PostDoc Journal
Vol. 1 No. 1, January 2013

Journal of Postdoctoral Research
www.postdocjournal.com

The Role of Microglia in Alzheimer's Disease

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Abstract
Alzheimer's disease is a devastating neurological condition for which there is no known cure. Inflammation in the brain has been implicated in development of pathology, and of particular interest are the microglia, a major phagocytic cell population. Microglia are able to bind to and engulf amyloid-β (Aβ), a protein that forms fibrils that accumulate as plaques. However, despite increased numbers of activated microglia surrounding Aβ plaques, there is continued neuronal destruction. Indeed, in the ageing brain, microglia have increased activation levels and express higher levels of inflammatory markers. This may account for the microglial dysfunction exhibited as disease progresses. Microglia express various cell surface receptors for Aβ, including the scavenger receptors SCARA-1 and CD36, involved in phagocytosis of Aβ and intracellular signaling through Aβ binding respectively. In addition, the formyl peptide receptors complement receptors and the toll-like receptors are all expressed on microglia and are capable of interacting with Aβ. These Aβ-receptor interactions contribute to induction of inflammatory signaling pathways and disease progression. In addition to binding Aβ, microglia also secrete the Aβ degrading enzymes neprilysin, endothelin-converting enzyme (ECE), insulin-degrading enzyme (IDE) and matrix-metalloprotease-9 (MMP-9). However, microglia in aged brains have been shown to have lower levels of these enzymes, which may contribute to pathology. The important role that microglia play in the development of disease makes them an attractive target for anti-inflammatory therapies utilizing their surface receptors and secreted cytokines, such as TNFα. However, microglia work in concert with other cells in the brain, including astrocytes and the endothelial cells of the blood-brain-barrier. A multi-modal approach will be required in the future to develop efficient therapy for this devastating condition.

Introduction

Alzheimer's disease (AD) is described as a neurological condition manifesting itself in memory loss, dementia, neuropsychiatric symptoms and eventually death. There is no known cure at present, and it is projected that over the next 40 years approximately 13 million people in the USA will be affected [1]. The need for an effective therapy is of importance as the population ages. Of great interest are microglia, monocytic-derived cells that induce inflammation in the brain. This review will focus on the function of microglia in ageing and the pathology of AD.

Alzheimer's disease pathology

AD pathology exhibits as amyloid-β (Aβ) plaques, and neurofibrillary tangles (NFT) of tau protein in the brain. Tau protein accumulates in NFT inside of neuronal axons in AD brains [2], and contains a high number of phosphorylation sites. Upon phosphorylation tau dissociates from the microtubules, as observed in AD [3]. Tau protein then undergoes conformational changes which form fibrils [4]. Tau NFT have been shown to be intimately associated with microglia [5,6]. It has previously been shown that activated microglia secreting cytokines may contribute to the hyperphosphorylation and promotion of aggregation of tau protein [7].

Aβ levels in the brain are regulated by several mechanisms: phagocytic clearance by microglia [8] and astrocytes [9], production or destruction by enzymes [10] or transport across the blood-brain-barrier by various cell

surface receptors, including the scavenger receptor RAGE (receptor for advanced glycation end products) expressed on brain endothelium [11].

Amyloid-β protein is cleaved from amyloid precursor protein (APP) by β-secretase and γ-secretase enzymes [10]. In healthy brains a dynamic equilibrium is thought to exist, where the levels of Aβ are controlled by Aβ degrading enzymes. However in the disease state, Aβ accumulates instead of being removed. These deposits of Aβ become fibrillar over time, resulting in Aβ plaque deposition in the brain [5,12,13].

What are microglia?

Microglia are cells derived from the macrophage cell lineage and are the major phagocytic cells of the brain. These cells are hematopoietic in origin [14] and constitute the first line of defense against invading pathogens, and also recognize various host-derived ligands. Microglia become activated during the development of pathology such as AD, surrounding dead and damaged cells and clear these areas in a similar fashion to macrophages in the periphery [15]. Studies with the BV-2 microglial cell line showed microglia are capable of generating specific macrophage-like inflammatory responses *in vitro* when stimulated with various cytokines and chemokines [16].

Microglia and ageing

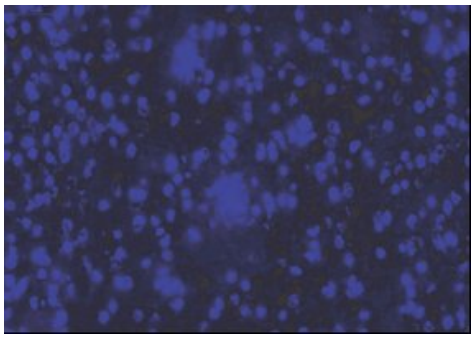
A microarray study of age-related immune changes showed that as humans age there is an upregulation of immune and inflammation genes, with a large change associated with AD development. Notably, microglial activation genes were upregulated including TLR (toll-like receptor) and inflammasome signaling genes, whereas genes involved in downregulation of microglial responses were decreased [17]. In a separate study, upregulation of RAGE (receptor for advanced glycation end products) and MHCII (major histocompatibility complex class 2) [18] and increased basal production of the inflammatory cytokines TNFα and IL-1β were also observed in aged brains [19]. Microglia also exhibit morphological changes and degeneration during normal ageing leading to the possibility of impaired Aβ clearance in aged patients, which could lead to the promotion of neurodegeneration [20]. This suggests that even during normal ageing there is a dysregulation of microglia and associated immune responses, and that this is increased during AD.

When microglia come into contact with Aβ, they become activated and release inflammatory chemokines and cytokines into the local environment [21]. Initially, microglia are able to clear Aβ plaques, but become overwhelmed as AD progresses, and release toxic cytokines, chemokines and reactive oxygen species, these agents act upon surrounding neurons contributing to their death [22]. Aβ plaques increase in size and number despite the presence of increased microglia [23], and ultimately the toxic environment causes neuronal cell death and advancement of disease [24]. Figure 1 shows microglia (stained red) around Aβ plaques (stained green) in the mouse model of AD, PS1APP, at 365 days of age.

Microglial Aβ Receptors

ApoE

Variation in the ApoE (apolipoprotein E) allele, along with ageing in humans is a major risk factor for AD. ApoE is the main cholesterol transporter in the brain and intracellular degradation of Aβ by microglia was found to be enhanced in the presence of ApoE [25]. ApoE expression is regulated by PPAR-γ (peroxisome proliferator–activated receptor gamma) and LXR (liver X receptors) which are ligand-activated nuclear receptors. PPAR-γ and LXR form heterodimers with retinoid X receptors, these heterodimers induce the expression of ApoE on microglia which stimulates their ability to phagocytose soluble Aβ. Agonist treatment of LXR and PPAR-γ improved cognitive functions and reduced Aβ levels in mouse brains [26].



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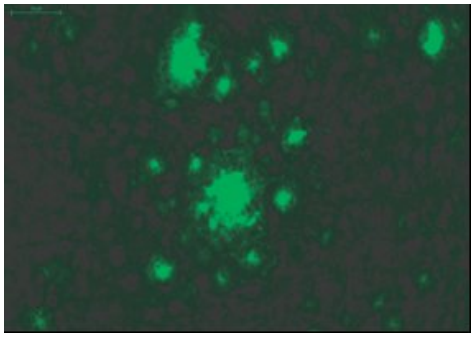

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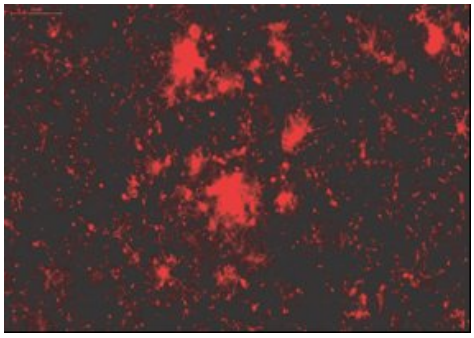
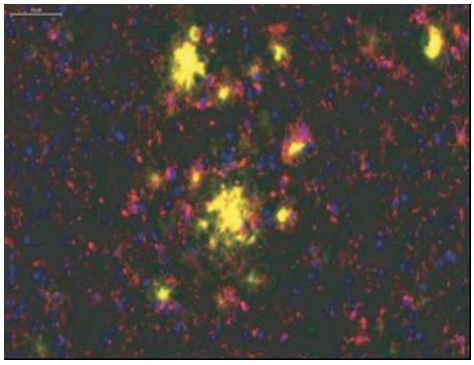


CD11b



MERGED

Figure 1. PS1APP mouse brain section at 365 days of age (100X magnification) stained for microglia (CD11b), Aβ plaques (THIO-S) and cell nuclei (DAPI), showing microglial accumulation around Aβ plaques (MERGED).

Scavenger receptors

Microglia express various receptors for Aβ on their cell surface, including scavenger receptors (SR). The scavenger receptors belong to the family of pattern recognition receptors (PRR), which also include toll-like receptors. SR were first described as receptors expressed on macrophages that bound and internalized modified low-density lipoprotein, and contributed to foam cell formation during atherosclerosis [27]. Other ligands for SRs have been discovered since then, and the definition of scavenger receptors has expanded to define a large family of molecules that bind various self and non- self ligands, and are evolutionarily conserved, highlighting their important roles in innate defense against pathogens.

Most SRs are grouped into classes A through F, with the receptors RAGE, SR-PSOX and CD163 remaining unclassified. Class A includes SCARA-1, SCARA-2 and MARCO, all of which have a coiled coil domain believed to be the ligand binding domain of these receptors. The class B receptors SCARB-1, SCARB-2 and CD36 have an N and C terminal membrane spanning region and a large extracellular loop. SCARC, the class C receptor has been discovered in *Drosophila* but no mammalian ortholog has been identified to date. CD68, the most well characterized class D SR has a mucin-like domain and SCARE (Class E receptors) includes LOX-1, with a C-type lectin domain. SREC, a member of the class F receptors is characterized by multiple epidermal growth-factor like repeats [28].

Aβ phagocytosis by SCARA-1

SCARA-1 (scavenger receptor A-1) is a well-characterized multi-ligand binding receptor. SCARA-1 binds modified lipoproteins, lipopolysaccharide (LPS) [29] and lipoteichoic acid (LTA) [30, 31], and also is involved in phagocytosis of bacteria [31, 32].

SCARA-1 expressed on microglia has previously been shown to be a receptor for Aβ in mice and humans whose function is to facilitate phagocytosis of Aβ [33]. SCARA-1 knock-out microglia were greatly reduced in

their ability to engulf Aβ when compared with cells isolated from wild-type mice [34]. When a SCARA-1 knock-out mouse was crossed with a transgenic AD model mouse, there was surprisingly no reduction in Aβ plaque number in the brains of these animals [35]. This study shows a redundancy in SCARA-1 function and suggests other receptors are involved in clearing Aβ.

Aβ mediated inflammation through CD36

CD36 is a member of the scavenger receptor class B family, initially described as a receptor for thrombospondin [36], and for red blood cells infected with malaria [37]. CD36 is expressed on microglia and is a receptor for Aβ [38]. Aβ binding to CD36 triggers an intracellular signaling cascade involving the Src family members Fyn and Lyn and MAPK (mitogen-activated protein kinase) activation which then activates the microglia and leads to production of cytokines, chemokines and reactive oxygen species (ROS) [39]. It was recently shown that the activation of CD36 by Aβ requires the toll-like receptors TLR-4 and TLR-6; this heterotrimeric complex activates the inflammasome by causing an increase in IL-1β mRNA in microglia [40].

Formyl peptide receptors

The formyl peptide receptors (FPR) bind bacterial ligands and are expressed on microglia. FPRL-1 (formyl peptide receptor like-1) is also capable of binding Aβ 1-42 [41]. and CD11b mediate uptake of fibrillar Aβ and Aβ taken from AD patient brains, and transport it to the lysosomes. Inhibiting phagocytosis by knocking down C3 decreased Aβ uptake by microglia. When fluorescent fibrillar Aβ was injected into C3 deficient mice, there was an increase in Aβ in the brain when compared with control animals, suggesting complement components play an important role in the clearance of Aβ [47].

Aβ binding to microglia can induce the generation of complement components, C1q, C3, C4 and C5a, and stimulate microglia to express complement receptors including C5R. Inhibiting C5R with pharmacological

Aβ interactions with FPRL-1 lead to internalization and cell activation leading to the release of pro-inflammatory cytokines [42], in addition FPRL-1 has been shown to be expressed at a high level on phagocytes surrounding fibrillar Aβ plaques in AD patient brain sections, which suggests FPRL-1 is involved in the uptake of Aβ by microglia, and their subsequent activation [43].

Toll-like Receptors

Members of the family of these evolutionarily conserved receptors expressed on microglia have been shown to bind Aβ, most recently TLR-4 and TLR-6 in combination with CD36 [40]. An AD mouse model also deficient in TLR-4 has an increase in Aβ deposition and cognitive decline, [44] demonstrating a role for TLRs in Aβ removal from the brain. TLR-2 and TLR-4 coupled with CD14 was also shown to bind to fibrillar Aβ, and initiate intracellular signaling through the Src-Vav-Rac pathway leading to ROS production and phagocytosis by microglia [45]. A recent study in human patients showed an increase in TLR-2 and TLR-4 expression and protein levels on circulating monocytes in AD affected individuals compared with controls [46].

Complement receptors

Complement components are part of the phagocytic defense against invading pathogens. Studies with microglia *in vitro* showed the complement component C3 agents inhibited Aβ plaque formation and reduced microglial activation around 50% in a recent study. These finding also correlated with improvements in cognitive functions in two different AD models in mice [48]. Inhibitors of complement also contribute to the development of AD. CD59, which can prevent formation of the membrane attack complex (MAC), was decreased in AD patients compared with controls. In contrast, C9, the terminal MAC component was increased in AD brains [49]. CD59 mRNA expression can also be downregulated by Aβ, suggesting a mechanism by which Aβ regulates complement activity in the AD brain [50].

Aβ degrading enzymes released by microglia
There have been several enzymes identified that degrade Aβ, these include the zinc metalloendopeptidases neprilysin, ECE-1 and ECE-2 (endothelin-converting enzyme), and the related enzymes, IDE (insulin-degrading enzyme) and MMP9 (matrix metalloendopeptidase-9) [51]. Cathepsin B, a cysteine protease has also been shown to degrade Aβ within the endosomes, where internalization and processing of APP occurs [52].

Neprilysin is the most potent Aβ degrading enzyme expressed in the brain [53], capable of degrading monomers and oligomers of Aβ [54]. In an AD mouse model deficient in neprilysin, an increase in Aβ plaques, AD-like pathology and memory loss were observed [55]. However, in one study when neprilysin was overexpressed there was a reduction in overall soluble Aβ levels by 50%, but no prevention of cognitive decline [56]. In a separate study where neprilysin was overexpressed by neurons in a transgenic mouse model, an improvement in memory over transgenic mice expressing normal levels of neprilysin was observed [57]. The distinction between the two studies may be due to the differences in neprilysin overexpression constructs.

Matrix metalloprotease-9 (MMP-9) is an enzyme secreted by microglia that degrades fibrillar Aβ *in vitro* and Aβ plaques in mouse brains [58]. The enzyme is initially secreted as a proenzyme and is cleaved into active MMP-9 upon its release [59]. In the brains of AD patients, levels of MMP-9 have also been shown to increase. The activity of MMP-9 is regulated by tissue inhibitors of MMPs (TIMPs) [60], and MMP-9 is induced by the actions of inflammatory cytokines [61]. Microglia from transgenic PS1APP AD mice showed a general decrease in Aβ degrading enzymes, including MMP-9 at 14 months of age, suggesting microglia in aged mice may have a defect in their ability to clear Aβ [62].

Endothelin-converting enzyme- 1 (ECE-1) has been shown previously to degrade Aβ [63]. ECE-1 is also expressed by microglia [64], and overexpression of ECE-1 in the transgenic mouse model of AD, PS1APP had decreased Aβ plaque levels in their brains [65]. A human study by Funalot et al [66] showed a decrease in ECE-1 levels in AD brains compared with control patient brains. However, a more recent study comparing control and AD patients showed no difference in ECE-1 mRNA or protein expression, nor was there any difference in ECE-1 activity in AD patients. In contrast, neprilysin mRNA and protein expression and enzyme activity was decreased in the AD patients, suggesting that neprilysin is the major protease involved in Aβ degradation [67].

Insulin-degrading enzyme (IDE) is expressed by microglia [68], and has been shown to decrease in AD patient brains compared with control subjects. Transfection of human cells expressing APP, with IDE, upregulated the cell's ability to degrade Aβ 1-42 soluble and fibrillar forms [69]. Another recent study showed activated microglia, in addition to having an increased ability to phagocytose Aβ 1-42 through FPR-2, also upregulated IDE at the protein level to degrade Aβ [70]. In a mouse model of AD deficient in IDE, a 50% reduction in Aβ degradation was observed, with a concomitant increase of the intracellular signaling domain of APP [71]. Patients with sporadic AD were found to have doubled the amount of IDE containing plaques compared to those patients with familial AD which demonstrates potential different etiologies between the two forms of AD [72].

More recently a novel role for Cathepsin-B, a protease found in secretory vesicles, has been described relating to the destruction of oligomeric Aβ. In a study with mouse primary microglia, internalization of oligomeric Aβ mediated by SRA-1 trafficked oligomeric Aβ to the endosomes, where Cathepsin-B facilitated its destruction [73].

Targeting microglia for therapy

Since the microglia surrounding Aβ plaques are activated and engaged in an inflammatory response, they could provide a target for therapies aimed at reducing inflammation, thus lessening the damage to surrounding neurons by the release of cytotoxic mediators. Non-steroidal anti-inflammatory drug (NSAID) use was epidemiologically studied in humans with AD. Although there was no difference in Aβ plaque number between NSAID-treated and controls, there was a significant decrease in microglial activation in the NSAID-treated patients; interestingly NSAID-treated non-diseased individuals also had a reduction in microglial activation [74]. To further explore the role of NSAID in reducing microglial activation, a transgenic mouse model of AD was treated with the NSAID ibuprofen over a 6 month period. NSAID-treated mice had decreased IL-1β protein and Aβ plaques, and fewer activated microglia surrounding Aβ plaques compared with untreated animals [75]. However, experimental dosing of NSAID in human cohorts of AD and control patients did not decrease microglia activation in NSAID-treated patients, suggesting that the activation stage or age of the patients may influence the microglial response to treatments [76].

Another target for AD therapy is TNFα, a cytokine produced by microglia upon stimulation with Aβ [77]. In AD brains, TNFα overexpression has been shown and treatment of transgenic AD model mice with an anti-TNFα antibody decreased TNFα, and Aβ plaque deposition [78]. However, a study using triple transgenic AD mice deficient in TNFα receptors showed an overall decrease in phagocytic activity of microglia, demonstrating a critical role for TNFα in uptake of Aβ. In fact, the total ablation of TNFα may contribute to pathogenesis of AD [79]. A more specifically directed approach to examine the role of TNFα was taken with a study of transgenic AD mice injected either with a lentivirus expressing a dominant-negative TNFα inhibitor, or a pharmacological TNFα inhibitor. Both chronic inhibition with a lentivirus and short-term inhibition with a drug decreased Aβ accumulation in the brains of these mice. The timing of TNFα inhibition was such that AD-like pathology was already established, in contrast to a systemic knocking out of TNFα receptors which could have wider-ranging effects systemically [80].

Controlling microglial actions through their surface receptors is also currently being explored. Inhibiting the CD36-mediated inflammatory signaling pathway initiated by Aβ binding to CD36 could prevent the release of cytotoxic chemokines, and thus reduce neurodegeneration. A recent study by our group discovered ursolic acid acts as a CD36-Aβ inhibitor through development and use of a high-content drug screen. Ursolic acid inhibited the inflammation caused by CD36 intracellular signaling and reduced ROS production by microglia without effecting the ability of the cells to engulf Aβ, it remains to be determined if ursolic acid has an effect of the development of AD *in vivo* [81]. The microglial protein ANXA-1 (annexin A1) is expressed by microglia, and is upregulated during AD. ANXA-1 acts as a bridge between apoptotic neurons expressing phosphatidylserine, and FPRL-2 on the microglia. ANXA-1 restores the ability of microglia to discriminate between healthy and dying cells, the capability of which is reduced in the inflammatory state [82].

Other Cells in the Brain

Microglia interact with a number of other cell types in the brain, and development of AD therapies must take these interactions into account. Of particular interest are interactions with neurons. We have previously shown co-culture of Aβ-stimulated wild-type microglia with CAD neuronal cells lead to significant neuronal cell death, due to release of cytotoxic nitric oxide species by the microglia [40]. The release of ROS by microglia activated by Aβ has also been shown to have an effect on the endothelial cells of the BBB. ROS production caused loss of the tight junction proteins claudin-5 and occludin in brain sections from AD patients,

and a decrease in the BBB integrity [89]. Astrocytes, the most abundant cell in the brain [90] are also capable of binding Aβ and and controlling the actions of microglia. The interplay between microglia and astrocyte secretions may co-ordinate the function of both cell types, particularly through the release of IL-1β and TNFα, which induces other cytokines such as TGFβ. TGFβ acts as an antagonist to IL-1β and TNFα, serving as a negative feedback to control the inflammatory state [92].

Summary

Microglia are an essential cell of the brain, not only do they provide a first line of defense against invading micro-organisms, but they also recognize pathogenic host ligands such as Aβ and tau proteins. The role of microglia in AD is double-edged, initially microglia are activated and are able to clear away deposits of Aβ via SRs such as SRA-1, but become overwhelmed and Aβ plaques are allowed to form. Perhaps this is due to a change in phenotype as we age, resulting in a microglial population that becomes dysregulated. Figure 2 shows a summary of the receptors involved in Aβ clearance and signaling on microglia, and the enzymes involved in Aβ degradation.

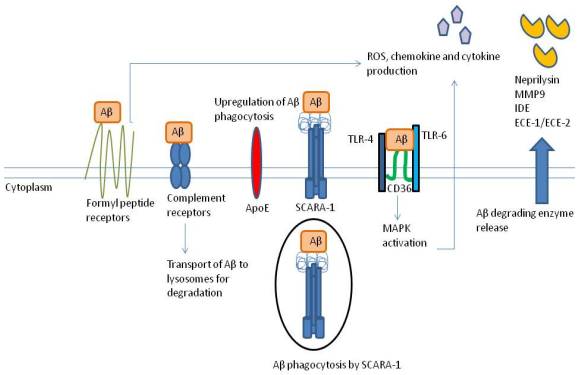


Figure 2. Receptors expressed on microglia involved in Aβ binding and clearance showing the signaling pathways involved and the Aβ degrading enzymes released by microglia.

In addition to microglia, there are other cells in the brain that play an important role in the becoming activated. These cells surround senile plaques and release cytokines, maintaining the inflammatory response [91] development of AD pathology, and could be targets for therapy. Astrocytes are also capable of binding and internalizing Aβ, with a preference for oligomeric Aβ over fibrillar [83]. These cells are the most abundant in the central nervous system and become activated, release cytotoxic chemokines, cytokines and reactive oxygen species (ROS) [84]. TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) induced by ROS has previously been shown to cause neuronal apoptosis through the binding of death receptor 5 and the activation of caspase 8 [85]. Brain endothelial cells form the blood-brain-barrier (BBB), of which the transport of molecules across is regulated by tight junctions. Aβ can disrupt tight junctions and has been shown to decrease the permeability of endothelial cells in culture [86]. The scavenger receptor RAGE expressed on endothelial cells has been shown to bind Aβ [87], allowing Aβ to cross the BBB and enter the brain. In an AD mouse model 5XFAD, Aβ bound to RAGE was shown to perturb tight junctions through calcineurin signaling and MMP-9 secretion [88]. A multi-modal approach considering microglia, astrocytes and endothelial cells will be required to fully understand AD, and to develop therapies against this devastating disorder.

Acknowledgements

I would like to thank Joseph El Khoury M.D. for critical reading of the manuscript

References

1. Jalbert JJ, Daiello LA, Lapane KL (2008) Dementia of the Alzheimer type. Epidemiol Rev 30: 15-34.

2. Matus A (1990) Microtubule-associated proteins and the determination of neuronal form. J Physiol (Paris) 84: 134-137.

3. Jicha GA, Bowser R, Kazam IG, Davies P (1997) Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. J Neurosci Res 48: 128-132.

4. Harper JD, Lansbury PT, Jr. (1997) Models

of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 66: 385-407.
5. McGeer PL, Itagaki S, Tago H, McGeer EG (1987) Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. Neuroscience letters 79: 195-200.
6. Fonseca MI, Ager RR, Chu SH, Yazan O, Sanderson SD, et al. (2009) Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. J Immunol 183: 1375-1383.
7. Bhaskar K, Konerth M, Kokiko-Cochran ON, Cardona A, Ransohoff RM, et al. (2010) Regulation of tau pathology by the microglial fractalkine receptor. Neuron 68: 19-31.
8. McGeer PL, Itagaki S, Tago H, McGeer EG (1987) Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. Neurosci Lett 79:195-200.
9. Thal DR (2012) The role of astrocytes in amyloid beta-protein toxicity and clearance. Exp Neurol 236:1-5.
10. Thinakaran G, Koo EH (2008) Amyloid precursor protein trafficking, processing, and function. The Journal of biological chemistry 283: 29615-29619.
11. Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, et al. (2003) RAGE mediates amyloid- beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med 9:907-913.
12. El Khoury J, Hickman SE, Thomas CA, Cao L, Silverstein SC, et al. (1996) Scavenger receptor-mediated adhesion of microglia to beta-amyloid fibrils. Nature 382: 716-719.
13. Wilkinson K, Boyd JD, Glicksman M, Moore KJ, El Khoury J (2011) A high content drug screen identifies ursolic acid as an inhibitor of amyloid beta protein interactions with its receptor CD36. J Biol Chem 286: 34914-34922.
14. Chan WY, Kohsaka S, Rezaie P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53: 344-354.
15. Fetler L, Amigorena S (2005) Neuroscience. Brain under surveillance: the microglia patrol. Science 309: 392-393.
16. Wilcock DM (2012) A Changing Perspective on the Role of Neuroinflammation in Alzheimer's Disease. Int J Alzheimers Dis 2012: 495243.
17. Cribbs DH, Berchtold NC, Perreau V, Coleman PD, Rogers J, et al. (2012) Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. J Neuroinflammation 9: 179.
18. Perry VH, Matyszak MK, Fearn S (1993) Altered antigen expression of microglia in the aged rodent CNS. Glia 7: 60-67.
19. Ye SM, Johnson RW (1999) Increased interleukin-6 expression by microglia from brain of aged mice. J Neuroimmunol 93: 139-148.
20. Streit WJ, Miller KR, Lopes KO, Njie E (2008) Microglial degeneration in the aging brain--bad news for neurons? Front Biosci 13: 3423-3438.
21. Fiala M, Zhang L, Gan X, Sherry B, Taub D, et al. (1998) Amyloid-beta induces chemokine secretion and monocyte migration across a human blood--brain barrier model. Molecular medicine 4: 480-489.
22. Hale C, Veniant M, Wang Z, Chen M, McCormick J, et al. (2008) Structural characterization and pharmacodynamic effects of an orally active 11beta-hydroxysteroid dehydrogenase type 1 inhibitor. Chem Biol Drug Des 71: 36-44.
23. Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, et al. (2008) Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 4283-4292.
24. Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 8354-8360.

25. Jiang Q, Lee CY, Mandrekar S, Wilkinson B, Cramer P, et al. (2008) ApoE promotes the proteolytic degradation of Abeta. Neuron 58: 681-693.
26. Mandrekar-Colucci S, Landreth GE (2011) Nuclear receptors as therapeutic targets for Alzheimer's disease. Expert Opin Ther Targets 15: 1085-1097.
27. Goldstein JL, Ho YK, Basu SK, Brown MS (1979) Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci U S A 76: 333-337.
28. Wilkinson K, El Khoury J (2012) Microglial scavenger receptors and their roles in the pathogenesis of Alzheimer's disease. Int J Alzheimers Dis 2012: 489456.
29. Hampton RY, Golenbock DT, Penman M, Krieger M, Raetz CR (1991) Recognition and plasma clearance of endotoxin by scavenger receptors. Nature 352: 342-344.
30. Greenberg JW, Fischer W, Joiner KA (1996) Influence of lipoteichoic acid structure on recognition by the macrophage scavenger receptor. Infect Immun 64: 3318-3325.
31. Thomas CA, Li Y, Kodama T, Suzuki H, Silverstein SC, et al. (2000) Protection from lethal gram- positive infection by macrophage scavenger receptor-dependent phagocytosis. J Exp Med 191: 147-156.
32. Peiser L, De Winther MP, Makepeace K, Hollinshead M, Coull P, et al. (2002) The class A macrophage scavenger receptor is a major pattern recognition receptor for Neisseria meningitidis which is independent of lipopolysaccharide and not required for secretory responses. Infect Immun 70:5346-5354.
33. Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, et al. (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proceedings of the National Academy of Sciences of the United States of America 94: 13287-13292.
34. Chung H, Brazil MI, Irizarry MC, Hyman BT, Maxfield FR (2001) Uptake of fibrillar beta-amyloid by microglia isolated from MSR-A (type I and type II) knockout mice. Neuroreport 12: 1151-1154.
35. Huang F, Buttini M, Wyss-Coray T, McConlogue L, Kodama T, et al. (1999) Elimination of the class A scavenger receptor does not affect amyloid plaque formation or neurodegeneration in transgenic mice expressing human amyloid protein precursors. The American journal of pathology 155: 1741-1747.
36. Asch AS, Barnwell J, Silverstein RL, Nachman RL (1987) Isolation of the thrombospondin membrane receptor. J Clin Invest 79: 1054-1061.
37. Ockenhouse CF, Chulay JD (1988) Plasmodium falciparum sequestration: OKM5 antigen (CD36) mediates cytoadherence of parasitized erythrocytes to a myelomonocytic cell line. J Infect Dis 157: 584-588.
38. El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, et al. (2003) CD36 mediates the innate host response to beta-amyloid. The Journal of experimental medicine 197: 1657-1666.
39. Moore KJ, El Khoury J, Medeiros LA, Terada K, Geula C, et al. (2002) A CD36-initiated signaling cascade mediates inflammatory effects of beta-amyloid. The Journal of biological chemistry 277: 47373-47379.
40. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, et al. (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nat Immunol 11:155-161.
41. Iribarren P, Chen K, Hu J, Zhang X, Gong W, et al. (2005) IL-4 inhibits the expression of mouse formyl peptide receptor 2, a receptor for amyloid beta1-42, in TNF-alpha-activated microglia. J Immunol 175: 6100-6106.
42. Pan XD, Zhu YG, Lin N, Zhang J, Ye QY, et al. (2011) Microglial phagocytosis induced by fibrillar beta- amyloid is attenuated by oligomeric beta-amyloid: implications for Alzheimer's disease. Mol Neurodegener 6: 45.
43. Le Y, Murphy PM, Wang JM (2002) Formyl-peptide receptors revisited. Trends Immunol 23: 541-548.
44. Tahara K, Kim HD, Jin JJ, Maxwell JA, Li L,

et al. (2006) Role of toll-like receptor signalling in Abeta uptake and clearance. Brain 129: 3006-3019.
45. Reed-Geaghan EG, Savage JC, Hise AG, Landreth GE (2009) CD14 and toll-like receptors 2 and 4 are required for fibrillar Abeta stimulated microglial activation. J Neurosci 29: 11982-11992.
46. Zhang W, Wang LZ, Yu JT, Chi ZF, Tan L (2012) Increased expressions of TLR2 and TLR4 on peripheral blood mononuclear cells from patients with Alzheimer's disease. J Neurol Sci 315: 67-71.
47. Fu H, Liu B, Frost JL, Hong S, Jin M, et al. (2012) Complement component C3 and complement receptor type 3 contribute to the phagocytosis and clearance of fibrillar Abeta by microglia. Glia 60: 993-1003.
48. Fonseca MI, Ager RR, Chu S-H, Yazan O, Sanderson SD, et al. C5aR Antagonist Decreases Pathology and Enhances Behavioral Performance in Murine Models of Alzheimer's Disease. The Journal of Immunology 183: 1375-1383.
49. Bonifati DM, Kishore U (2007) Role of complement in neurodegeneration and neuroinflammation. Molecular Immunology 44: 999-1010.
50. Shen Y, Li R, McGeer EG, McGeer PL (1997) Neuronal expression of mRNAs for complement proteins of the classical pathway in Alzheimer brain. Brain Res 769: 391-395.
51. Miners JS, Barua N, Kehoe PG, Gill S, Love S (2011) Abeta-degrading enzymes: potential for treatment of Alzheimer disease. J Neuropathol Exp Neurol 70: 944-959.
52. Mueller-Steiner S, Zhou Y, Arai H, Roberson ED, Sun B, et al. (2006) Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease. Neuron 51: 703-714.
53. Shirotani K, Tsubuki S, Iwata N, Takaki Y, Harigaya W, et al. (2001) Neprilysin degrades both amyloid beta peptides 1-40 and 1-42 most rapidly and efficiently among thiorphan- and phosphoramidon-sensitive endopeptidases. J Biol Chem 276: 21895-21901.
54. Kanemitsu H, Tomiyama T, Mori H (2003) Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form. Neurosci Lett350: 113-116.
55. Huang SM, Mouri A, Kokubo H, Nakajima R, Suemoto T, et al. (2006) Neprilysin-sensitive synapse- associated amyloid-beta peptide oligomers impair neuronal plasticity and cognitive function. J Biol Chem 281: 17941-17951.
56. Meilandt WJ, Cisse M, Ho K, Wu T, Esposito LA, et al. (2009) Neprilysin overexpression inhibits plaque formation but fails to reduce pathogenic Abeta oligomers and associated cognitive deficits in human amyloid precursor protein transgenic mice. J Neurosci 29: 1977-1986.
57. Poirier R, Wolfer DP, Welzl H, Tracy J, Galsworthy MJ, et al. (2006) Neuronal neprilysin overexpression is associated with attenuation of Abeta-related spatial memory deficit. Neurobiol Dis 24: 475-483.
58. Yan P, Hu X, Song H, Yin K, Bateman RJ, et al. (2006) Matrix Metalloproteinase-9 Degrades Amyloid-β Fibrils in Vitro and Compact Plaques in Situ. Journal of Biological Chemistry 281: 24566-24574.
59. Nagase H, Woessner JF, Jr. (1999) Matrix metalloproteinases. J Biol Chem 274: 21491-21494.
60. Wilcock DM, Morgan D, Gordon MN, Taylor TL, Ridnour LA, et al. (2011) Activation of matrix metalloproteinases following anti-Abeta immunotherapy; implications for microhemorrhage occurrence. J Neuroinflammation 8: 115.
61. Chakrabarti S, Patel KD (2005) Regulation of matrix metalloproteinase-9 release from IL-8-stimulated human neutrophils. Journal of Leukocyte Biology 78: 279-288.
62. Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J Neurosci 28: 8354-8360.
63. Eckman EA, Eckman CB (2005) Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. Biochem Soc Trans 33: 1101-1105.
64. Davenport A, Kuc R, Plumpton C,

Mockridge J, Barker P, et al. (1998) Endothelin-converting enzyme in human tissues. The Histochemical Journal 30: 359-374.
65. Carty NC, Nash K, Lee D, Mercer M, Gottschall PE, et al. (2008) Adeno-associated viral (AAV) serotype 5 vector mediated gene delivery of endothelin-converting enzyme reduces Abeta deposits in APP + PS1 transgenic mice. Mol Ther 16: 1580-1586.
66. Funalot B, Ouimet T, Claperon A, Fallet C, Delacourte A, et al. (2004) Endothelin-converting enzyme-1 is expressed in human cerebral cortex and protects against Alzheimer's disease. Mol Psychiatry 9: 1122-1128, 1059.
67. Wang S, Wang R, Chen L, Bennett DA, Dickson DW, et al. (2010) Expression and functional profiling of neprilysin, insulin-degrading enzyme, and endothelin-converting enzyme in prospectively studied elderly and Alzheimer's brain. J Neurochem 115: 47-57.
68. Tundo G, Ciaccio C, Sbardella D, Boraso M, Viviani B, et al. (2012) Somatostatin modulates insulin- degrading-enzyme metabolism: implications for the regulation of microglia activity in AD. PLoS ONE 7: e34376.
69. Sudoh S, Frosch MP, Wolf BA (2002) Differential effects of proteases involved in intracellular degradation of amyloid beta-protein between detergent-soluble and -insoluble pools in CHO-‘
695 cells. Biochemistry 41: 1091-1099.
70. Kong Y, Ruan L, Qian L, Liu X, Le Y (2010) Norepinephrine promotes microglia to uptake and degrade amyloid beta peptide through upregulation of mouse formyl peptide receptor 2 and induction of insulin-degrading enzyme. J Neurosci 30: 11848-11857.
71. Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, et al. (2003) Insulin-degrading enzyme
regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci U S A 100: 4162-4167.
72. Dorfman VB, Pasquini L, Riudavets M, Lopez-Costa JJ, Villegas A, et al. (2010) Differential cerebral deposition of IDE and NEP in sporadic and familial Alzheimer's disease. Neurobiol Aging 31:1743-1757.
73. Yang CN, Shiao YJ, Shie FS, Guo BS, Chen PH, et al. (2011) Mechanism mediating oligomeric Abeta clearance by naive primary microglia. Neurobiol Dis 42: 221-230.
74. Mackenzie IR, Munoz DG (1998) Nonsteroidal anti-inflammatory drug use and Alzheimer-type pathology in aging. Neurology 50: 986-990.
75. Lim GP, Yang F, Chu T, Chen P, Beech W, et al. (2000) Ibuprofen suppresses plaque pathology andinflammation in a mouse model for Alzheimer's disease. J Neurosci 20: 5709-5714.
76. Alafuzoff I, Overmyer M, Helisalmi S, Soininen H (2000) Lower Counts of Astroglia and Activated Microglia in Patients with Alzheimer's Disease with Regular Use of Non-Steroidal Anti- Inflammatory Drugs. J Alzheimers Dis 2: 37-46.
77. Medeiros LA, Khan T, El Khoury JB, Pham CL, Hatters DM, et al. (2004) Fibrillar amyloid protein
present in atheroma activates CD36 signal transduction. J Biol Chem 279: 10643-10648.
78. Shi JQ, Shen W, Chen J, Wang BR, Zhong LL, et al. (2011) Anti-TNF-alpha reduces amyloid plaques and tau phosphorylation and induces CD11c-positive dendritic-like cell in the APP/PS1 transgenic mouse brains. Brain Res 1368: 239-247.
79. Montgomery SL, Mastrangelo MA, Habib D, Narrow WC, Knowlden SA, et al. (2011) Ablation of TNF- RI/RII expression in Alzheimer's disease mice leads to an unexpected enhancement of pathology: implications for chronic pan-TNF-alpha suppressive therapeutic strategies in the brain. Am J Pathol 179: 2053-2070.
80. McAlpine FE, Lee JK, Harms AS, Ruhn KA, Blurton-Jones M, et al. (2009) Inhibition of soluble TNF signaling in a mouse model of Alzheimer's disease prevents pre-plaque amyloid-associated neuropathology. Neurobiol Dis 34: 163-177.
81. Wilkinson K, Boyd JD, Glicksman M, Moore KJ, El Khoury J (2011) A high-content drug screen identifies ursolic acid as an inhibitor of amyloid-beta interactions with its

receptor CD36. The Journal of biological chemistry.
82. McArthur S, Cristante E, Paterno M, Christian H, Roncaroli F, et al. (2010) Annexin A1: a central player in the anti-inflammatory and neuroprotective role of microglia. J Immunol 185: 6317-6328.
83. Nielsen HM, Mulder SD, Belien JA, Musters RJ, Eikelenboom P, et al. (2010) Astrocytic A beta 1-42 uptake is determined by A beta-aggregation state and the presence of amyloid-associated proteins. Glia 58: 1235-1246.
84. Li C, Zhao R, Gao K, Wei Z, Yin MY, et al. (2011) Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. Curr Alzheimer Res 8: 67-80.
85. Kwon D, Choi K, Choi C, Benveniste EN (2008) Hydrogen peroxide enhances TRAIL-induced cell death through up-regulation of DR5 in human astrocytic cells. Biochem Biophys Res Commun 372: 870-874.
86. Strazielle N, Ghersi-Egea JF, Ghiso J, Dehouck MP, Frangione B, et al. (2000) In vitro evidence that beta-amyloid peptide 1-40 diffuses across the blood-brain barrier and affects its permeability. J Neuropathol Exp Neurol 59: 29-38.
87. Yan SD, Chen X, Fu J, Chen M, Zhu H, et al. (1996) RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature 382: 685-691.
88. Kook SY, Hong HS, Moon M, Ha CM, Chang S, et al. (2012) Abeta1-42-RAGE Interaction Disrupts Tight Junctions of the Blood-Brain Barrier Via Ca2+-Calcineurin Signaling. J Neurosci 32: 8845-8854.
89. Carrano A, Hoozemans JJM, van der Vies SM, van Horssen J, de Vries HE, Rozemuller AJM (2012). Neuroinflammation and Blood-Brain Barrier Changes in Capillary Amyloid Angiopathy. Neurodegenerative Dis 10:329-331.
[90] Sofroniew, M, Vinters, H (2010). Astrocytes: biology and pathology. Acta Neuropathologica 119:7-35.
[91]. Medeiros R, LaFerla FM (2013). Astrocytes: Conductors of the Alzheimer disease neuroinflammatory symphony. Experimental Neurology 239:133-138.
[92]. Von Bernhardi R, Ramirez G (2001). Microglia astrocyte interaction in Alzheimer's disease: friends or foes for the nervous system? Biol. Res. 34:123-128.