## The Role of the Complement System in Microglial-Mediated Aβ Clearance

The complement system provides another line of defense to invading microbes and pathogens. In the CNS both neurons and glia are capable of making and secreting proteins involved in the complement cascade. This cascade results in the formation of a membrane attack complex and the opsonization of pathogens resulting in their clearance *via* complement receptors. Complement pathway activation can occur through three distinct mechanisms: the classical pathway, the alternative pathway and the lectin pathway. These cascades are quite complex and are comprised of over 40 proteins [108]. In the postmortem AD brain, complement proteins from both the alternative and classical pathways are found in close association with Aβ deposits and neurons expressing neurofibrillary tangles [109, 110]. *In vitro* studies have also shown that β-amyloid fibrils can activate both the classical and alternative pathways *via* interactions with C1q and C3, respectively [111–113]. Thus, accumulation of complement factors such as C1q, mannose binding lectin and C3 are thought to result in a local inflammatory response in the brain and contribute to AD progression.

Complement activation can also result in the recruitment of microglia to amyloid plaques and the release of inflammatory signaling proteins. Activation of this pathway has been associated with Aβ clearance. Supporting this idea, *in vivo* mouse models have been established to examine the role of the complement system in AD. Inhibition of C3 by over-expression of soluble complement receptor related protein y, an inhibitor of complement C3, resulted in a 2-to-3-fold increase in Aβ deposition as well as increased neurodegeneration at 10–12 months of age [114]. Similarly, C3 deficiency in an APP transgenic mouse model did not result in significant neuropathological differences in younger transgenic animals between the ages of 8–12 months. However, the authors did find that older mice (17 month old animals) had a 2-fold increase in plaque burden, elevated Aβ plasma levels, and a loss of hippocampal neurons. This study also discovered that the microglia in C3-deficient APP transgenic mice displayed an ‘alternative activation’ phenotype, as evidenced by elevated levels of brain IL-10 and IL-4 and decreased nitric oxide synthase 2 (NOS2) and TNFα levels [115]. These data suggest that while the complement system may aid in the inflammatory environment associated with AD, some components of the complement cascade may also play a beneficial role in microglia-mediated clearance mechanisms of Aβ.

However, conflicting reports found that inhibition of the complement system also ameliorated AD pathology. AD transgenic mice deficient in the complement protein C1q (a component of the classical complement cascade) displayed a 50–60% reduction in glial activation and an increase in neuronal markers in the hippocampus [116]. While there was no reduction in amyloid pathology in this study, the authors concluded that glial activation in response to amyloid fibril-stimulated complement activation played an important role in neuronal death, thus inhibiting this response promoted neuronal survival [116]. Similarly, in another study, Tg2576 transgenic animals treated with a small molecule inhibitor (PMX205) of the complement activation product C5a resulted in decreased levels of fibrillar amyloid and reactive microglia and improvement in the passive avoidance behavioral task [117].

Receptors for C5a are located on microglial cells and activation of this receptor leads to the chemotactic recruitment of microglia [118, 119]. It should be noted that all the animal studies have focused on a widespread inhibition or activation of the complement pathway. In summary, the outcomes of these studies has yielded contradictory results, thus making it is difficult to make any conclusive remarks on the role of complement in AD.

## ALTERNATIVE ACTIVATION OF MICROGLIAL CELLS

Traditionally, the microglial cell was classified into two primary phenotypic states; 'quiescent' or 'activated'. The transformation of 'quiescent' microglia to an 'activated' phenotype was associated with inflammation and disease. It is now clear that the phenotypic and functional heterogeneity of microglial cells in the CNS is far more prevalent than previously appreciated and it is slowly being recognized that these cells are highly plastic and play diverse roles in the brain. Several recent studies have highlighted the inherent diversity of microglia and have discovered that they are able to conform into not just two but a variety of different activation states. Past decades of research in AD have focused on the classical, pro-inflammatory activation of microglia. While classical activation is an important host-defense mechanism, it is important to down-regulate this response and initiate tissue repair to attain homeostasis in the CNS after insult or injury [15, 120]. Recent studies suggest that this may be achieved by modulating the activation states of local or infiltrating microglial cells. This appreciation of the phenotypic heterogeneity of microglia has had its basis in studies of peripheral macrophage biology. Gordon and colleagues have proposed a classification system that describes the 'classical' proinflammatory activation state as M1 and 'alternative' activation states as M2 [121]. More recent attempts at defining the diverse macrophage phenotypes have employed other nomenclatures, in an attempt to reflect the plasticity and heterogeneity of these cells [122,123]. Indeed, it is now widely appreciated that there is a spectrum of activation states that defy easy classification. Characteristics of the individual cells are a function of local environmental influences and the capacity of these cells to initiate and resolve the tissue response to pathogens or injury. It is not clear whether these phenotypic distinctions are appropriate for describing the brain resident populations of myeloid lineage cells [121, 124].

Classical activation (M1 activation) of microglial cells results in the production of Th1-proinflammatory cytokines, NOS2 expression and is characterized by the ability of microglia to present antigen [120]. In peripheral organs, tissue macrophages can acquire non-classical, or 'alternative' activation phenotypes. This has been postulated to be an evolutionary adaptation to parasitic infections in which the immune system is unable to remove the activating pathogen. An analogous situation may exist in the brain owing to the inability of microglia to remove Aβ plaques. Alternative activation (or M2 activation) of microglia is typified by the expression of Th2 anti-inflammatory cytokines including IL-10, TGF-β, IL-4 and IL-13, and suppressed expression of Th1-proinflammatory cytokines and NOS2. Alternatively activated microglia have typically been thought to play a role in tissue repair and express genes such as YM1, YM2, RELMα (FIZZ1), CD206 (mannose receptor) and arginase 1(Arg1) [125].

It is important to note, however, that M1 and M2 activation states represent only two different phenotypes in a continuum of macrophage activation which may also encompass "mixed" microglial phenotypes as well [123]. The M2 alternative activation state has been further subdivided into three distinct categories, M2a, M2b and M2c. M2a macrophages are induced by IL-4 and IL-13 and exhibit an anti-inflammatory phenotype. M2b macrophages are unique in that they express high levels of pro-inflammatory cytokines, characteristic of M1 activation, but also express high levels of the anti-inflammatory cytokine IL-10 [126]. The M2b phenotype is induced by exposure to immune complexes, agonists for IL-1

receptor and TLRs. M2c macrophages are considered to be in an “acquired deactivation” state induced by IL-10, TGFβ, glucocorticoids or contact with apoptotic cells, and are associated with the suppression of the innate immune response. Macrophages exhibiting the M2c phenotype play a role in the phagocytosis of cellular debris and apoptotic cells without the induction of a classical immune response allowing for normal tissue maintenance and repair [120].

While the M2a, M2b and M2c macrophage activation phenotype has been classified in the periphery, it is unclear whether these states occur in microglia in the brain. Expression of alternative markers have been found it the brain, but it remains to be determined whether the microglial population can shift between activation phenotypes from classical to alternative activation or vice versa, although this seems probable. It is also unclear to what extent both microglial subpopulations coexist in the brain and their distribution in relation to the plaque pathology. *In vitro* studies have revealed that cultured monocyte-derived macrophages that were polarized to a classical activation state were able to switch their activation status in response to IL-4 and IL-10 treatment [127].

Recent studies have shown that AD mouse models display a switch in microglial activation status in response to disease progression. A study conducted by Colton *et al*. [125] demonstrated that two mouse models of AD (Tg2576 and Tg-SwDI) expressed increased levels of mRNA for the alternative activation markers Arg1, mannose receptor and YM1 in comparison to wild-type controls. Brain samples obtained from AD patients showed a similar increase in levels of M2 markers [125]. Transgenic human APP mice deficient for C3 also exhibited an alternative activation phenotype, displaying reduced NOS2 production and an increase in levels of IL-4 and IL-10 [115]. However, a contradictory study conducted by Jimenez *et al*. [128] described an age-dependent switch in microglial activation in the hippocampus of the APP/PS1 mouse model. They provided evidence that microglial cells exhibit an M2 alternative activation phenotype early in disease progression which then switches to a classical phenotype in older transgenic mice. Interestingly, the authors found that in young APP/PS1 mice, microglial cells surrounding amyloid plaques displayed an alternative activation state and expressed IL-1β and YM1. At later times, following plaque deposition, the microglia alter their activation status and display an increase in TNFα production while down-regulating expression of IL-4 and YM1 [128]. Furthermore, the authors went on to show that the soluble brain extracts containing small oligomeric species of Aβ from 18 month old APP/PS1 mice, stimulated the production of TNFα by astroglial cells. This suggests that recognition of oligomeric Aβ by microglial cells is a key component in programming these cells towards M1 activation. The idea that microglial cells exhibit an age and disease-associated change in activation state is of importance, and the significance of this phenotypic change in ameliorating disease pathology needs to be studied in more depth.

Recently, it has been shown that activation of the nuclear receptor PPARγ controls the activation status of macrophages, promoting acquisition of an M2 phenotype. Similarly, PPARγ and PPARδ activation resulted in induction of M2 activation marker Arg1 and the pro-inflammatory cytokine IL-4 [129, 130]. This induction was lost in macrophages from PPARγ and PPAR δ knockout mice results in elevated brain levels of IL-4 and Arg1. However, this study was carried out in peripheral monocytes and it is unknown if PPARγ agonists can induce M2 state in microglial cells within the brain. Since microglia play such an integral role in AD pathophysiology, understanding role of microglial activation states and their subsequent effect on AD pathology is of importance, for not only understanding disease mechanisms, but also for developing new therapies.

## INFILTRATION OF PERIPHERAL MONOCYTES INTO THE BRAIN

In the periphery, injury results in the recruitment of circulating monocytes to the site of injury, their infiltration into the tissue and their differentiation into macrophages. These cells then phagocytose debris and dying cells as well as secrete pro-inflammatory cytokines and other immune effector molecules. These cells are attracted to the site of injury by the local release of chemokines and cytokines, most prominently CCL2. Infiltrating macrophages then exit the tissue and are removed through the lymphatic system as well as the peripheral circulation, allowing the site of insult to return to pre-injury homeostasis. While this mechanism is useful to remove inflammatory macrophages in the injured tissue, the blood-brain barrier, separating the CNS from the rest of the body, poses as a problem for circulating monocytes to enter and exit the brain [131].

It is widely accepted that in the CNS microglial cells have a low turnover rate and are capable of self renewal [132]. However, in AD, the appearance of amyloid plaques is associated with a parallel increase in the number of microglia and astrocytes [133]. This had previously been believed to be the result of migration of endogenous microglia and their proliferation *in situ*. Recently, the question has been reexamined following several reports that peripheral monocytes can traffic into the brain. Moreover, there is evidence that suggests that infiltrating bone marrow-derived microglia may be more competent and efficient at restricting amyloid plaques in comparison to their endogenous counterparts [134, 135].

A number of recent studies have challenged the idea that the blood-brain barrier is impermeable and have suggested that infiltrating monocytes could invade the CNS and ameliorate AD-related pathology. A study by Simard *et al*. [135] reported that bone marrow derived monocytes transversed the blood-brain barrier from the periphery, invaded the brain parenchyma and associated with and cleared Aβ deposits. Specifically, they reported that 1% of the brain microglial population was derived from peripherally recruited monocytes ($CD11b^+$, $Iba1^+$ cells). These infiltrating cells were found to be associated with about 20% of the β-amyloid plaques found in the AD brain and were able to internalize Aβ deposits [32, 135, 136]. This latter observation is of importance because resident CNS microglia were unable to mediate the clearance of Aβ [135]. However, two studies published in 2007, challenged this idea when they demonstrated that under physiological conditions, peripherally-derived monocytes were unable to cross the blood-brain barrier and infiltrate the brain, suggesting that the infiltration of monocytes into the brain of the AD models was an artifact of the experimental techniques utilized [137, 138].

Recently, a couple of elegant studies have demonstrated that CCL2 (formerly known as monocyte chemotactic protein-1) may play a key role in the attraction and infiltration of peripheral monocytes into the brain. CCL2 is a member of the β chemokine family; it is a ligand for the G-protein coupled receptor, CCR2. CCL2 functions as a chemo-attractant for microglia and monocytes and its expression is elevated in both activated astrocytes as well as cells of monocytic origin [139, 140]. Stimulation of both microglia and astrocytes with Aβ increases expression of CCL2 [50, 51]. In the AD brain, CCL2 is found associated with senile plaques as well as in activated microglia isolated from APP transgenic mice [141, 142]. CCR2 has been shown to be involved in the migration of macrophages to sites of axonal injury [143, 144]. Over-expression of CCL2 in the Tg2576 mouse model of AD resulted in an increased accumulation of microglial cells and diffuse plaques in the mouse brain [145]. CCL2 overexpression was also shown to accelerate memory deficits in the Tg2576 mouse model and facilitated Aβ oligomer formation *in vitro* by microglial cells [146]. Delivery of a dominant negative CCL2 by administration by AAV1/2 hybrid virus to an AD mouse model resulted in a reduction of microgliosis and amyloid deposition [147].

A compelling study by El Khoury and colleagues [141] demonstrated that loss of CCR2 in an AD mouse model was associated with a significant reduction in microglial number and increased levels of brain Aβ. This was paralleled with a decrease in levels of neprilysin, a key Aβ protease. In this study the authors distinguished between peripherally-derived monocytes/microglia and endogenous microglia by the expression levels of CD45. Endogenous microglial cells exhibit lower levels of CD45 in comparison to infiltrating cells. Tg2576 animals display a 12-fold increase in levels of $CD45^{hi}$ microglia; however, CCR2 knockout animals had wild-type levels of CD45 expressing microglia, suggesting that infiltration of peripheral monocytes/microglia is impaired in the knockout animals [141]. Additionally, a study by D'Mello *et al.* [41] demonstrated that hepatic inflammation resulted in an increase in cerebral CCL2 levels and well as an increase in circulating CCR2-positive monocytes. They were able to show that hepatic inflammation induced the expression of CCL2 by cerebral microglia prior to monocyte infiltration into the brain and that infiltration of monocytes was dependent on TNFα signaling [41]. These studies provide compelling evidence that this chemokine and its receptor may play an influential role in the infiltration of peripheral monocytes into the brain.

To determine whether monocytes can infiltrate the brain several other studies have looked at manipulating the expression of CD11c-expressing myeloid cells. CD11c is a cell surface integrin and is most notably expressed by antigen presenting dendritic cells. Dendritic cells are part of the mononuclear phagocytic system and were initially thought to be distinct from macrophages. However, it was recently reported that CD11c co-localized within a subset of cells that were positive for the microglial markers Iba1, F4/80 and CDllb [148]. It has been suggested that dendritic cells are "immature macrophages", however this remains controversial [149].

Butovsky *et al.* [150] utilized diptheria toxin to selectively ablate dendritic-like innate immune cells in a mouse model of AD. This model was genetically engineered to target toxicity towards bone marrow-derived cells of myeloid origin that express CD11c. Depletion of $CD11c^{+}$ cells resulted in a significant increase in amyloid deposition in the brain parenchyma [150]. Town and colleagues have provided additional evidence supporting the concept that monocytes can infiltrate the blood-brain barrier and traffic into the brain [151]. They demonstrated that TGF-β receptor signaling through Smad2/3 plays an important role in inhibiting the infiltration of peripheral monocytes/microglia into the brain. TGF-β signaling was disrupted in Tg2576 animals by expression of a dominant negative TGF-β receptor in cells of myeloid lineage using the CD11c promoter. Blockade of TGF-β signaling resulted in decreased plaque pathology (both cerebral and vascular), improved behavior, and an increased number of peripheral $CD45^{hi}$ expressing macrophages surrounding amyloid plaques and cerebral vessels [151]. These infiltrating cells display the cell surface markers $CD45^{+}CD11b^{+}Ly\text{-}6C^{-}$ [151] and correspond to a population of monocytes that home to inflamed tissues [152].

However, the evaluation of whether peripheral monocytes can enter the CNS remains problematic. The lack of specific markers to distinguish between CNS-resident microglia and peripheral monocytes has made this a difficult question to answer. Greissmann and colleagues have described two populations of circulating monocytes, a "inflammatory subset" that is short-lived and $CX3CR1^{lo}CCR2^{+}GR1^{+}$ and a "homeostatic subset" that is $CX3CR1^{hi}CCR2^{-}GR1^{-}$ [152]. It is though that this "inflammatory subset" of monocytes, which express CCR2, are able to infiltrate the diseased brain. Furthermore, a number of current studies utilize $CD45^{hi}$ and CD11c expression as markers that are limited to infiltrating monocytes. While no definitive studies have been carried out to show that this is in fact the case, a general consensus in the field supports the idea that $CD45^{hi}$ microglia in the brain are derived from peripheral monocytes.

Similarly, another study looked at the ability of perivascular macrophages to clear cerebral amyloid angiopathy in an AD transgenic mouse model [153]. The authors utilized a liposome-encapsulated clodronate, an intracellular toxin, to deplete perivascular macrophages in a TgCRND8 mouse model of AD and demonstrated a 5-fold increase in cerebral amyloid angiopathy pathology as a result of the removal of perivascular macrophages [153].

This avenue of research is important because it has been suggested that infiltrating monocytes may represent a phenotypically distinct group of microglia, capable of ameliorating disease pathology, while endogenous microglial cells may maybe arrested in a classically activated state promoting disease progression. If this theory is correct, enhancing infiltration of peripheral monocytes may be beneficial in mediating the suppression of AD-related inflammation.

## CONCLUSIONS

The past few years have revealed many new roles and functions of microglial cells in the CNS. While we have gained an understanding of the complexity of this cell type we are far from understanding its role in disease related pathophysiology. In this review, we have explored the functional role of microglia in the CNS, as well as summarized the positive and negative effects of these cells and their role in AD-mediated inflammatory responses. Specifically, the dynamic nature of microglial cells as well as their vast phenotypic diversity leads to the fascinating possibility that alleviating disease-related pathology may be possible by manipulating microglial activation status.

## Acknowledgments

This work was supported by grants from the NIH (AG16740), the Alzheimer's Association and the Blanchett Hooker Rockefeller Foundation. SM was supported by a Ruth L. Kirschstein NRSA individual fellowship (5F31NS061445-02).

## ABBREVIATIONS

| | |
|---|---|
| **Aβ** | Amyloid-β |
| **AD** | Alzheimer's disease |
| **Arg1** | Arginase 1 |
| **APP** | Amyloid precursor protein |
| **CNS** | Central nervous system |
| **CpG ODN** | Unmethylated cytosine-guanosineoligodeoxynucleotides |
| **IFNγ** | Interferon γ |
| **IL-1β** | Interleukin-1β |
| **IL-6** | Interleukin-6 |
| **NOS2** | Nitric oxide synthase 2 |
| **PPAR** | Peroxisome proliferator-activated receptor |
| **PS1** | Presenilin 1 |
| **ROS** | Reactive oxygen species |
| **TGFβ** | Transforming growth factor β |

| | |
|---|---|
| **TLRs** | Toll-like receptors |
| **TNFα** | Tumor necrosis factor α |
| **TNFR1** | Tumor necrosis factor receptor 1 |

## References


1. Cole SL, Vassar R. The Alzheimer's disease beta-secretase enzyme, BACE1. Mol Neurodegener. 2007; 2:22. [PubMed: 18005427]
2. Perlmutter LS, Barron E, Chui HC. Morphologic association between microglia and senile plaque amyloid in Alzheimer's disease. Neurosci Lett. 1990; 119(1):32–36. [PubMed: 2097581]
3. Wisniewski HM, Wegiel J, Wang KC, Lach B. Ultrastructural studies of the cells forming amyloid in the cortical vessel wall in Alzheimer's disease. Acta Neuropathol. 1992; 84(2):117–127. [PubMed: 1381856]
4. Frackowiak J, Wisniewski HM, Wegiel J, Merz GS, Iqbal K, Wang KC. Ultrastructure of the microglia that phagocytose amyloid and the microglia that produce beta-amyloid fibrils. Acta Neuropathol. 1992; 84(3):225–233. [PubMed: 1414275]
5. Wisniewski HM, Barcikowska M, Kida E. Phagocytosis of beta/A4 amyloid fibrils of the neuritic neocortical plaques. Acta Neuropathol. 1991; 81(5):588–590. [PubMed: 1858487]
6. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000; 21(3):383–421. [PubMed: 10858586]
7. Rezaie P, Male D. Mesoglia & microglia--a historical review of the concept of mononuclear phagocytes within the central nervous system. J Hist Neurosci. 2002; 11(4):325–374. [PubMed: 12557654]
8. Morris L, Graham CF, Gordon S. Macrophages in haemopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. Development. 1991; 112(2):517–526. [PubMed: 1794320]
9. Dheen ST, Kaur C, Ling EA. Microglial activation and its implications in the brain diseases. Curr Med Chem. 2007; 14(11):1189–1197. [PubMed: 17504139]
10. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience. 1990; 39(1):151–170. [PubMed: 2089275]
11. Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. Neuroscience. 1992; 48(2):405–415. [PubMed: 1603325]
12. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury *in vivo*. Nat Neurosci. 2005; 8(6): 752–758. [PubMed: 15895084]
13. Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience. 1985; 15(2):313–326. [PubMed: 3895031]
14. Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y, Kohsaka S. Microglia-specific localisation of a novel calcium binding protein, Iba1. Brain Res Mol Brain Res. 1998; 57(1):1–9. [PubMed: 9630473]
15. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3(1):23–35. [PubMed: 12511873]
16. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science. 2005; 308(5726):1314–1318. [PubMed: 15831717]
17. Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci. 2006; 9(12): 1512–1519. [PubMed: 17115040]

18. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. J Neurosci. 2009; 29(13):3974–3980. [PubMed: 19339593]
19. Akiyama H, Mori H, Saido T, Kondo H, Ikeda K, McGeer PL. Occurrence of the diffuse amyloid beta-protein (Abeta) deposits with numerous Abeta-containing glial cells in the cerebral cortex of patients with Alzheimer's disease. Glia. 1999; 25(4):324–331. [PubMed: 10028915]
20. Mackenzie IR, Hao C, Munoz DG. Role of microglia in senile plaque formation. Neurobiol Aging. 1995; 16(5):797–804. [PubMed: 8532113]
21. Akiyama H, McGeer PL. Brain microglia constitutively express beta-2 integrins. J Neuroimmunol. 1990; 30(1):81–93. [PubMed: 1977769]
22. Stalder M, Phinney A, Probst A, Sommer B, Staufenbiel M, Jucker M. Association of microglia with amyloid plaques in brains of APP23 transgenic mice. Am J Pathol. 1999; 154(6):1673–1684. [PubMed: 10362792]
23. Sasaki A, Shoji M, Harigaya Y, Kawarabayashi T, Ikeda M, Naito M, Matsubara E, Abe K, Nakazato Y. Amyloid cored plaques in Tg2576 transgenic mice are characterized by giant plaques, slightly activated microglia, and the lack of paired helical filament-typed, dystrophic neurites. Virchows Arch. 2002; 441(4):358–367. [PubMed: 12404061]
24. Wegiel J, Imaki H, Wang KC, Wronska A, Osuchowski M, Rubenstein R. Origin and turnover of microglial cells in fibrillar plaques of APPsw transgenic mice. Acta Neuropathol. 2003; 105(4): 393–402. [PubMed: 12624793]
25. Wegiel J, Wang KC, Imaki H, Rubenstein R, Wronska A, Osuchowski M, Lipinski WJ, Walker LC, LeVine H. The role of microglial cells and astrocytes in fibrillar plaque evolution in transgenic APP(SW) mice. Neurobiol Aging. 2001; 22(1):49–61. [PubMed: 11164276]
26. Stalder M, Deller T, Staufenbiel M, Jucker M. 3D-Reconstruction of microglia and amyloid in APP23 transgenic mice: no evidence of intracellular amyloid. Neurobiol Aging. 2001; 22(3):427–434. [PubMed: 11378249]
27. Bornemann KD, Wiederhold KH, Pauli C, Ermini F, Stalder M, Schnell L, Sommer B, Jucker M, Staufenbiel M. Abeta-induced inflammatory processes in microglia cells of APP23 transgenic mice. Am J Pathol. 2001; 158(1):63–73. [PubMed: 11141480]
28. Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. J Neuroimmunol. 1989; 24(3):173–182. [PubMed: 2808689]
29. Frautschy SA, Yang F, Irrizarry M, Hyman B, Saido TC, Hsiao K, Cole GM. Microglial response to amyloid plaques in APPsw transgenic mice. Am J Pathol. 1998; 152(1):307–317. [PubMed: 9422548]
30. Yan P, Bero AW, Cirrito JR, Xiao Q, Hu X, Wang Y, Gonzales E, Holtzman DM, Lee JM. Characterizing the appearance and growth of amyloid plaques in APP/PS1 mice. J Neurosci. 2009; 29(34):10706–10714. [PubMed: 19710322]
31. Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A, Rozkalne A, Koenigsknecht-Talboo J, Holtzman DM, Bacskai BJ, Hyman BT. Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature. 2008; 451(7179):720–724. [PubMed: 18256671]
32. Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, Klunk WE, Kohsaka S, Jucker M, Calhoun ME. Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. J Neurosci. 2008; 28(16):4283–4292. [PubMed: 18417708]
33. Maeda J, Suhara T, Zhang MR, Okauchi T, Yasuno F, Ikoma Y, Inaji M, Nagai Y, Takano A, Obayashi S, Suzuki K. Novel peripheral benzodiazepine receptor ligand [11C]DAA1106 for PET: an imaging tool for glial cells in the brain. Synapse. 2004; 52(4):283–291. [PubMed: 15103694]
34. Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, Jones T, Banati RB. In-vivo measurement of activated microglia in dementia. Lancet. 2001; 358(9280):461–467. [PubMed: 11513911]
35. Mankowski JL, Queen SE, Tarwater PJ, Adams RJ, Guilarte TR. Elevated peripheral benzodiazepine receptor expression in simian immunodeficiency virus encephalitis. J Neurovirol. 2003; 9(1):94–100. [PubMed: 12587072]

36. Ji B, Maeda J, Sawada M, Ono M, Okauchi T, Inaji M, Zhang MR, Suzuki K, Ando K, Staufenbiel M, Trojanowski JQ, Lee VM, Higuchi M, Suhara T. Imaging of peripheral benzodiazepine receptor expression as biomarkers of detrimental versus beneficial glial responses in mouse models of Alzheimer's and other CNS pathologies. J Neurosci. 2008; 28(47):12255–12267. [PubMed: 19020019]
37. Versijpt JJ, Dumont F, Van Laere KJ, Decoo D, Santens P, Audenaert K, Achten E, Slegers G, Dierckx RA, Korf J. Assessment of neuroinflammation and microglial activation in Alzheimer's disease with radiolabelled PK11195 and single photon emission computed tomography. A pilot study. Eur Neurol. 2003; 50(1):39–47. [PubMed: 12824711]
38. Edison P, Archer HA, Gerhard A, Hinz R, Pavese N, Turkheimer FE, Hammers A, Tai YF, Fox N, Kennedy A, Rossor M, Brooks DJ. Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study. Neurobiol Dis. 2008; 32(3):412–419. [PubMed: 18786637]
39. Wiley CA, Lopresti BJ, Venneti S, Price J, Klunk WE, DeKosky ST, Mathis CA. Carbon 11-labeled Pittsburgh Compound B and carbon 11-labeled (R)-PK11195 positron emission tomographic imaging in Alzheimer disease. Arch Neurol. 2009; 66(1):60–67. [PubMed: 19139300]
40. Venneti S, Lopresti BJ, Wang G, Hamilton RL, Mathis CA, Klunk WE, Apte UM, Wiley CA. PK11195 labels activated microglia in Alzheimer's disease and *in vivo* in a mouse model using PET. Neurobiol Aging. 2009; 30(8):1217–1226. [PubMed: 18178291]
41. D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. J Neurosci. 2009; 29(7):2089–2102. [PubMed: 19228962]
42. Patel NS, Paris D, Mathura V, Quadros AN, Crawford FC, Mullan MJ. Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. J Neuroinflamm. 2005; 2(1):9.
43. Herber DL, Mercer M, Roth LM, Symmonds K, Maloney J, Wilson N, Freeman MJ, Morgan D, Gordon MN. Microglial activation is required for Abeta clearance after intracranial injection of lipopolysaccharide in APP transgenic mice. J Neuroimmune Pharmacol. 2007; 2(2):222–231. [PubMed: 18040847]
44. Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. J Neuroimmunol. 2007; 184(1–2):69–91. [PubMed: 17222916]
45. Rojo LE, Fernandez JA, Maccioni AA, Jimenez JM, Maccioni RB. Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. Arch Med Res. 2008; 39(1):1–16. [PubMed: 18067990]
46. Hoozemans JJ, Veerhuis R, Rozemuller JM, Eikelenboom P. Neuroinflammation and regeneration in the early stages of Alzheimer's disease pathology. Int J Dev Neurosci. 2006; 24(2–3):157–165. [PubMed: 16384684]
47. Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med. 2006; 12(9):1005–1015. [PubMed: 16960575]
48. Lucin KM, Wyss-Coray T. Immune activation in brain aging and neurodegeneration: too much or too little? Neuron. 2009; 64(1):110–122. [PubMed: 19840553]
49. Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol. 2008; 9(8):857–865. [PubMed: 18604209]
50. Smits HA, Rijsmus A, van Loon JH, Wat JW, Verhoef J, Boven LA, Nottet HS. Amyloid-beta-induced chemokine production in primary human macrophages and astrocytes. J Neuroimmunol. 2002; 127(1–2):160–168. [PubMed: 12044988]
51. El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, Freeman MW, Luster AD. CD36 mediates the innate host response to beta-amyloid. J Exp Med. 2003; 197(12):1657–1666. [PubMed: 12796468]
52. Lue LF, Brachova L, Civin WH, Rogers J. Inflammation, A beta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. J Neuropathol Exp Neurol. 1996; 55(10):1083–1088. [PubMed: 8858005]

53. He P, Zhong Z, Lindholm K, Berning L, Lee W, Lemere C, Staufenbiel M, Li R, Shen Y. Deletion of tumor necrosis factor death receptor inhibits amyloid beta generation and prevents learning and memory deficits in Alzheimer's mice. J Cell Biol. 2007; 178(5):829–841. [PubMed: 17724122]
54. Yamamoto M, Kiyota T, Horiba M, Buescher JL, Walsh SM, Gendelman HE, Ikezu T. Interferon-gamma and tumor necrosis factor-alpha regulate amyloid-beta plaque deposition and beta-secretase expression in Swedish mutant APP transgenic mice. Am J Pathol. 2007; 170(2):680–692. [PubMed: 17255335]
55. Shaftel SS, Kyrkanides S, Olschowka JA, Miller JN, Johnson RE, O'Banion MK. Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. J Clin Invest. 2007; 117(6):1595–1604. [PubMed: 17549256]
56. Chakrabarty P, Jansen-West K, Beccard A, Ceballos-Diaz C, Levites Y, Verbeeck C, Zubair AC, Dickson D, Golde TE, Das P. Massive gliosis induced by interleukin-6 suppresses A{beta} deposition *in vivo*: evidence against inflammation as a driving force for amyloid deposition. FASEB J. 2009; 24(2):548–559. [PubMed: 19825975]
57. Ransohoff RM. The MHP36 line of murine neural stem cells expresses functional CXCR1 chemokine receptors that initiate chemotaxis *in vitro*. J Neuroimmunol. 2007; 186(1–2):199. [PubMed: 17482680]
58. Duan RS, Yang X, Chen ZG, Lu MO, Morris C, Winblad B, Zhu J. Decreased fractalkine and increased IP-10 expression in aged brain of APP(swe) transgenic mice. Neurochem Res. 2008; 33(6):1085–1089. [PubMed: 18095157]
59. Walker DG, Dalsing-Hernandez JE, Campbell NA, Lue LF. Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. Exp Neurol. 2009; 215(1):5–19. [PubMed: 18938162]
60. Lyons A, Downer EJ, Crotty S, Nolan YM, Mills KH, Lynch MA. CD200 ligand receptor interaction modulates microglial activation *in vivo* and *in vitro*: a role for IL-4. J Neurosci. 2007; 27(31):8309–8313. [PubMed: 17670977]
61. Mott RT, Ait-Ghezala G, Town T, Mori T, Vendrame M, Zeng J, Ehrhart J, Mullan M, Tan J. Neuronal expression of CD22: novel mechanism for inhibiting microglial proinflammatory cytokine production. Glia. 2004; 46(4):369–379. [PubMed: 15095367]
62. Hsieh CL, Koike M, Spusta SC, Niemi EC, Yenari M, Nakamura MC, Seaman WE. A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. J Neurochem. 2009; 109(4):1144–1156. [PubMed: 19302484]
63. Frank S, Burbach GJ, Bonin M, Walter M, Streit W, Bechmann I, Deller T. TREM2 is upregulated in amyloid plaque-associated microglia in aged APP23 transgenic mice. Glia. 2008; 56(13):1438–1447. [PubMed: 18551625]
64. Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47-SIRPalpha signalling pathway. Trends Cell Biol. 2009; 19(2):72–80. [PubMed: 19144521]
65. Tan J, Town T, Paris D, Mori T, Suo Z, Crawford F, Mattson MP, Flavell RA, Mullan M. Microglial activation resulting from CD40-CD40L interaction after beta-amyloid stimulation. Science. 1999; 286(5448):2352–2355. [PubMed: 10600748]
66. Calingasan NY, Erdely HA, Altar CA. Identification of CD40 ligand in Alzheimer's disease and in animal models of Alzheimer's disease and brain injury. Neurobiol Aging. 2002; 23(1):31–39. [PubMed: 11755016]
67. Togo T, Akiyama H, Kondo H, Ikeda K, Kato M, Iseki E, Kosaka K. Expression of CD40 in the brain of Alzheimer's disease and other neurological diseases. Brain Res. 2000; 885(1):117–121. [PubMed: 11121537]
68. Tan J, Town T, Crawford F, Mori T, DelleDonne A, Crescentini R, Obregon D, Flavell RA, Mullan MJ. Role of CD40 ligand in amyloidosis in transgenic Alzheimer's mice. Nat Neurosci. 2002; 5(12):1288–1293. [PubMed: 12402041]
69. Mandrekar S, Jiang Q, Lee CY, Koenigsknecht-Talboo J, Holtzman DM, Landreth GE. Microglia mediate the clearance of soluble Abeta through fluid phase macropinocytosis. J Neurosci. 2009; 29(13):4252–4262. [PubMed: 19339619]
70. Koenigsknecht J, Landreth G. Microglial phagocytosis of fibrillar beta-amyloid through a beta1 integrin-dependent mechanism. J Neurosci. 2004; 24(44):9838–9846. [PubMed: 15525768]

71. Bamberger ME, Harris ME, McDonald DR, Husemann J, Landreth GE. A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. J Neurosci. 2003; 23(7):2665–2674. [PubMed: 12684452]
72. Paresce DM, Chung HY, Maxfield FR. Slow degradation of aggregates of the Alzheimer's disease amyloid beta-protein by microglial cells. J Biol Chem. 1997; 272(46):29390–29397. [PubMed: 9361021]
73. Chung H, Brazil MI, Soe TT, Maxfield FR. Uptake, degradation, and release of fibrillar and soluble forms of Alzheimer's amyloid beta-peptide by microglial cells. J Biol Chem. 1999; 274(45):32301–32308. [PubMed: 10542270]
74. Grathwohl SA, Kalin RE, Bolmont T, Prokop S, Winkelmann G, Kaeser SA, Odenthal J, Radde R, Eldh T, Gandy S, Aguzzi A, Staufenbiel M, Mathews PM, Wolburg H, Heppner FL, Jucker M. Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. Nat Neurosci. 2009; 12(11):1361–1363. [PubMed: 19838177]
75. Koenigsknecht-Talboo J, Landreth GE. Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. J Neurosci. 2005; 25(36):8240–8249. [PubMed: 16148231]
76. Zelcer N, Khanlou N, Clare R, Jiang Q, Reed-Geaghan EG, Landreth GE, Vinters HV, Tontonoz P. Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. Proc Natl Acad Sci USA. 2007; 104(25):10601–10606. [PubMed: 17563384]
77. Yamamoto M, Kiyota T, Walsh SM, Liu J, Kipnis J, Ikezu T. Cytokine-mediated inhibition of fibrillar amyloid-beta peptide degradation by human mononuclear phagocytes. J Immunol. 2008; 181(6):3877–3886. [PubMed: 18768842]
78. Wisniewski T, Konietzko U. Amyloid-beta immunisation for Alzheimer's disease. Lancet Neurol. 2008; 7(9):805–811. [PubMed: 18667360]
79. Combs CK, Bates P, Karlo JC, Landreth GE. Regulation of beta-amyloid stimulated proinflammatory responses by peroxisome proliferator-activated receptor alpha. Neurochem Int. 2001; 39(5–6):449–457. [PubMed: 11578780]
80. Shie FS, Breyer RM, Montine TJ. Microglia lacking E Prostanoid Receptor subtype 2 have enhanced Abeta phagocytosis yet lack Abeta-activated neurotoxicity. Am J Pathol. 2005; 166(4):1163–1172. [PubMed: 15793296]
81. Liang X, Wang Q, Hand T, Wu L, Breyer RM, Montine TJ, Andreasson K. Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. J Neurosci. 2005; 25(44):10180–10187. [PubMed: 16267225]
82. McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology. 1996; 47(2):425–432. [PubMed: 8757015]
83. Vlad SC, Miller DR, Kowall NW, Felson DT. Protective effects of NSAIDs on the development of Alzheimer disease. Neurology. 2008; 70(19):1672–1677. [PubMed: 18458226]
84. Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, Tran T, Ubeda O, Ashe KH, Frautschy SA, Cole GM. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci. 2000; 20(15):5709–5714. [PubMed: 10908610]
85. Lim GP, Yang F, Chu T, Gahtan E, Ubeda O, Beech W, Overmier JB, Hsiao-Ashec K, Frautschy SA, Cole GM. Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. Neurobiol Aging. 2001; 22(6):983–991. [PubMed: 11755007]
86. McKee AC, Carreras I, Hossain L, Ryu H, Klein WL, Oddo S, LaFerla FM, Jenkins BG, Kowall NW, Dedeoglu A. Ibuprofen reduces Abeta, hyperphosphorylated tau and memory deficits in Alzheimer mice. Brain Res. 2008; 1207:225–236. [PubMed: 18374906]
87. Salminen A, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T. Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence *via* pattern recognition receptors. Prog Neurobiol. 2009; 87(3):181–194. [PubMed: 19388207]
88. Cui Y, Le Y, Yazawa H, Gong W, Wang JM. Potential role of the formyl peptide receptor-like 1 (FPRL1) in inflammatory aspects of Alzheimer's disease. J Leukoc Biol. 2002; 72(4):628–635. [PubMed: 12377930]

89. Cameron B, Landreth GE. Inflammation, microglia, and alzheimer's disease. Neurobiol Dis. 2009 In Press.

90. Combs CK, Karlo JC, Kao SC, Landreth GE. β-Amyloid stimulation of microglia and monocytes results in TNFalpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. J Neurosci. 2001; 21(4):1179–1188. [PubMed: 11160388]

91. Combs CK, Johnson DE, Cannady SB, Lehman TM, Landreth GE. Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of beta-amyloid and prion proteins. J Neurosci. 1999; 19(3):928–939. [PubMed: 9920656]

92. Hickman SE, Allison EK, El Khoury J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J Neurosci. 2008; 28(33):8354–8360. [PubMed: 18701698]

93. Reed-Geaghan EG, Savage JC, Hise AG, Landreth GE. CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation. J Neurosci. 2009; 29(38):11982–11992. [PubMed: 19776284]

94. Kawai T, Akira S. TLR signaling. Semin Immunol. 2007; 19(1):24–32. [PubMed: 17275323]

95. Jack CS, Arbour N, Manusow J, Montgrain V, Blain M, McCrea E, Shapiro A, Antel JP. TLR signaling tailors innate immune responses in human microglia and astrocytes. J Immunol. 2005; 175(7):4320–4330. [PubMed: 16177072]

96. Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of Toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol. 2002; 61(11):1013–1021. [PubMed: 12430718]

97. Frank S, Copanaki E, Burbach GJ, Muller UC, Deller T. Differential regulation of toll-like receptor mRNAs in amyloid plaque-associated brain tissue of aged APP23 transgenic mice. Neurosci Lett. 2009; 453(1):41–44. [PubMed: 19429012]

98. Fassbender K, Walter S, Kuhl S, Landmann R, Ishii K, Bertsch T, Stalder AK, Muehlhauser F, Liu Y, Ulmer AJ, Rivest S, Lentschat A, Gulbins E, Jucker M, Staufenbiel M, Brechtel K, Walter J, Multhaup G, Penke B, Adachi Y, Hartmann T, Beyreuther K. The LPS receptor (CD14) links innate immunity with Alzheimer's disease. FASEB J. 2004; 18(1):203–205. [PubMed: 14597556]

99. Liu Y, Walter S, Stagi M, Cherny D, Letiembre M, Schulz-Schaeffer W, Heine H, Penke B, Neumann H, Fassbender K. LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. Brain. 2005; 128(Pt 8):1778–1789. [PubMed: 15857927]

100. Walter S, Letiembre M, Liu Y, Heine H, Penke B, Hao W, Bode B, Manietta N, Walter J, Schulz-Schuffer W, Fassbender K. Role of the toll-like receptor 4 in neuroinflammation in Alzheimer's disease. Cell Physiol Biochem. 2007; 20(6):947–956. [PubMed: 17982277]

101. Tahara K, Kim HD, Jin JJ, Maxwell JA, Li L, Fukuchi K. Role of toll-like receptor signalling in Abeta uptake and clearance. Brain. 2006; 129(Pt 11):3006–3019. [PubMed: 16984903]

102. DiCarlo G, Wilcock D, Henderson D, Gordon M, Morgan D. Intrahippocampal LPS injections reduce Abeta load in APP+PS1 transgenic mice. Neurobiol Aging. 2001; 22(6):1007–1012. [PubMed: 11755009]

103. Sheng JG, Bora SH, Xu G, Borchelt DR, Price DL, Koliatsos VE. Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice. Neurobiol Dis. 2003; 14(1):133–145. [PubMed: 13678674]

104. Richard KL, Filali M, Prefontaine P, Rivest S. Toll-like receptor 2 acts as a natural innate immune receptor to clear amyloid beta 1-42 and delay the cognitive decline in a mouse model of Alzheimer's disease. J Neurosci. 2008; 28(22):5784–5793. [PubMed: 18509040]

105. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov. 2006; 5(6):471–484. [PubMed: 16763660]

106. Ribes S, Ebert S, Czesnik D, Regen T, Zeug A, Bukowski S, Mildner A, Eiffert H, Hanisch UK, Hammerschmidt S, Nau R. Toll-like receptor prestimulation increases phagocytosis of Escherichia coli DH5alpha and Escherichia coli K1 strains by murine microglial cells. Infect Immun. 2009; 77(1):557–564. [PubMed: 18981243]

107. Scholtzova H, Kascsak RJ, Bates KA, Boutajangout A, Kerr DJ, Meeker HC, Mehta PD, Spinner DS, Wisniewski T. Induction of toll-like receptor 9 signaling as a method for ameliorating

Alzheimer's disease-related pathology. J Neurosci. 2009; 29(6):1846–1854. [PubMed: 19211891]

108. Kolev MV, Ruseva MM, Harris CL, Morgan BP, Donev RM. Implication of complement system and its regulators in Alzheimer's disease. Curr Neuropharmacol. 2009; 7(1):1–8. [PubMed: 19721814]
109. Stoltzner SE, Grenfell TJ, Mori C, Wisniewski KE, Wisniewski TM, Selkoe DJ, Lemere CA. Temporal accrual of complement proteins in amyloid plaques in Down's syndrome with Alzheimer's disease. Am J Pathol. 2000; 156(2):489–499. [PubMed: 10666378]
110. Strohmeyer R, Shen Y, Rogers J. Detection of complement alternative pathway mRNA and proteins in the Alzheimer's disease brain. Brain Res Mol Brain Res. 2000; 81(1–2):7–18. [PubMed: 11000474]
111. Jiang H, Burdick D, Glabe CG, Cotman CW, Tenner AJ. beta-Amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain. J Immunol. 1994; 152(10):5050–5059. [PubMed: 8176223]
112. Rogers J, Cooper NR, Webster S, Schultz J, McGeer PL, Styren SD, Civin WH, Brachova L, Bradt B, Ward P. Complement activation by beta-amyloid in Alzheimer disease. Proc Natl Acad Sci USA. 1992; 89(21):10016–10020. [PubMed: 1438191]
113. Bradt BM, Kolb WP, Cooper NR. Complement-dependent proinflammatory properties of the Alzheimer's disease beta-peptide. J Exp Med. 1998; 188(3):431–438. [PubMed: 9687521]
114. Wyss-Coray T, Yan F, Lin AH, Lambris JD, Alexander JJ, Quigg RJ, Masliah E. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. Proc Natl Acad Sci USA. 2002; 99(16):10837–10842. [PubMed: 12119423]
115. Maier M, Peng Y, Jiang L, Seabrook TJ, Carroll MC, Lemere CA. Complement C3 deficiency leads to accelerated amyloid beta plaque deposition and neurodegeneration and modulation of the microglia/macrophage phenotype in amyloid precursor protein transgenic mice. J Neurosci. 2008; 28(25):6333–6341. [PubMed: 18562603]
116. Fonseca MI, Zhou J, Botto M, Tenner AJ. Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. J Neurosci. 2004; 24(29):6457–6465. [PubMed: 15269255]
117. Fonseca MI, Ager RR, Chu SH, Yazan O, Sanderson SD, LaFerla FM, Taylor SM, Woodruff TM, Tenner AJ. Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. J Immunol. 2009; 183(2):1375–1383. [PubMed: 19561098]
118. Monk PN, Scola AM, Madala P, Fairlie DP. Function, structure and therapeutic potential of complement C5a receptors. Br J Pharmacol. 2007; 152(4):429–448. [PubMed: 17603557]
119. Nolte C, Moller T, Walter T, Kettenmann H. Complement 5a controls motility of murine microglial cells *in vitro via* activation of an inhibitory G-protein and the rearrangement of the actin cytoskeleton. Neuroscience. 1996; 73(4):1091–1107. [PubMed: 8809827]
120. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004; 25(12):677–686. [PubMed: 15530839]
121. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol. 2009; 27:451–483. [PubMed: 19105661]
122. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008; 8(12):958–969. [PubMed: 19029990]
123. Town T, Nikolic V, Tan J. The microglial "activation" continuum: from innate to adaptive responses. J Neuroinflamm. 2005; 2:24.
124. Morgan D, Gordon MN, Tan J, Wilcock D, Rojiani AM. Dynamic complexity of the microglial activation response in transgenic models of amyloid deposition: implications for Alzheimer therapeutics. J Neuropathol Exp Neurol. 2005; 64(9):743–753. [PubMed: 16141783]
125. Colton CA, Mott RT, Sharpe H, Xu Q, Van Nostrand WE, Vitek MP. Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. J Neuroinflamm. 2006; 3:27.

126. Mosser DM. The many faces of macrophage activation. J Leukoc Biol. 2003; 73(2):209–212. [PubMed: 12554797]

127. Gratchev A, Kzhyshkowska J, Kothe K, Muller-Molinet I, Kannookadan S, Utikal J, Goerdt S. Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. Immunobiology. 2006; 211(6–8):473–486. [PubMed: 16920487]

128. Jimenez S, Baglietto-Vargas D, Caballero C, Moreno-Gonzalez I, Torres M, Sanchez-Varo R, Ruano D, Vizuete M, Gutierrez A, Vitorica J. Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic. J Neurosci. 2008; 28(45):11650–11661. [PubMed: 18987201]

129. Loane DJ, Deighan BF, Clarke RM, Griffin RJ, Lynch AM, Lynch MA. Interleukin-4 mediates the neuroprotective effects of rosiglitazone in the aged brain. Neurobiol Aging. 2009; 30(6):920–931. [PubMed: 17950491]

130. Gallardo-Soler A, Gomez-Nieto C, Campo ML, Marathe C, Tontonoz P, Castrillo A, Corraliza I. Arginase I induction by modified lipoproteins in macrophages: a peroxisome proliferator-activated receptor-gamma/delta-mediated effect that links lipid metabolism and immunity. Mol Endocrinol. 2008; 22(6):1394–1402. [PubMed: 18323470]

131. van Rossum D, Hilbert S, Strassenburg S, Hanisch UK, Bruck W. Myelin-phagocytosing macrophages in isolated sciatic and optic nerves reveal a unique reactive phenotype. Glia. 2008; 56(3):271–283. [PubMed: 18069669]

132. Chan WY, Kohsaka S, Rezaie P. The origin and cell lineage of microglia: new concepts. Brain Res Rev. 2007; 53(2):344–354. [PubMed: 17188751]

133. Barron KD. The microglial cell. A historical review. J Neurol Sci. 1995; 134(Suppl):57–68. [PubMed: 8847546]

134. Malm TM, Koistinaho M, Parepalo M, Vatanen T, Ooka A, Karlsson S, Koistinaho J. Bone-marrow-derived cells contribute to the recruitment of microglial cells in response to beta-amyloid deposition in APP/PS1 double transgenic Alzheimer mice. Neurobiol Dis. 2005; 18(1):134–142. [PubMed: 15649704]

135. Simard AR, Soulet D, Gowing G, Julien JP, Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron. 2006; 49(4): 489–502. [PubMed: 16476660]

136. Stalder AK, Ermini F, Bondolfi L, Krenger W, Burbach GJ, Deller T, Coomaraswamy J, Staufenbiel M, Landmann R, Jucker M. Invasion of hematopoietic cells into the brain of amyloid precursor protein transgenic mice. J Neurosci. 2005; 25(48):11125–11132. [PubMed: 16319312]

137. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci. 2007; 10(12):1538–1543. [PubMed: 18026097]

138. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Bruck W, Priller J, Prinz M. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. Nat Neurosci. 2007; 10(12):1544–1553. [PubMed: 18026096]

139. Calvo CF, Yoshimura T, Gelman M, Mallat M. Production of monocyte chemotactic protein-1 by rat brain macrophages. Eur J Neurosci. 1996; 8(8):1725–1734. [PubMed: 8921263]

140. Glabinski AR, Balasingam V, Tani M, Kunkel SL, Strieter RM, Yong VW, Ransohoff RM. Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain. J Immunol. 1996; 156(11):4363–4368. [PubMed: 8666808]

141. El Khoury J, Toft M, Hickman SE, Means TK, Terada K, Geula C, Luster AD. Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. Nat Med. 2007; 13(4):432–438. [PubMed: 17351623]

142. Ishizuka K, Kimura T, Igata-yi R, Katsuragi S, Takamatsu J, Miyakawa T. Identification of monocyte chemoattractant protein-1 in senile plaques and reactive microglia of Alzheimer's disease. Psychiatry Clin Neurosci. 1997; 51(3):135–138. [PubMed: 9225377]

143. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. J Exp Med. 2000; 192(7):1075–1080. [PubMed: 11015448]

144. Babcock AA, Kuziel WA, Rivest S, Owens T. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. J Neurosci. 2003; 23(21):7922–7930. [PubMed: 12944523]

145. Yamamoto M, Horiba M, Buescher JL, Huang D, Gendelman HE, Ransohoff RM, Ikezu T. Overexpression of monocyte chemotactic protein-1/CCL2 in beta-amyloid precursor protein transgenic mice show accelerated diffuse beta-amyloid deposition. Am J Pathol. 2005; 166(5): 1475–1485. [PubMed: 15855647]

146. Kiyota T, Yamamoto M, Xiong H, Lambert MP, Klein WL, Gendelman HE, Ransohoff RM, Ikezu T. CCL2 accelerates microglia-mediated Abeta oligomer formation and progression of neurocognitive dysfunction. PLoS One. 2009; 4(7):e6197. [PubMed: 19593388]

147. Kiyota T, Yamamoto M, Schroder B, Jacobsen MT, Swan RJ, Lambert MP, Klein WL, Gendelman HE, Ransohoff RM, Ikezu T. AAV1/2-mediated CNS gene delivery of dominant-negative CCL2 mutant suppresses gliosis, beta-amyloidosis, and learning impairment of APP/PS1 mice. Mol Ther. 2009; 17(5):803–809. [PubMed: 19277012]

148. Bulloch K, Miller MM, Gal-Toth J, Milner TA, Gottfried-Blackmore A, Waters EM, Kaunzner UW, Liu K, Lindquist R, Nussenzweig MC, Steinman RM, McEwen BS. CD11c/EYFP transgene illuminates a discrete network of dendritic cells within the embryonic, neonatal, adult, and injured mouse brain. J Comp Neurol. 2008; 508(5):687–710. [PubMed: 18386786]

149. Hume DA. Macrophages as APC and the dendritic cell myth. J Immunol. 2008; 181(9):5829–5835. [PubMed: 18941170]

150. Butovsky O, Kunis G, Koronyo-Hamaoui M, Schwartz M. Selective ablation of bone marrow-derived dendritic cells increases amyloid plaques in a mouse Alzheimer's disease model. Eur J Neurosci. 2007; 26(2):413–416. [PubMed: 17623022]

151. Town T, Laouar Y, Pittenger C, Mori T, Szekely CA, Tan J, Duman RS, Flavell RA. Blocking TGF-beta-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. Nat Med. 2008; 14(6):681–687. [PubMed: 18516051]

152. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 2003; 19(1):71–82. [PubMed: 12871640]

153. Hawkes CA, McLaurin J. Selective targeting of perivascular macrophages for clearance of beta-amyloid in cerebral amyloid angiopathy. Proc Natl Acad Sci USA. 2009; 106(4):1261–1266. [PubMed: 19164591]