

Microglia-Mediated Neuroprotection, *TREM2*, and Alzheimer's Disease: Evidence From Optical Imaging

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ABSTRACT

Recent genetic studies have provided overwhelming evidence of the involvement of microglia-related molecular networks in the pathophysiology of Alzheimer's disease (AD). However, the precise mechanisms by which microglia alter the course of AD neuropathology remain poorly understood. Here we discuss current evidence of the neuroprotective functions of microglia with a focus on optical imaging studies that have revealed a role of these cells in the encapsulation of amyloid deposits ("microglia barrier"). This barrier modulates the degree of plaque compaction, amyloid fibril surface area, and insulation from adjacent axons thereby reducing neurotoxicity. We discuss findings implicating genetic variants of the microglia receptor, triggering receptor expressed on myeloid cells 2, in the increased risk of late onset AD. We provide evidence that increased AD risk may be at least partly mediated by deficient microglia polarization toward amyloid deposits, resulting in ineffective plaque encapsulation and reduced plaque compaction, which is associated with worsened axonal pathology. Finally, we propose possible avenues for therapeutic targeting of plaque-associated microglia with the goal of enhancing the microglia barrier and potentially reducing disease progression.

Keywords: Alzheimer's disease, Axonal dystrophy, Microglia barrier, Neuroprotection, Optical imaging, *TREM2*

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Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, is characterized by slowly progressive cognitive decline that has devastating personal and socioeconomic implications. Despite significant efforts to develop treatments the field has seen little success, partly due to an incomplete understanding of the mechanisms underlying disease pathogenesis. The defining neuropathological criteria for AD are extracellular deposits of aggregated amyloid- β ($A\beta$) peptides and intracellular neurofibrillary tangles composed of hyperphosphorylated microtubule-associated protein (MAP) tau. A consensus is that $A\beta$ deposition occurs first, triggering intracellular neurofibrillary tangles formation, synapse loss, cell death, and cognitive decline (1,2). However, the sequence of events leading to protein aggregation and the relative contributions of $A\beta$ and tau aggregates to neural toxicity and glial reactions remain incompletely understood.

$A\beta$ peptides are produced by enzymatic cleavage of the extracellular domain of the amyloid precursor protein (3) and are continuously released into the interstitial space. Peptides with different lengths are produced, with $A\beta$ 40 being the most abundant isoform but $A\beta$ 42 having the highest propensity to aggregate and greatest potential cytotoxicity (1). These peptides can polymerize into oligomers and fibrils, with low-molecular-weight species (e.g., dimers, trimers) being water soluble and diffusible (4), while protofibrils and fibrils become insoluble and have a tendency to coalesce into aggregates that deposit within the brain parenchyma and microvasculature (5).

Once extracellular aggregation occurs, these deposits become a sink where newly formed $A\beta$ monomers bind with high affinity, causing gradual plaque enlargement over very long intervals (6–9). Postmortem clinical-pathological correlations and positron emission tomography (PET) imaging of AD patients has revealed that the buildup of $A\beta$ precedes cognitive deficits by decades (10,11). This suggests that a critical threshold of $A\beta$ burden must be reached to instigate cognitive decline. However, there is a relatively weak correlation between plaque load and cognitive scores (12,13), suggesting that additional factors contribute to modulating neural injury caused by amyloid deposits.

One such factor may be the microglial and astrocytic responses that occur around amyloid deposits. Microglia are yolk sac-derived cells (14) that share functional and molecular features with tissue macrophages (15–17), and function as resident immune cells in the central nervous system. Microglia are found throughout the central nervous system where they are tiled into nonoverlapping domains. Under homeostatic conditions, microglia are highly branched and motile, constantly extending and retracting while their cell body remains stationary (18,19). The function of this dynamic behavior is poorly understood, but it may serve a surveillance role for detection of tissue homeostatic and pathological changes (20,21). Recent work suggested that microglia may have additional physiological roles in the healthy brain such as refining synapses (22,23), monitoring synapse activity (24), and

providing trophic support for neuronal plasticity (25). In response to pathogenic stimuli, cell debris, and physical injury, microglia rapidly transform into activated phenotypes involving proliferation, increased phagocytosis, and production of proinflammatory cytokines (20). In AD, microglia cluster around A β deposits and adopt a polarized morphology with hypertrophic processes extending toward plaques (26–28). Microglia are thought to regulate the degree of amyloid deposition by phagocytosis of amyloid aggregates with potentially protective impact on AD progression (29,30). However, chronic microglial activation may be associated with production of neurotoxic inflammatory cytokines and reactive oxygen species (31), and microglia have been suggested to phagocytose synapses under pathological conditions (32,33); therefore, they could exert deleterious effects that contribute to disease pathogenesis. Thus, it remains unclear if microglia have a net protective or harmful effect.

The role of microglia in AD has recently gained renewed impetus owing to the identification of rare coding variants associated with AD in genes highly expressed in these cells (34,35), providing strong evidence that microglia may contribute directly to the pathogenesis of this disorder. The strongest of these associations are variants in *TREM2*, a gene that in the brain is virtually exclusively expressed in microglia (36). Recent evidence suggests that microglia exert neuroprotective functions that are impaired in individuals with *TREM2* variants resulting in increased AD risk (34,35,37). Here, we review the biology of microglial neuroprotection in AD, with special emphasis on a previously unrecognized role for these cells in the encapsulation of amyloid plaques, which has marked effects on the conformation and toxicity of amyloid deposits and their insulation from adjacent neuronal processes (27,38). We discuss studies using high-resolution optical imaging in live mice and postmortem human brain that have provided supporting evidence for these neuroprotective functions and the modulatory role by *TREM2*. Finally, we discuss the implications of these findings regarding therapeutic interventions and diagnostic imaging.

***TREM2* VARIANTS HIGHLIGHT A PROTECTIVE MICROGLIAL FUNCTION IN AD PATHOGENESIS**

Although microglia could play a significant role in AD pathogenesis through A β phagocytosis or secretion of proinflammatory cytokines, evidence supporting their involvement in AD has not been definitive. AD patients on chronic anti-inflammatory treatment did not show any cognitive benefits (39), suggesting that neuroinflammation was not a major disease driver. In addition, mutations in genes expressed by microglia (*CR1*, *HLA-DRB1*, *CD33*, *MS4A6A*) modified AD risk only modestly ($0.9 < \text{odds ratio} < 1.1$) (40). In contrast, single nucleotide polymorphisms in a microglia-specific gene, *TREM2*, were found to be strongly associated with late-onset AD (odds ratio = ~ 3 ; see meta-analysis in Figure 1) (34,35). Moreover, mutations in the *TREM2*-signaling partner (*TYROBP*; also known as *DAP12*) also increased AD risk (37). Although *TREM2* is also expressed in peripheral monocytes (41), these cells appear to play a limited role in AD pathogenesis because they do not significantly enter the normal (42) or neurodegenerative brain (42,43) in mice, or humans with AD

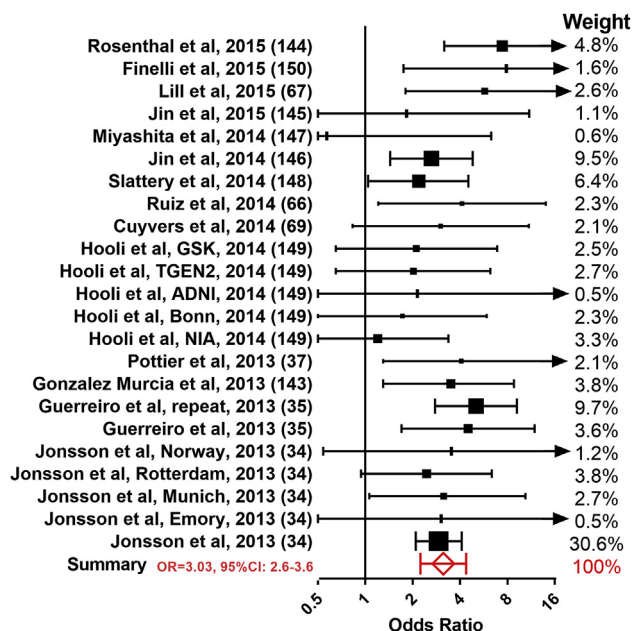


Figure 1. Meta-analysis on the association of R47H *TREM2* mutation and the risk of developing Alzheimer's disease (AD). Studies (34,35,37,66,67,69,143–150) were selected from the combined search results of *rs75932628 Alzheimer* and *TREM2 R47H Alzheimer* on PubMed (total of 64 search results as of September 2017), with the following criteria: 1) case-control studies examining the risk for late-onset AD associated with the single nucleotide polymorphism *rs75932628* (19 studies); and 2) the studies have at least one *TREM2* R47H subject in the case and control groups (14 studies). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by combining raw data from all studies. Arrows in the graph indicate values exceeding the axis limits. The heterogeneity among studies does not reach statistical significance with Cochran's Q test.

(15,43–45). Therefore, for the first time, there is unequivocal evidence that certain microglial functions are robustly involved in AD pathogenesis.

Triggering receptor expressed on myeloid cells 2 (*TREM2*) is a single-pass transmembrane protein that was known to regulate immune responses in peripheral macrophages (41,46) by means of lipopolysaccharide binding and bacterial phagocytosis (47). In addition, *TREM2* attenuates inflammatory cytokine release from macrophages (41), promotes their differentiation and survival, and stimulates phagocytosis (48). *TREM2* knockout (*TREM2*^{−/−}) microglia exhibit reduced clearance of dying cells (49), or myelin debris (50,51); and loss of *TREM2* increases tumor necrosis factor alpha, interleukin-1 β , and interleukin-6 release upon lipopolysaccharide stimulation (52). Individuals with severe loss of *TREM2* function due to homozygous mutations develop polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu-Hakola disease) (53), possibly owing to abnormalities in microglia-oligodendrocyte interactions (54,55).

However, it remains unclear how *TREM2* mutations contribute to increased AD risk. Given that mutations in *TREM2* (i.e., R62H, R47H, and D87N) are all associated with increased AD risk, it is likely that they cause loss rather than gain of function. Indeed, expression of mutant *TREM2* in cultured cells led to disruption in its translocation to the cell membrane, and

diminished ligand binding (56,57). Therefore, mutations in *TREM2* lead to a loss-of-function phenotype like *TREM2* knockout, with apparent reduced phagocytosis and inflammation. However, there is conflicting data on whether *Trem2* knockout increases or decreases overall plaque burden (58–60), even though *TREM2*^{−/−} microglia have reduced phagocytic capacity in culture (56,61). Furthermore, *Trem2* deficiency does not seem to increase inflammation given that cytokines were reduced in AD mice lacking *Trem2* (60,62). The extracellular domain of *TREM2* can be cleaved into a soluble fragment (s*TREM2*), which can stimulate cytokine release (63), but the R47H mutation may reduce its capacity for inducing cytokine secretion (63). Therefore, *TREM2* mutations are unlikely to increase the risk of AD through an inflammatory mechanism.

While the changes observed in phagocytosis and cytokine production are subtle, loss of *TREM2* dramatically disrupts microglial engagement with amyloid plaques. Several groups have reported that *TREM2* deficiency in AD mice leads to reduced microglial numbers around amyloid deposits (59,60,62) owing to lower proliferation rates (38,43,64), reduced metabolic fitness (65), and increased death (60). Furthermore, the polarization of microglial processes toward the plaque surface is markedly reduced in mice with *Trem2* haploinsufficiency (38). A similar polarization deficiency, albeit to a lower degree, occurs in humans carrying a single allele of the R47H *TREM2* mutation (38). The reduction in polarization and plaque encapsulation observed in *TREM2* deficiency suggests that this microglial function may play important roles in AD pathogenesis. However, human *TREM2* variants appear to also modestly increase the risk of non- $A\beta$ -based disorders such as amyotrophic lateral sclerosis, frontotemporal dementia (66–70), and Nasu-Hakola disease in homozygous mutants (53,71). This suggests that *TREM2* deficiency may affect additional mechanisms independent of amyloid phagocytosis or plaque encapsulation, such as efficient corpse removal of dying cells (52,72) or degenerating myelin (50,51), or additional unknown *TREM2* functions.

$A\beta$ PHAGOCYTOSIS: WHAT DO MICROGLIA REALLY EAT IN VIVO?

Given that $A\beta$ can disrupt synaptic transmission, induce oxidative stress and trigger cell death in vitro (4), microglial phagocytosis of $A\beta$ could be a neuroprotective function. Microglia have been shown to internalize fluorescently tagged synthetic $A\beta$ in vitro or after infusion into the mouse brain in vivo (73,74). Imaging of mouse or AD human tissue reveals some $A\beta$ inside microglial phagolysosomes (73,75), consistent with their ability to phagocytose $A\beta$ in vivo. Moreover, their role in $A\beta$ clearance has been demonstrated by genetic manipulation of chemokine or pattern recognition receptors. Loss of *CCR2* (76), *CD45* (77), or *TLR4* (78) in microglia exacerbated amyloid load, while *CX3CR1* or *NLRP3* deficiency increased microglial phagocytosis and reduced amyloid burden (73,79,80). Moreover, passive immunization with anti- $A\beta$ antibodies reduced fibrillar amyloid deposition in transgenic mice (81–83), and potentially in humans as assessed by PET scanning (84,85) and postmortem histology (86,87). Thus, under certain conditions microglial phagocytosis of $A\beta$ can reduce the overall amyloid burden.

However, the exact $A\beta$ species that microglia can gobble up remains controversial. $A\beta$ exists in a variety of conformations and sizes, with nascent $A\beta$ polymers forming dimers, oligomers and protofibrils, and plaques, which are composed of β -sheet rich fibrils (3). One possibility is that microglia are not selective and phagocytose all $A\beta$ species, including mature fibrillar plaques (29,30). However, immunohistochemistry with conformation specific antibodies revealed that microglial lysosomes contain oligomers and protofibrils, but not β -sheet rich amyloid fibrils (73). Furthermore, time-lapse in vivo imaging of individual plaques labeled by a single pulse of a β -sheet binding dye showed no change in plaque shape over months (73), indicating no significant removal of $A\beta$ fibrils by adjacent microglia. Consistently, in vivo studies that relabeled plaques before each imaging time-point observed gradual growth and no disappearance of plaques (6–9), even after anti- $A\beta$ immunization (88). Moreover, using a β -secretase inhibitor (89) or a regulatable transgene to turn off amyloid precursor protein expression (82,90) during $A\beta$ immunotherapy remained ineffective in clearing preexisting plaque cores, although they did appear to reduce the diffuse protofibrillar $A\beta$ halo surrounding them (82). In contrast, experiments tracking fluorescently tagged $A\beta_{42}$ monomers infused into the subarachnoid space, which rapidly bound to the protofibrillar plaque halo, did not show significant removal over intervals up to 90 days (27). Collectively, these experiments indicate that under normal circumstances microglia do not efficiently remove $A\beta$ fibrils from compact plaques or protofibrillar halos, but may be able to phagocytose nascent $A\beta$ polymers. Therefore, microglial phagocytosis has the potential to reduce seeding of new plaques (73,74) but may have a limited effect once seeding has occurred. Consistent with this, ablation of microglia for 1 month did not change either soluble $A\beta$ levels or plaque numbers in aged mice (91–94). However, these studies followed animals for short intervals and in advanced stages of amyloidosis, which may have led to underestimating phagocytosis. Indeed, a recent paper demonstrated that ablation of microglia led to modest growth of the plaque halo (95). Although this could be due to a loss of ongoing microglial phagocytosis leading to plaque growth (27,82), it is also possible that growth is due to the loss of microglial encapsulation, which restricts $A\beta$ polymerization and outward fibril extension (see discussion below).

MICROGLIAL PROCESSES FORM A NEUROPROTECTIVE BARRIER AROUND PLAQUES

Microglial processes are highly intertwined, with fibrils protruding from the plaque core (26,28). Intravital imaging in mice revealed that as amyloid deposits form, microglia concurrently cluster around and polarize their processes toward the plaque surface (96). Once enveloped around plaques, microglial processes can remain anchored to the plaque with little motility over weeks (27), in contrast to their constant motility in the normal brain (18,19) or in microglia distant from plaques (73). Close inspection revealed that plaque regions wrapped by microglial processes appeared compact as evidenced by intense labeling with thioflavin S, while those microregions not covered by microglia appeared more diffuse (27). Interestingly, small molecule dyes like curcumin and THK-265 preferentially

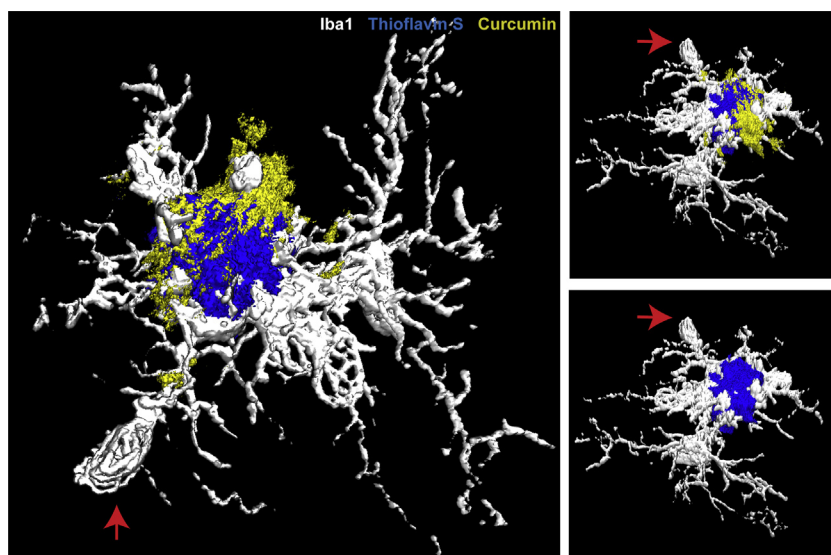


Figure 2. Microglial barrier and protofibrillar A β hotspot around amyloid plaques. Example three-dimensional reconstruction of a confocal image stack showing microglial coverage around an amyloid deposit in a 5xFAD mouse. Curcumin-labeled protofibrillar A β accumulates in the plaque regions not covered by microglial processes (27).

labeled plaque regions not covered by microglia (Figure 2) (27), possibly reflecting their affinity for protofibrillar A β conformation (97). These data are consistent with the possibility that the tight wrapping of microglia behaves as a physical barrier that limits the outgrowth of fibrils and compacts them into a conformation with high affinity for β -sheet binding dyes.

What are the consequences of the presence of these hotspots of protofibrillar A β in areas not covered by microglia? To test this, several measures of axonal dystrophy were quantified. Remarkably, plaque microregions not compacted by microglia were associated with a greater extent of dystrophic axons (27). One possible reason for this is that the freely extending fibrils not insulated by microglia protrude into the parenchyma and cause physical damage to neurites. Alternatively, the protofibrillar A β conformation in areas not covered by microglia may be more neurotoxic, consistent with *in vitro* data (98). These and other findings prompted a new hypothesis for the role of plaque-associated microglial processes. We postulated that the tight envelopment of microglia around the amyloid surface constitutes a neuroprotective barrier that limits fibril outgrowth and plaque-associated toxicity. Consistent with this, depletion of microglia in an AD mouse model showed increased plaque outgrowth and dendritic spine loss and shaft atrophy in adjacent neurons (95). Given that plaque-associated axonal dystrophy has been shown to be a good correlate of cognitive dysfunction (99,100), this pathology may be a significant contributor to neural circuit disruption in AD. Thus, the microglial encapsulation function may play a role in preventing AD-associated neural dysfunction.

In AD mouse models haploinsufficient for *Trem2* (38,59,60,62) or DAP12 (38,101), microglial clustering around plaques was found to be significantly reduced. Importantly, microglial process polarization was also dramatically diminished, leading to a near-complete loss of plaque encapsulation (38). As a consequence, plaques became much more diffuse, with their morphology shifting from one with compact borders to one with outward projecting fibers resembling a sea urchin (38,43). Furthermore, superresolution optical

microscopy revealed that in *Trem2*-deficient mice, individual A β filaments appeared to have a greater number of side branches (38). The increase in outwardly projecting fibers with greater number of branches would be predicted to significantly increase the total A β fibrils surface area that can be exposed to surrounding neural structures, with potentially damaging effects. Consistent with this view, the extent of plaque-associated axonal dystrophy was exacerbated in mice lacking *Trem2* or DAP12, supporting the hypothesis that the microglial barrier is a neuroprotective function. Importantly, heterozygous human carriers of the R47H *TREM2* mutation also had disrupted microglial clustering and barrier formation around plaques. Similar to *Trem2*-haplodeficient mice, R47H mutants exhibited an increase in the number of less compact and filamentous deposits as well as a greater extent of dystrophic axons and neuronal processes with hyperphosphorylated tau protein (38). The phenotypic resemblance in humans and mice suggests that the microglial barrier is a shared mechanism to limit plaque-associated neural damage. However, the conformational plaque phenotype in R47H human carriers is less dramatic than in mice. One likely explanation is that the R47H variants constitute only a partial loss of function, with less severe barrier disruption. Alternatively, the markedly faster rates of amyloid accumulation in mice may overwhelm the capacity of microglia, leading to a more severe phenotype. Interestingly, however, humans with R47H variants did have a robust disruption of axons as evidenced by the degree of axonal dystrophy and tau protein hyperphosphorylation. Thus, in humans the main protective function of microglia may be their ability to insulate plaques from the surrounding tissue, while their role in plaque compaction may be more limited.

CELLULAR MECHANISMS INVOLVED IN THE MICROGLIAL BARRIER FUNCTION

Microglial sensing of amyloid deposits and their polarization toward plaques are likely important steps in the formation of an

effective barrier. *TREM2* may serve as both the receptor for recognizing plaque components and the trigger for downstream cytoskeleton reorganization that is required for process polarization. *TREM2* does not bind to A β per se, but has affinity for lipids found on plaques (60). *TREM2*-lipid mediated signaling may be critical for barrier formation. A single point mutation in the arginine-47 (R47) residue within the *TREM2* ligand-binding domain (102) leads to reduced lipid affinity (60) and disruption of microglial clustering and plaque encapsulation (38). Intriguingly, microglia do not form barrier processes around diffuse plaques. Unlike compact plaques, diffuse deposits are not decorated with lipids (103,104), suggesting that lipidation of A β is a key step in *TREM2*-mediated microglial polarization. Upon lipid binding in vitro, the intracellular domain of *TREM2* can trigger downstream DAP12-mediated immunoreceptor tyrosine-based activation motif (ITAM)-signaling cascade, PI3K pathway activation, and cytoskeletal reorganization (52). DAP12 and phosphorylated tyrosine are upregulated and colocalized with *TREM2* in the polarized microglial processes, and disrupting this signaling pathway in mice by deletion of DAP12 abolished the microglial barrier (38). However, single-cell RNA sequencing (15) or immunohistochemistry (38,44) has shown that *TREM2* signaling appears to be activated only after microglia have fully engaged around plaques. This raises questions as to how *TREM2* sensing of the plaque can occur prior to its upregulation (38) and precisely at what stage of plaque engagement *TREM2* signaling becomes critical. Nevertheless, evidence argues that *TREM2*-DAP12 signaling mediates plaque sensing and is likely involved in the microglial process polarization necessary for barrier formation.

The exact process by which microglia induce plaque compaction remains unknown. One possibility is that microglial processes clear the diffuse protofibrillar A β at the plaque halo through phagocytosis and/or secretion of proteolytic enzymes, leading to the appearance of a more compact core in areas covered by microglia. However, these microglial processes are not optimally positioned to remove prefibrillar A β because they are closely anchored to the perimeter of the compact plaque core (27) rather than at the site of the less compact plaque halo and under normal conditions have been shown to have a limited capacity for phagocytosis of protofibrillar A β (73,82). Therefore, instead of phagocytosis, we propose that microglial processes wrapping around deposits create an insulated and crowded macromolecular environment that leads to accelerated A β aggregation (105), resulting in plaque compaction. Also, compact plaque regions have decreased affinity for monomeric A β 42, which may further limit the outward outgrowth of protofibrils once deposits become compact. The reduced affinity for A β 42 could be due to microglia exerting a direct force on outwardly growing amyloid fibrils leading to their bending, which may mask their growing ends and prevent their elongation. Microglia may reduce fibrillization by physically preventing the entry of monomeric A β 42 into the core region. However, in vivo infusion of A β 40, which has a molecular weight similar to A β 42, demonstrates full penetration throughout the plaque. This and other experiments (27,73), suggest that microglia do not prevent entry of monomeric A β 42 but rather change the affinity to A β 42 in areas covered by microglial processes,

likely by altering the conformation and compaction of accumulating protofibrils.

FAILURE OF THE MICROGLIAL BARRIER AS A GENERAL MECHANISM IN THE DEVELOPMENT OF AD

While *TREM2* loss-of-function mutations are found in a small percentage of AD patients (~0.5%) (34), a defective microglial barrier could also be a risk factor for the development of late-onset AD. Indeed, like the defective barrier seen in *Trem2*- or DAP12-deficient mice (38), comparison of plaques of similar size between young and old wild-type AD mice revealed that microglial coverage was significantly reduced in aging, and as predicted this was associated with enlarged protofibrillar A β halos as well as greater axonal dystrophy (27). Multiple mechanisms may contribute to defective microglial encapsulation, given that aging is associated with complex molecular and cellular changes (106), including reduced cell proliferation (107). Indeed, bromodeoxyuridine incorporation in plaque-associated microglia is significantly reduced in aging (27), suggesting that reduced proliferation limits the number of microglia available for plaque encapsulation. In addition, microglia in aging display tortuous processes and focal swellings (21,107), suggesting cellular dysfunction that may impair polarization toward plaques. In addition, aging microglia may be less phagocytic and adopt an activated phenotype with release of proinflammatory cytokines (108), and a reduction in anti-inflammatory cytokines such as transforming growth factor- β (109). Thus, as protective microglial functions such as phagocytosis and plaque encapsulation fail in aging, their chronic activation and changes in cytokines may increase their neurotoxic potential.

Recent studies have suggested potential modulation of microglia by mechanisms involving apolipoprotein E (APOE). APOE is known for its role in brain lipid and cholesterol transport. The *APOE* gene has three polymorphic alleles (ϵ 2, ϵ 3, and ϵ 4), and genome-wide association studies have shown that carriers of one ϵ 4 allele have two to three times increased risk of AD, while ϵ 4/ ϵ 4 have ~15 times increased risk (110). The *APOE* ϵ 4 allele is also the most prevalent AD risk factor, estimated to be present in ~14% of the global population and 37% of AD patients (111). Recent studies uncovered an interaction between APOE and *TREM2* in vitro, where recombinant *TREM2* exhibited high binding affinity to APOE (112,113). Interestingly, this affinity was reduced in mutated *TREM2* (R46A, R47A, R47E, and R47H), suggesting a correlation between loss of APOE-*TREM2* interaction and increased AD risk. Because APOE can be found on the plaque surface (114), it is possible that APOE provides a targeting signal for *TREM2*-expressing microglial processes. However, it remains unknown whether the different APOE isoforms bind to *TREM2* with the same affinity. Interestingly, APOE ϵ 4, but not APOE ϵ 3, was shown to activate toll-like receptors in microglial cultures, leading to reduced *TREM2* expression (115), and thus it is possible that APOE ϵ 4 could disrupt *TREM2*-mediated microglial polarization toward plaques in vivo. Consistent with this, in APOE ϵ 4 isoform-specific knockin mice, microglia adopted an inflammatory phenotype and displayed abnormal processes around plaques compared with ϵ 2 and ϵ 3 knockin mice

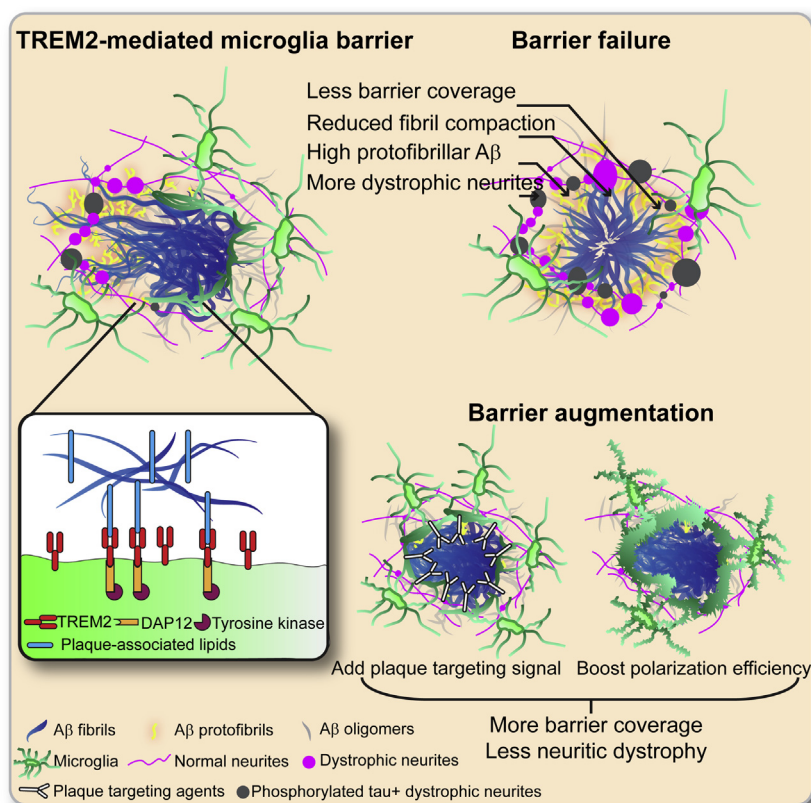


Figure 3. Modulating microglial barrier alters plaque-associated protofibrillar amyloid- β (A β) content, axonal dystrophy, and tau protein hyperphosphorylation. Triggering receptor expressed on myeloid cells 2 (TREM2) on microglial processes binds to the lipid and lipoprotein components of amyloid plaque, triggering the downstream signaling cascade involving DAP12 and Syk tyrosine kinase. This signal leads cytoskeletal reorganization and to the polarization and expansion of the microglial processes, and the formation of microglial barrier, which increases the compaction of adjacent amyloid fibrils. Deficiency in the microglial barrier results in loosely organized amyloid fibrils, protofibrillar A β accumulation, increased axonal dystrophy formation, and neuronal process tau protein hyperphosphorylation. Augmenting the microglial barrier may have therapeutic benefits in AD. Two potential strategies are 1) plaque-targeted signaling ligands that activate *TREM2* or other receptors and 2) modulators of downstream signals that boost the microglial barrier.

(116,117). Therefore, in addition to the better-studied differential effects of APOE on A β metabolism (117–120), it is possible that APOE within amyloid deposits plays a role in signaling to adjacent microglia (121) for the polarization of their processes and encapsulation of plaques. However, additional microglial functions mediated through APOE, which are independent of amyloid, may also be at play, as it has recently been shown that APOE isoforms in mice directly modulate tau pathology and cell death (122,123). Thus, the potential involvement of APOE in the microglial encapsulation of plaques combined with the fact that this function diminishes with aging, suggests that failure of the microglial barrier could constitute a general mechanism involved in AD pathogenesis.

MICROGLIA-MEDIATED NEUROPROTECTION AS A TARGET FOR AD THERAPIES

Experimental strategies to enhance the microglial encapsulation of plaques have been demonstrated with the chemokine receptor *CX3CR1* genetic deletion or by passive anti-A β immunization in mice (27). These manipulations led to reduced axonal dystrophy formation around plaques, indicating a possible neuroprotective effect of microglia. Importantly, in humans carrying the R47H *TREM2* variant, diminished microglial encapsulation not only worsened axonal dystrophy, but also exacerbated neuronal phospho-tau protein accumulation around plaques (38). This suggests that boosting the microglial

barrier not only may reduce axonal dystrophy, but also could limit plaque-associated tau protein pathology. Given that the degree of tau protein hyperphosphorylation negatively correlates with cognitive function (124), it is plausible that enhancing microglial encapsulation of plaques could slow disease progression.

Although it is likely that most molecular manipulations will have a variety of effects on microglial function including on phagocytosis and cytokine production, ongoing research into mechanisms of microglial process polarization may suggest novel strategies to specifically manipulate their ability to encapsulate amyloid deposits (Figure 3). The following are potential strategies.

Anti-A β Immunization

Several groups have shown that this treatment increases microglial clustering around plaques in AD mouse models (27,82,84). Anti-A β immunization increases plaque encapsulation probably by activating Fc receptors that trigger downstream signaling overlapping with that of *TREM2* (125). It is worth noting that microglia only express a subset of Fc receptors and different Fc receptor subtypes activate different downstream pathways (126). Particularly, while Fc gamma receptor I and III activate ITAM signaling that converges with *TREM2* activation, Fc gamma receptor IIB inhibits ITAM signaling. Therefore, anti-A β immunoglobulin G antibodies with high Fc gamma receptor I and low Fc gamma receptor IIB affinity may be the most effective in

boosting microglial barrier function due to their potential net activation of ITAM signaling.

Anti-APOE Immunization

Anti-APOE immunotherapy has been shown to increase microglial recruitment around plaques in mice (127). Because APOE can bind A β aggregates (114), it is likely that anti-APOE antibodies have affinity toward plaques, similar to anti-A β antibodies. Likewise, anti-APOE antibodies may be able to promote microglial process polarization by activating Fc receptors and their downstream signaling. Furthermore, given recent results showing that APOE knockout is neuroprotective against tau pathology (122), antibodies sequestering APOE may have a dual beneficial effect.

CX3CR1 Inhibition

Genetically deleting *CX3CR1* in microglia leads to reduced plaque load (73,79) and enhanced microglial barrier (27). Neutralization of *CX3CR1* could thus be an approach to enhance microglial encapsulation of plaques. This may be achieved by neutralizing antibodies against *CX3CR1* or its ligand fractalkine, or by small molecule antagonist such as AZD-8797 (128). However, systemic suppression of *CX3CR1* signaling may disrupt bacterial clearance by the peripheral immune system (129), while *CX3CR1* deficiency may exacerbate tau hyperphosphorylation (130,131). Thus, suppression of *CX3CR1* should ideally be confined to plaque-associated microglia to increase encapsulation with minimal systemic side effects.

Bispecific Antibodies

Antibodies with two different antigen-binding domains can simultaneously bind to two separate epitopes. One of the two domains may be against fibrillar A β (84), which would lead to enrichment around plaques, providing an approach to engage the second target only in the vicinity of the plaque. If the second antigen binding domain is antifractalkine or anti-*CX3CR1*, then such approach may achieve simultaneous suppression of *CX3CR1* signaling and activation of ITAM signaling in plaque-associated microglia, potentially leading to enhancement of the microglial barrier and/or phagocytosis.

In terms of suitability for human use of these therapeutic strategies, based on previous mouse data, it appears to be clear that the barrier function is most effective when plaques are still relatively small (27). Thus, it is likely that therapies would have to target early preclinical AD. Furthermore, as with all attempts to translate therapies based on mouse models, there is a significant uncertainty that targeting amyloid will be sufficient in the absence of direct modulation of other disease hallmarks such as tau protein pathology.

POTENTIAL IMPLICATIONS FOR CLINICAL HUMAN IMAGING

Current amyloid PET tracers are limited in their utility as predictive biomarkers owing to their poor dynamic range and linearity. When patients present with mild cognitive impairment, their PET signals are near maximal and correlate poorly with the degree of cognitive impairment (10,132,133). Interestingly, our data show that plaque compaction inversely correlates with the degree of axonal injury (27,38). However,

PET tracers, which are derivatives of thioflavin T or Congo red, have the greatest affinity for compact plaques, which likely prevents detection of the potentially most neurotoxic protofibrillar species of A β (27,134). In contrast, small molecule dyes such as curcumin and THK-265 preferentially bind protofibrillar A β (27,97). Given that protofibrillar plaque regions are associated with more severe axonal dystrophy and neuronal process tau protein hyperphosphorylation (38), it is possible that novel PET probes based on compounds with affinity to protofibrillar A β could constitute better biomarkers of neurotoxicity. Indeed, improved brain-penetrant curcumin analogs have been developed, which may have potential as amyloid PET tracers in humans (135,136).

Current PET imaging approaches to monitor microglial activation utilize small-molecule ligands that bind the translocator protein 18 kDa in mice (137–139) and humans (140,141). Translocator protein 18 kDa is found in the outer mitochondrial membrane in various cell types, including neurons, astrocytes, and endothelial cells (142). Thus, while translocator protein 18 kDa is upregulated in microglia in AD (138,139), it lacks sufficient specificity for proper quantification of microglial activation. Novel PET tracers targeting microglial receptors such as *TREM2* may have greater potential because of their cell specificity and their marked upregulation in microglia surrounding amyloid deposits, which would greatly enhance the signal to noise ratios. Such probes may also provide information about the robustness of the microglial barrier and may thus be an indicator of the neuroprotective microglia that encapsulate plaques and reduce axonal dystrophy. Overall, such a strategy may offer greater resolution for tracking and interpreting the progression of AD-related neuroinflammation in parallel with existing amyloid and tau protein PET tracers.

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ARTICLE INFORMATION

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