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Clearance of amyloid-β peptides by microglia and macrophages: the issue of what, when and where

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Abstract

Accumulation of senile plaques consisting of amyloid-β peptide (Aβ) aggregates is a prominent pathological feature in Alzheimer's disease. Effective clearance of Aβ from the brain parenchyma is thought to regulate the development and progression of the disease. Macrophages in the brain play an important role in Aβ clearance by a variety of phagocytic and digestive mechanisms. Subpopulations of macrophages are heterogeneous such that resident microglia in the parenchyma, blood macrophages infiltrating from the periphery, and perivascular macrophages residing along cerebral vessels make functionally distinct contributions to Aβ clearance. Despite phenotypic similarities between the different macrophage subsets, a series of *in vivo* models have been derived to differentiate their relative impacts on Aβ dynamics as well as the molecular mechanisms underlying their activities. This review discusses the key findings from these models and recent research efforts to selectively enhance macrophage clearance of Aβ.

Keywords

Alzheimer's disease; a myloid- β peptide; microglia; macrophages; perivascular macrophages; phagocytosis

Alzheimer's disease (AD) is the most common form of dementia and one of the leading causes of death in the elderly population [1]. The disease is clinically characterized by loss of episodic memory progressing toward global cognitive decline [1]. Observations from post-mortem AD brains reveal neuropathlogical changes that include loss of neurons and synapses in cortical and subcortical regions, as well as formation of senile plaques and neurofibrillary tangles consisting of hyper-phosphorylated Tau protein in these regions [2]. Amyloid-β peptides (Aβ) constitute the primary component of senile plaques [2]. Aβ are small soluble peptides commonly 40 or 42 amino acids long produced from a membrane-embedded precursor, amyloid precursor protein (APP), through sequential cleavage by β- and γ-secretases [3]. Aβ can aggregate to form soluble oligomers, large insoluble fibrils and,

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ultimately, plaques [2]. Aβ oligomers are neurotoxic both *in vitro* and *in vivo* [4]. Transgenic mouse lines that produce abnormally high levels of Aβ aggregation and deposition display neurological and cognitive deficits similar to that observed in AD patients [5]. Soluble Aβ including Aβ dimers and oligomers inhibit synaptic plasticity and memory more readily than larger more insoluble Aβ aggregates and may be the primary Aβ species responsible for Aβ-mediated neurotoxicity [6,7]. Although questions remain as to whether Aβ aggregation is a direct cause of AD development, reducing Aβ deposition and aggregation in the brain as a therapeutic means to treat AD has been a central research theme in the field.

One of the major cell types thought to participate in clearing Aβ deposits are brain macrophages. Reports noting the spatial association between senile plaques and brain macrophages in AD date back two decades [8–10]. Such observations prompted debate with regard to the role of these cells in regulating Aβ deposition and neuronal survival [11]. Macrophages are bone marrow-derived cells classically considered as phagocytes and are an integral component of the innate immune system [12]. They are released into the circulation as precursor-like monocytes, which differentiate into tissue macrophages upon extravasation through the endothelium [12]. The primary function of macrophages is phagocytosis of pathogens and cellular debris, although physiologically macrophages also play a role in immunosurveillance and tissue homeostasis [12]. The CNS hosts a unique population of resident myeloid cells termed microglia that are isolated from circulation by the blood–brain barrier (BBB) during early development [13]. Due to the relative absence of other immune cells in the CNS, microglia exhibit several phenotypic and functional differences compared with peripheral macrophages [13,14]. A third population of myeloid cells termed perivascular macrophages line the walls of CNS blood vessels associated with the BBB [14,15]. They are phenotypically similar to circulating peripheral macrophages and are conventionally regarded as a population of migratory blood macrophages [14]. Due to their unique location, however, perivascular macrophages possess functions distinct from both microglia and circulating macrophages [14,15]. The phenotypic and functional differences between these myeloid populations and the implications of these differences with regard to Aβ deposition and clearance are discussed below.

Microglia versus peripheral macrophages

Under physiological conditions, microglia have a ramified morphology and express a low level of CD45 [14,16], a surface marker highly expressed in peripheral macrophages. The ramified morphology is generally associated with a quiescent state where microglia actively survey and maintain homeostatic functions of surrounding cells [17]. Upon insult or stress microglia can transform into an ‘activated’ state that phenotypically resembles peripheral macrophages [13,14,17]. Activated microglia are capable of performing macrophage-like immune functions including cytokine release and phagocytosis [13,17]. The term ‘activated microglia’ is a rather general term. In reality, microglia, like peripheral macrophages, consist of heterogeneous populations with distinct immunological and functional characteristics [12,17,18]. Even under physiological conditions, microglia isolated from different regions of the brain express different levels of phenotypic markers [18]. Although activated microglia

are indistinguishable from peripheral macrophages in many ways, in the context of AD, they appear to have separate functions.

Since the initial discovery of the spatial association between microglia and Aβ plaques, several hypotheses have been formed to explain this distinctive pathology. A series of epidemiological studies in the early 1990s revealed that the incidence of dementia in elderly patients with arthritis was lower compared with a general population of the same age [19]. A twin study later confirmed that taking NSAIDs may account for the difference in AD prevalence [20]. Along with the finding that Aβ plaques in AD brains colocalize with components of the complement cascade, which are products of innate immunity [21–23], the epidemiological observations led to the hypothesis that microglia are proinflammatory in AD and primarily have a detrimental role. Microglia may adopt a proinflammatory phenotype upon attempting to phagocytose and digest Aβ, producing reactive oxygen species and proinflammatory cytokines that induce neurotoxicity [19]. Numerous clinical trials have taken place in which NSAIDs were used to treat AD, with mixed and inconclusive results [19].

As an alternative hypothesis in the early 1990s, Wisniewski *et al.* proposed that resident brain microglia and peripheral macrophages may have different roles in AD [24]. He noted that in AD patients comorbid with stroke, peripheral macrophages infiltrate through the more permeable BBB and were found to have Aβ immunoreactivity within their lysosomes by immuno-electron microscopy [24]. By contrast, microglia that spatially associated with Aβ plaques contained Aβ immunoreactivity in the endoplasmic reticulum, suggesting that they do not actively participate in Aβ phagocytosis but are instead producers of Aβ [25–27]. Observations from a later study by Akiyama and colleagues supported Wisniewski's hypothesis that infiltrating peripheral macrophages and not resident microglia were the primary cells responsible for Aβ phagocytosis and degradation [28]. Akiyama and colleagues noted that the infiltrating macrophages post-stroke contained Aβ that were immunoreactive for C-terminal anti-Aβ antibodies but immuno-negative for N-terminal antibodies, suggesting that Aβ phagocytosed by these macrophages was being digested and degraded [28]. In later studies using mouse models of AD, reconstructed 3D ultrastructural analyses demonstrated that microglia-associated Aβ aggregates were often incompletely surrounded by microglial membranes and that microglia with phagocytic characteristics associate with dystrophic neurites more frequently than Aβ aggregates, instilling further doubt for the role of resident microglia as Aβ phagocytes [29,30].

Since activated microglia are in many ways indistinguishable from infiltrating macrophages, the notion that one is less capable of clearing Aβ than the other becomes an intriguing quandary. Studies examining macrophages derived from AD patients provide supportive evidence for the argument that peripheral macrophages have a major role in Aβ clearance as well as AD etiology. Compared with macrophages derived from healthy individuals, AD macrophages are poorly phagocytic for Aβ, and more susceptible to apoptosis upon Aβ exposure *in vitro* [31,32]. Transcription of several macrophage genes including *MGAT3* and toll-like receptors (TLRs) are also downregulated [33]. Incubation of isolated macrophages with AD brain sections found association of fibrillar Aβ (fAβ) with apoptotic macrophages in congophilic microvessels, suggesting release of fAβ by macrophages as a result of

defective phagocytosis [32]. Phagocytic deficiencies in AD macrophages can be rescued by either curcuminoids or vitamin D3 [33,34]. In contrast to peripheral macrophages, human microglia derived from healthy brains less readily uptake Aβ and display a different cytokine profile in response to Aβ exposure [35]. Furthermore, numerous *in vitro* studies have reported that although cultured microglia phagocytose Aβ, the degradation of fAβ after phagocytosis is inefficient compared with that of peripheral macrophages [27,36–38]. Frackowiak *et al.* reported that Aβ ingested by cultured microglia could remain nondegraded in phagosomes for up to 20 days [27]. A subsequent study by Chung *et al.* observed a similar trend where microglia stopped degrading ingested fAβ after 3 days [37]. Additionally, the authors showed that microglial degradation of soluble Aβ (sAβ) was also poor such that a large portion of ingested sAβ was released by microglia undegraded. In a later study [38], the same research group augmented Aβ degradation by cultured microglia using exogenous targeting of lysosomal enzymes, suggesting that low hydrolytic activity in the lysosomes may account for poor digestion of Aβ by these cells. Other studies revealed that stimulating microglia with the proinflammatory cytokine macrophage colony stimulating factor (M-CSF) can also increase the acidity and hydrolytic activity of microglial lysosomes and subsequently more efficient degradation of fAβ [39–41].

Are microglia immunoincompetent in AD?

The finding that microglial degradation of Aβ can be 'boosted' by endogenous cytokines has important implications. First, the CNS has a limited number of resident bone marrow-derived cells responsible for producing potent proinflammatory cytokines, such as M-CSF. Even in mouse models of AD, the level of these cytokines is minute compared with that observed in other models of inflammation, such as acute microbial challenge [42]. Second, the CNS also lacks regular residence of antibody-producing lymphocytes and professional antigen-presenting cells that augment innate immune responses, such as macrophage phagocytosis [13]. The lack of support and 'priming' from other components of the immune system may account for the apparent poor clearance of Aβ by microglia. Observations from several preclinical and clinical studies provide strong support for this hypothesis. In addition to M-CSF, CNS administration of both granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor (GM-CSF) to mouse models of AD improved clearance of brain Aβ as well as cognitive functions [43–45]. Not only did the treated mice have a higher ratio of microglia internalizing Aβ, the phagocytosed Aβ was localized in lysosomes [43] suggesting that, in line with *in vitro* findings, cytokine stimulation can boost microglial degradation of Aβ. In AD mice preconditioned with a single dose of lipopolysaccharide (LPS), a potent activator of proinflammatory cytokines, Aβ clearance was also increased [46,47], although chronic LPS administration resulted in decreased Aβ clearance and enhanced plaque formation [48].

Although GM-CSF is currently in clinical trials for treatment of AD, shifting the CNS toward a proinflammatory milieu runs the risk of side effects associated with over-production of neurotoxic inflammatory mediators. An alternative approach to enhance the immuno-competency within the CNS is through establishment of adaptive immunity. Schenk *et al.* pioneered the field of immunotherapy in AD in 1999, demonstrating in an AD mouse model that systemic treatment with a vaccine against Aβ prevented plaque formation in

younger mice and slowed plaque accumulation in aged mice [49]. These findings were soon replicated by several independent groups in different AD mouse models, leading to human clinical trials employing active immunization as treatment strategy [50]. Adverse effects associated with vaccination-induced immune responses, in particular aseptic meningoencephalopathy, halted the earlier trials [50]. Researchers have since experimented with other immunization strategies that minimize non-B cell responses, such as adoptive antibody transfer, also known as passive immunization [50]. The profound reduction in Aβ plaques after active or passive immunization came somewhat unexpectedly, considering that production of anti-Aβ antibodies by B cells occurs peripherally. A study using iodinated anti-Aβ antibodies showed a transfer rate of only 0.11% from the circulation to the brain [51]. Nonetheless, in AD brains, this percentage may be higher as several studies have documented AD-associated increase in BBB permeability in both patient and mouse models [52–56]. Despite a potentially low percentage of antibodies crossing the BBB, brains of immunized AD mice contain antibody-bound Aβ deposits [57,58], suggesting that opsonization of Aβ by anti-Aβ antibodies has taken place. Enhanced phagocytosis of Aβ by opsonization is not limited to antibodies. Other Aβ-binding molecules, such as derivatives of curcumin and *scyllo*-inositol, have been shown to promote phagocytosis of Aβ by microglia/macrophages [59–61], supporting the argument that microglial clearance of Aβ is normally inefficient but can be promoted by Aβ-binding molecules, including anti-Aβ antibodies. Although microglial phagocytosis provides one explanation for antibody-mediated Aβ clearance, microglia-independent mechanisms have also been suggested [50]. Collectively, observations from AD immunotherapy and from cytokine administration studies provide useful insights concerning the role of resident microglia in Aβ clearance. Cumulative evidence suggests that microglia without exogenous stimulation or facilitation by adaptive immunity do not appear to have a significant role in Aβ removal.

Lessons from selective ablation of microglia or macrophages

Given the hypothesis that microglia play a limited role in parenchymal clearance of Aβ, the close spatial association between these cells and senile plaques becomes a puzzling occurrence. Results from *in vitro* studies allow only limited interpretations as several phenotypic differences exist between *in vivo* and cultured microglia [13,17]. As an attempt to selectively target CNS microglia *in vivo*, two independent groups have developed transgenic mouse models expressing mutant thymidine kinase (TK) under the CD11b promoter [62,63]. Mutant TK acts as a suicide transgene that converts the antiviral drug ganciclovir into cytotoxic metabolites upon cell proliferation. In the healthy CNS, microglia are mostly nonproliferating and insensitive to ganciclovir treatment [62,63]. However, in AD mice expressing mutant TK, ganciclovir treatment eliminated a significant portion of the microglial population [64]. Since macrophages also express CD11b, by restricting ganciclovir treatment to the brain, only the resident microglia are ablated [64]. Using this approach, Grathwohl *et al.* showed that selective ablation of microglia had no impact on Aβ clearance or neuronal dystrophy in AD mice [64]. The possibility exists that infiltrating macrophages were also affected by ganciclovir upon entry to the CNS. To completely rule out contributions from peripheral macrophages, the authors developed a chimeric model where peripheral blood cells including macrophages were removed by irradiation, sparing

irradiation-insensitive parenchymal microglia [64]. The peripheral macrophage population was then replenished by transplantation of wild-type bone marrow, thus limiting ganciclovir toxicity within the CNS [64]. Again, the authors observed no significant effects on Aβ removal [64]. The study reinforces a bystander role for CNS microglia, suggesting that without exogenous manipulations, these cells are not primary mediators of Aβ dynamics. The experimental paradigm does have several limitations, one being the maximum treatment time of 4 weeks due to the lethal hemorrhages from intracerebroventricular injections of ganciclovir [64]. It has been argued that absence of microglia for a longer duration may be required to have an impact on plaque removal [65]. Additionally, ganciclovir may disrupt other CD11b-expressing CNS cells including pericytes, an integral component of the BBB [66]. Loss of pericytes has been shown to result in compromised BBB and major structural changes in the cerebral vasculature [15]. Age is also a factor; in an independent study, Simard and colleagues used the same treatment paradigm under similar transgenic conditions (APP/PS1:TK) but compared different ages of mice [67]. They also observed no differences in plaque distribution in younger mice less than 6 months of age [67]. In 6-month-old mice, however, ganciclovir treatment resulted in an increased number of plaques [67], suggesting that microglia may become more involved in Aβ removal in older individuals. Regardless, cumulative data from the ganciclovir studies argue against a role for resident microglia as participants in Aβ clearance.

Compared with resident microglia, experimental ablation of peripheral macrophages is considerably less difficult due to their sensitivity toward irradiation. Studies of macrophage involvement in Aβ clearance has benefited from the aforementioned bone marrow transplantation chimeric model where the transplanted macrophages that reconstitute the recipient's immune system are fluorescently labeled to be distinguishable from the host's resident microglia. Since originally derived by Hickey and Kimura in 1988 [68], the model has been widely used to investigate the role of peripheral macrophages in numerous acute and chronic CNS disorders. Several independent studies have transplanted bone marrow into irradiated AD mice and found that transplanted macrophages readily infiltrate into the CNS [67,69,70]. These studies collectively found that the infiltration rate in AD mice is much higher compared with nontransgenic controls, suggesting peripheral macrophages have more access to the CNS in AD conditions [67,69,70]. Stalder and colleagues reported that in irradiated APP23 mice, only a subpopulation of Aβ plaques associated with transplanted macrophages [70]. Ultrastructural analysis also revealed that these macrophages did not appear to phagocytose Aβ [70]. The authors thus propose that modulation of Aβ plaques by peripheral macrophages may be transient through nonphagocytic mechanisms [70]. By contrast, in the APP/PS1 model, Simard and colleagues [67] observed that Aβ immuno-reactivity colocalized with lysosomes in the transplanted macrophages implicating Aβ phagocytosis, in line with the results observed from that in AD patient-derived macrophages [33]. Compared with APP23 mice, the APP/PS1 model has an earlier onset and more aggressive plaque pathology [67,70], raising the possibility that more severe plaque deposition yields an inflammatory milieu favoring Aβ clearance by infiltrating macrophages. The irradiated chimeric model has also been challenged with regard to the effects of irradiation on vessel structure and BBB integrity [71]. Irradiation has also been shown to modify the CNS immune milieu and may therefore influence more pathways than simply

infiltration of peripheral phagocytic cells [72]. Mildner *et al.* reported that when the head is shield-protected during the irradiation procedure, almost no transplanted macrophages could infiltrate the CNS under physiological conditions [71]. Similarly, in a parabiosis model where the peripheral circulations of donor and recipient are linked without BBB disruption, peripheral macrophages also do not infiltrate the CNS [73]. Whether or not in AD patients and AD mouse models the BBB becomes permeable for significant macrophage infiltration remains inconclusive. Increased BBB permeability is a prominent feature in clinical cases of AD [52]. Several studies have also reported BBB disruption in AD mouse models on the basis of serum protein leakage and dye extravasation [53–56]. However, these studies also noted that BBB changes in these mice tend to be subtle and transient, raising doubts as to whether significant infiltration of macrophages can occur [53–56]. Ultimately, the extensive changes to the CNS milieu inflicted by experimental manipulations, such as irradiation and ganciclovir, hamper efforts for clinical translation. However, an alternate approach that is more readily translatable to AD patients is the use of MRI-guided focused ultrasound to transiently open the BBB for delivery of therapeutics or different cell populations [74–77]. This technology is presently in clinical trials for brain tumors [201].

Perivascular macrophages: the missing link?

One subset of peripheral macrophages that may play a crucial role in Aβ clearance are the perivascular macrophages that line the walls of cerebral blood vessels. Despite the unique physical location, they are phenotypically more similar to circulating macrophages than to microglia [14]. The BBB, where perivascular macrophages reside, mediates the efflux of brain Aβ into the peripheral Aβ pool [78]. This pathway, termed the ‘sink effect’, provides an efficient clearance mechanism for CNS Aβ in addition to phagocytosis by microglia/macrophages [78]. Shunting of parenchymal Aβ into the circulation contributes to the development of cerebral amyloid angiopathy, where Aβ deposits accumulate in cerebral blood vessels resulting in vascular abnormalities [79]. Efflux of Aβ is thought to be mediated by cells of the BBB, namely astrocytes, endothelial cells, and pericytes via the ATP-dependent P-glycoprotein pump and chaperone-mediated binding of Aβ to surface receptors, such as lipoprotein receptor-related protein 1 [78]. Being professional phagocytes, perivascular macrophages may complement the BBB in removing larger aggregates of Aβ. Using a phagocyte-specific toxin to selectively ablate macrophages near cerebral vessels, Hawkes and McLaurin showed that depletion of perivascular macrophages in AD mice resulted in increased deposition of Aβ plaques in cerebral vessels [80]. Conversely, stimulation of perivascular macrophage turnover with chitin yielded the opposite effect on plaque accumulation [80]. The authors also noted a lack of colocalization between Aβ and astrocytes or parenchymal microglia near the vessels [80]. These results point toward a role for perivascular macrophages in removing Aβ from the parenchyma. A subsequent study by Mildner and colleagues reached similar conclusions using a different approach [72]. The authors generated transplanted irradiated AD mice with either wild-type or chemokine (C-C motif) receptor 2 (CCR2)-knockout bone marrow. Peripheral macrophages lacking CCR2 do not infiltrate the CNS and yet, Aβ clearance was unaffected [72]. The authors then performed the same transplantation experiment but shielded the head region from irradiation [72]. This paradigm limited the infiltration of peripheral macrophages into the parenchyma

such that macrophages in the perivascular space constitute the majority of the transplanted macrophage population near the brain. The authors could then selectively knockout CCR2 in perivascular macrophages. They found that without head irradiation, AD mice transplanted with CCR2-deficient bone marrow had increased accumulation of Aβ in the brain lysates compared with those grafted with wild-type bone marrow [72], suggesting that normal functioning of perivascular macrophages, which requires CCR2, is essential for brain Aβ removal. To corroborate this result, the authors also generated double transgenic mice harboring both AD pathology and CCR2 knockout [72]. These mice demonstrated increased deposition of Aβ in cerebral vessels as well as more Aβ accumulation within perivascular macrophages compared with control AD mice [72]. Collectively, these results suggest that phagocytic activity of perivascular macrophages is crucial not only for Aβ clearance in cerebral vessels but also in the brain parenchyma. The relative contribution of different macrophage subsets and of other non-phagocytic mechanisms to Aβ clearance remain intriguing scientific questions in mouse models and the potential relevance to AD patients must be carefully examined.

Molecular players involved in Aβ clearance by macrophages

The finding that CCR2 is required for Aβ clearance by perivascular macrophages is intriguing since these cells can be active without parenchymal infiltration. CCR2 is the receptor for MCP-1 (also termed CCL2), a chemokine involved in migration of immune cells including macrophages [81]. As mentioned earlier, stimulation of perivascular macrophage turnover, most likely from circulating monocytes, promoted vascular Aβ clearance [80]. Migration and homing of new macrophages to the sites of plaque accumulation would require chemokine mechanisms, which may explain the requirement for functional CCR2 by perivascular macrophages. Nonspecific deletion of CCR2 in AD mice resulted in increased accumulation of Aβ and less microglia/macrophages surrounding Aβ plaques, as well as worsened cognitive deficits and shortened lifespan [72,82,83]. Notably, *in vitro* phagocytic activity of microglia and peripheral macrophages was unaffected by CCR2 deletion [72], suggesting that migratory and degradation functions are independent but both essential for Aβ clearance. Overexpression of MCP-1 resulted in increased Aβ accumulation in AD mice despite a higher number of microglia/macrophages [84,85]. Although contradictory to *in vivo* deletion studies, this finding confirms *in vitro* observations that the migratory functions of macrophages are independent of their phagocytic activity.

A series of studies examined the role of another chemokine receptor, CX3C chemokine receptor 1 (CX3CR1) in Aβ clearance. Interaction between CX3CR1 and its ligand CX3CL1 (also known as fractalkine) is essential in the communication between neurons and microglia [86–88]. Deletion of CX3CR1 in AD mice, however, yielded opposite results compared with CCR2 deletion, such that CX3CR1-deficient mice had reduced Aβ plaques and increased number of microglia/macrophages surrounding the plaques [86–88]. In contrast to CCR2-knockout macrophages, CX3CR1-deficient macrophages exhibited higher phagocytic capacity for Aβ [86,87]. One explanation is that CX3CR1 signaling regulates both macrophage migration and phagocytosis. Compensation by other stimulation pathways and an overall change in the inflammatory milieu due to genetic manipulation should also be considered. Still, the possibility exists that CCR2-expressing and CX3CR1-expressing

macrophages represent different populations of microglia/macrophages with one preferentially recruited to Aβ plaques.

Genetic manipulations of proteins involved in macrophage phagocytosis and degradation of Aβ provide more consistent results. Aβ plaques in AD brains frequently associate with complement proteins that may opsonize the plaques for phagocytosis [21–23]. Disruption of the complement system by genetic deletion of complement 3 (C3) resulted in enhanced Aβ accumulation in AD mice [89]. Overexpression of soluble complement receptor-related protein y, which inhibits C3 activation, yielded comparable results [90]. With regard to Aβ degradation, deletion of the Aβ-degrading enzyme, neprilysin, in AD mice resulted in increased brain Aβ levels and worsened AD pathology [91–93]. Corroboratively, overexpression of neprilysin in AD mice through viral gene delivery led to decreased Aβ load [94,95]. Additional Aβ-degrading enzymes including insulysin (insulin-degrading enzyme), matrix metalloproteinases (MMPs), and endothelin-converting enzymes are also expressed by microglia/macrophages [96]. *Ex vivo* analysis of microglia isolated from aged AD mice showed decreased expression of neprilysin, insulysin and MMP9, compared with littermate controls [97], suggesting a role for these enzymes in clearance of Aβ by microglia/macrophages. Expression of Aβ-degrading enzymes is, for the most part, ubiquitous among CNS cell types [96]. Cell type-specific knockouts are needed to further clarify the role of these enzymes *in vivo*.

Macrophages express an array of surface receptors that bind Aβ, precursing its uptake and phagocytosis, including the scavenger receptors (SRs) and TLRs [98]. SRs are known for recognition of a wide variety of ligands, many of which are waste products of metabolism [98]. While deletion of class A SR in AD mice had no effect on Aβ levels [99], knockout of SR class B type 1 resulted in enhanced parenchymal and vascular Aβ deposition along with augmented cognitive deficits [100]. In the heterozygous mutant of SR class B type I, Aβ deposits co-localized with SRs in perivascular macrophages [100], implicating a role for SRs in this macrophage subset. Notably, Aβ phagocytsis by macrophages isolated from the knockout mice was unaffected despite increased accumulation of perivascular macrophages in the brain vessels [100]. This result is consistent with that observed in CCR2-deficient mice where migratory and phagocytic functions of macrophages are independent of each other [72]. Genetic manipulation of TLRs yielded similar observations. TLRs are a family of pattern recognition receptors that activate innate immune functions [13,17]. AD mice expressing an LPS inactive form of TLR4 showed exacerbated Aβ accumulation and AD pathology [101,102]. Similar results were observed in AD mice deficient in TLR2 [103]. Notably, activation of TLRs in addition to phagocytosis leads to production of proinflammatory cytokines and reactive oxygen species that promote macrophage infiltration but could also cause neurotoxicity [13,17]. The dual nature of TLR activation is illustrated in AD mice deficient for MyD88, a universal adaptor protein for the TLR family [104,105]. Heterozygous deletion of MyD88 in AD mice resulted in accelerated AD pathology [104], whereas homozygous deletion ameliorated plaque deposition [105], suggesting that the degree of TLR activation may determine whether Aβ clearance mechanisms are promoted or inhibited.

Ultimately, knowledge gathered from genetic manipulations may reveal only pieces of the puzzle as it becomes more apparent that inflammation in AD is a tightly controlled multifaceted system. Efforts have been made to modulate brain macrophages such that proinflammatory production of undesired neurotoxic products are suppressed without compromising migratory and phagocytic activities. The aforementioned CD45, a transmembrane protein tyrosine phosphatase, has also been explored as a potential target to shift microglia/macrophages toward favorable phenotypes [106–108]. Stimulation of CD45 signaling by specific antibodies has been shown to oppose both LPS- and Aβ-mediated activation of microglia, whereas dampening CD45 signaling with phosphatase inhibitors yields the opposite effect [106,107]. Interestingly, Zhu and colleagues demonstrated that in CD45-deficient AD mice, AD pathology is worsened in conjunction with microglia exhibiting decreased phagocytic activity and increased production of proinflammatory effectors [108]. Enhancement of CD45 signaling may in turn have therapeutic potentials in AD. The binding of microglial CD40 to CD40L has also garnered investigation. Classically, CD40–CD40L interaction promotes the transition of innate to adaptive immunity, such that in macrophages, cytokine production increases while phagocytosis decreases [109]. In AD mice, inhibition of CD40–CD40L binding ameliorates AD pathology [110,111]. Evidence from a subsequent culture study suggested that keeping microglia in an ‘innate’ phenotype may account for the amelioration; decreased CD40–CD40L interaction was shown to result in enhanced Aβ phagocytosis and dampened production of proinflammatory cytokines in cultured microglia [109]. In another study, Keene and colleagues derived mice with homozygous deletion in prostaglandin E2 receptor subtype 2 (EP2) [112]. Prior *in vitro* data had shown that microglia lacking EP2 have enhanced phagocytic activity and lowered expression of neurotoxic cytokines [113]. Interestingly, transplantation of bone marrow from EP2-deficient mice into irradiated AD mice yielded enhanced Aβ clearance compared with bone marrow from wild-type mice [112].

With regard to generating favorable macrophage phenotypes, Town *et al.* generated AD mice expressing a dominant-negative form of the TGF-β receptor under the control of the CD11c promoter (CD11c-DNR) [114]. TGF-β is conventionally considered a trophic cytokine that dampens microglial activation [114]. Increased TGF-β expression has been correlated with Aβ accumulation in AD mice [115]. Likewise, over-expression of TGF-β in astrocytes accelerated parenchymal clearance of Aβ into the vasculature [116]. Town *et al.* expected TGF-β signaling inhibition to produce the opposite effect, yet surprisingly, restricting TGF-β inhibition to CD11c-expressing macrophages showed drastic behavioral improvement along with a striking 90% decrease in brain Aβ load [114]. CD11c-DNR mice also had increased number of macrophages surrounding Aβ plaques and near cerebral vessels [114]. Most interestingly, flow cytometry revealed that most of the infiltrating macrophages, identified by high expression of CD45, were negative for Ly-6C, a phenotype characteristic of anti-inflammatory monocytes [114]. This suggests that the infiltrating macrophages were, for the most part, not producing proinflammatory mediators that could potentially induce neurotoxicity. The authors further demonstrated that Aβ phagocytosis in macrophages derived from CD11c-DNR mice was also more active [114]. The immense increase in Aβ clearance observed in the CD11c-DNR mice raises questions with regard to the specific pro- or anti-inflammatory molecules affected by TGF-β signaling inhibition. In the clinical

context, targeting macrophage-specific effectors may yield the best results. Nonetheless, the findings from the studies described above demonstrate that, despite a finely balanced inflammatory milieu in AD, there is potential to selectively target favorable aspects of macrophage activation without unwanted neurotoxicity.

The question of when: aging & dysfunctional microglia

Contrary to generating nontoxic Aβ-clearing macrophages, brains of AD patients harbor microglia that appear to be senescent and dysfunctional. Streit's group first proposed the hypothesis that aging of microglia progressively diminishes their normal function, potentially leading to insufficient Aβ clearance and subsequently AD pathology [117]. Morphological analyses revealed that in brains of aged individuals, some microglia display atrophic cell bodies as well as fragmented processes, morphologically distinct from ramified or activated microglia [117,118]. Streit *et al.* reported that in brains of AD patients, microglia dystrophy preceded the spread of Tau pathology suggesting a role in development of the disease. Interestingly, the dystrophic microglia did not associate with Aβ deposits, but co-localized with Tau structures including neurofibrillary tangles and neuritic plaques [119]. Aβ deposits instead associated with ramified nonactivated microglia [119]. The authors argue that neuroinflammation is not a major component of AD progression and that loss of neuroprotective functions in senescent microglia contributes to disease pathology [119]. This argument provides yet another explanation for the discrepancies between epidemiological and clinical data with regard to the beneficial effect of inhibiting neuroinflammation by NSAIDs [120]. Streit proposed that since microglia represent the major CNS cell type undergoing cell division postdevelopment, they are more susceptible to age-related telomere shortening and subsequent loss of cellular functions [121]. Flanary and Streit demonstrated *in vitro* that telomere shortening occurred in cultured microglia but not in cultured astrocytes [122]. Corroboratively, culture studies from independent groups have demonstrated decreased phagocytosis of Aβ in microglia derived from nontransgenic aged animals [97,123]. Hickman *et al.* reported that microglia isolated from aged AD mice expressed significantly lower levels of Aβ-binding receptors and Aβ-degrading enzymes compared with ones isolated from nontransgenic controls, a pattern not observed in microglia isolated from younger AD mice [97]. Observations *in vivo* appeared less straightforward. Rolyan *et al.* compared the effects of telomerase deletions between AD and normal mice and found that while telomere shortening yielded cognitive deficits in normal aging mice, it improved the deficits in AD mice [124]. In accordance with Streit's argument, telomerase deletion in AD mice resulted in less microglial activation [124]. However, Aβ accumulation also decreased in telomerase-deficient AD mice suggesting that microglial activation promotes Aβ deposition [124]. Since the telomeres of cell types other than microglia are also shortened in these models, it is difficult to conclude the precise role of telomere shortening in Aβ clearance by microglia and macrophages. The potential for generation of microglia/macrophage-specific telomerase deletions may provide important validations for the microglial senescence theory.

Conclusion & future perspective

The current review summarizes the experimental evidence for the relative contribution of different macrophage subsets in clearance of brain Aβ. Despite a high degree of phenotypic similarity between resident microglia and peripheral macrophages, innovative *in vivo* models that selectively ablate one population have yielded key insights. Cumulative evidence thus far point toward a limited role for resident microglia in Aβ removal despite having the most direct access to parenchymal Aβ. Although peripheral macrophages more readily phagocytose and degrade Aβ, questions remain as to whether they can significantly infiltrate the brain in clinical cases of AD. Perivascular macrophages in the cerebral blood vessels represent a third macrophage population and may function in concert with other phagocyte-independent efflux mechanisms shunting parenchymal Aβ to the periphery, complementing phagocytosis in the CNS. Dichotomous activity of cerebral and perivascular macrophages constitutes a tightly-controlled system for Aβ removal. Indeed, a series of gene deletion studies reveals a delicate balance between pro- and anti-inflammatory macrophage responses governed by complex molecular mechanisms. To achieve the optimistic therapeutic goal, such that macrophages efficiently clear Aβ without producing undesired neurotoxic effects, much more work is required in understanding the factors that control this balance.

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Executive summary

- Alzheimer's disease (AD) brains are characterized by deposition of senile plaques consisting of amyloid-β peptides (Aβs). Clinical and experimental evidence implicate a role for macrophages in Aβ clearance and overall course of the disease.
- Resident brain macrophages, or microglia, have a limited role in Aβ clearance unless further stimulated.
- Peripheral macrophages that infiltrate the brain during AD mediate Aβ clearance as shown by transplantation experiments in bone marrow-irradiated AD mice. It remains a question whether significant macrophage infiltration occurs in clinical cases of AD.
- Perivascular macrophages near cerebral vessels are important in clearing vascular Aβ deposited from the brain parenchyma and in turn may have a more significant role in parenchymal Aβ clearance compared with microglia and infiltrating macrophages.
- Aβ clearance by macrophages is dependent on migratory signals, Aβ-binding receptors, and activity of Aβ-degrading enzymes.
- Microglia/macrophage clearance of Aβ may diminish with age.