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New insights on the role of microglia in synaptic pruning in health and disease

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Abstract

Recent genome-wide association studies implicate microglia in Alzheimer's disease (AD) pathogenesis; however, their biological significance remains poorly understood. Synapse loss is a significant correlate of cognitive decline that serves as a critical hallmark of AD and other neurodegenerative diseases; however, mechanisms underlying synaptic vulnerability remain elusive. Emerging research on microglia function in the healthy brain is providing new insight into fundamental roles of microglia and immune molecules in brain wiring. Among their many roles, microglia prune developing synapses and regulate synaptic plasticity and function. Here, we review and discuss how this emerging work may provide new insight into how disruptions in microglia–synapse interactions could contribute to synapse loss and dysfunction, and consequently cognitive impairment, in AD.

Introduction

Alzheimer's disease (AD) is a progressive neurological disorder in which the earliest clinical symptoms involve impaired declarative memory and general cognitive impairment, such as compromised judgment, decision-making and orientation. The two pathological hallmarks of AD are extracellular neuritic plaques, in which the amyloid β-protein (Aβ) is the principal component, and intraneuronal tangles, which are non-membrane bound masses of paired helical filaments composed primarily of hyperphosphorylated tau. A frequent observation in brains of both AD patients and animal models of AD is the surrounding of neuritic amyloid plaques by highly reactive, phagocytic microglia. Thus, there has been extensive research to understand the role of microglia in the AD brain and their interaction with amyloid plaques and tangles, and their contributions to neuroinflammation and AD pathology (see comprehensive review in [1]). Recent genome-wide association studies (GWAS) and other integrated network studies have identified immune-related pathways as risk factors for late-onset AD, implicating microglia, the resident immune cells of the central nervous system (CNS), as central players in AD pathogenesis. Among these are *Trem2*, *CR1*, *ApoJ/Clusterin*, *CD33*, and immune-specific and microglia-specific network modules that include

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TYR-OBP [2–9]; however, the biological significance of these findings remains elusive. Recent research has focused on understanding how microglia and these immune-related molecules contribute to amyloid plaque deposition, maintenance and clearance, but little is known on what microglia do in earlier, pre-plaque stages of AD when synapses are already vulnerable. Emerging research implicates microglia and immune-related mechanisms in synapse elimination during development, raising the intriguing hypothesis that these developmental pruning mechanisms may be aberrantly reactivated in the aged brain to contribute to synapse loss and cognitive impairment in AD and other neurodegenerative diseases (NDDs) (Figure 1).

Microglia and their potential roles in Aβ plaque maintenance

One of the cardinal features of AD brains is the presence of reactive microglia surrounding senile Aβ plaques along with prominent activation of inflammatory processes and immune responses. Recent *in vivo* imaging and microglial ablation studies have provided insight into how microglia may impact plaque deposition, maintanence and clearance. Time lapse imaging in cerebral cortex of plaque-burdened AD mouse models revealed microglia to be recruited rapidly (within 48 hours) to newly formed plaques and that microglial processes remain very dynamic [10,11]. Microglia that surround plaques appear to constitute a barrier to not only restrict plaque growth, but also may prevent diffusion of synaptotoxic Aβ oligomers from plaques [10,11,12•]. Furthermore, microglia that surround plaques may ultimately become dysfunctional, displaying decreased process number and directed process motility and impaired phagocytic activity in a manner that temporally and spatially correlates with plaque deposition [13,14•]. However, microglial ablation studies suggest that microglia may have a minimal role in maintaining plaques: ablating microglia for up to four weeks in two mouse models of AD crossed with *CD11b-HSVTK* mice, which allows deletion of microglia following treatment with ganciclovir, had little to no impact on either plaque number or size or on amyloid-associated neuritic dystrophy, regardless of whether microglia were ablated before or after plaques deposit in these mice [15]. A recent study using PLX5622, an inhibitor against colony-stimulating factor 1 receptor (CSF1R) signaling upon which microglia are dependent on survival, induced a partial, chronic reduction of microglia in an AD mouse model, but failed to detect any alteration of plaque load [16•]. However, the PLX5622-treated AD mice performed better in learning and memory tests than control-treated AD mice, despite having similar levels of plaque load. Altogether, these studies suggest that microglia may have roles in the AD brain beyond plaque maintenance, with potential behavioral consequences impacting learning and memory.

Dynamic microglia–synapse interactions in the healthy brain

Microglia derive from myeloid progenitor cells in the yolk sac and colonize the brain early during embryonic development [17–19]. Once in the CNS, they adopt tissue-specific signatures and functions through local microenvironment signals [20–24]. Until a decade ago, microglia were studied mostly in the context of CNS injury and disease [25]. However, groundbreaking *in vivo* imaging studies revealed that microglia in the healthy brain have highly dynamic processes and continually survey their local microenvironment [26,27]. Moreover, microglial processes constantly contact dendritic spines, axons and synapses,

raising questions about whether and how microglia regulate synaptic structure and function [28–33,34••,35•].

Emerging research implicates microglia and immune-related proteins in the elimination and refinement of synaptic connections in the healthy developing brain [29–32,36,37••]. Microglial processes make direct and transient connections with neuronal synapses where, intriguingly, the frequency of these connections is related to basal neuronal activity and/or experience [28,29]. Direct evidence of microglia sculpting the brain came from electron microscopy and high resolution *in vivo* engulfment assays where presynaptic and postsynaptic elements were found inside microglial lysosomes [29,31]. Interestingly, neuronal activity regulated the microglial engulfment of synaptic input, where microglia preferentially phagocytosed less active inputs [31]. Furthermore, disruption of immune-related molecules or receptors expressed on microglia, such as complement proteins or complement and fractalkine receptors, resulted in synaptic and wiring abnormalities in both prenatal and postnatal brain development [30–33,35•], implicating microglia in sculpting synaptic connectivity. Interestingly, ablating microglia in the adult brain induced impairment in learning tasks that correlated with reduction in learning-induced synapse formation in the motor cortex [34••], suggesting that microglia regulate synaptic formation and/or elimination depending on context (*i.e.* different brain regions, developmental time points and behavior).

AD: a disorder of degenerating selected synapses

Region-specific degeneration and loss of synapses that precede neuronal death is an early hallmark that differentiates AD from normal aging [38]. Furthermore, synapse loss in the hippocampus and association cortices serves as a much stronger correlate of cognitive impairment in AD than do counts of amyloid plaques or neurofibrillary tangles [39]. Research in the last two decades has provided extensive evidence that soluble oligomers of Aβ, and not plaques *per se*, act as prime synaptotoxic agents (see comprehensive review in [40]). In multiple mouse models of AD, synapse numbers are significantly lower than in non-transgenic controls already at a very young age, long before these mice develop amyloid plaques [41–45]. In another striking example, mice expressing a familial APP mutation where the synaptotoxic Aβ oligomers are present and stabilized in the absence of fibrillar amyloid plaques display profound synaptic and neuronal loss in their cortex [46].

Despite the physical degeneration of synapses being an early, critical event in AD, whether microglia play a role in these early, pre-plaque stages of pathogenesis and how they may impact synaptic function remains little explored. As mentioned above, much of the research on the role of microglia has focused on their relationship with plaques. Although these are important studies that help us understand whether microglia may impact plaques and plaque-related neurodegeneration, there already exists a significant level of widespread neuroinflammation and associated gliosis at these late stages, clouding investigation of potential neuroimmune perturbations that may directly contribute to the initiation of synaptic and cognitive impairment. Therefore, it will be pertinent to shift the focus of investigation away from late stages of AD toward earlier stages when synapses are already vulnerable to explore potential dysfunction of microglia or immune-related pathways and how these may relate to synaptotoxic Aβ oligomers.

Possible mechanisms of microglia–synapse dysfunction in the AD brain

Recent GWAS and other integrated network studies in AD have revealed microglia-specific and immune-related genes [2–9], suggesting microglia could play a major role in AD pathogenesis. However, the biological significance remains elusive, and whether microglia become dysfunctional in the AD brain regions vulnerable to synapse loss prior to plaque accumulation is unknown.

Among the GWAS-identified variants are *CLU*, also known as complement lysis inhibitor or *APOJ*, and *CR1*, which encodes for the receptor for complement component C3b [3]. In the AD brain, complement proteins, in particular, C1q, C3 and C4, are often found upregulated and localized to neuritic plaques. Moreover, Aβ has been shown to bind and regulate the expression and localization of complement [1,47]. Genetic deletion of *C1qa* in an AD mouse model resulted in less plaque-related neuronal damage and gliosis, whereas genetic deletion of *C3* resulted in more plaques and increased plaque-related neurodegeneration [48–50]. These studies suggest that complement proteins may have multiple roles in plaque-related pathology; however, it is not yet known whether complement is dysregulated early in the AD brain or whether this immune pathway contributes to synaptic or cognitive impairment.

In the healthy developing brain, classical complement cascade proteins, C1q and C3, are widely expressed, localize to subsets of immature synapses, and mediate synapse elimination, a developmental process critical for precise synaptic connectivity [31,36,37••, 51]. Importantly, disruption of this pruning pathway results in sustained defects on synaptic connectivity and brain wiring. In the peripheral immune system, classical complement proteins are ‘eat me’ signals that promote the rapid clearance of invading pathogens or cellular debris by circulating macrophages that express complement receptors (C1qR and CR3) [52]. In the developing visual system, microglia phagocytose synapses that are undergoing pruning in a manner that is dependent on complement (C1q, C3 and CR3) and neuronal activity [31,37••]. Several complement components, including C3, are normally downregulated in the healthy adult brain [31,51] and complement-mediated synaptic pruning is regulated by several signals, including cytokine TGFβ [37••]. Interestingly, C1q is highly upregulated and deposits on synapses with normal aging in human and mouse brains, particularly in the hippocampus, one of the brain regions most vulnerable to synapse loss in AD [53••]. C3 has also been shown to contribute to synapse loss and dysfunction in the mouse hippocampus during normal aging [54••]. Altogether, these findings raise the intriguing question of whether C1q and/or C3 increases the susceptibility of the hippocampus to further insults such as accumulation of synaptotoxic Aβ oligomers. An important question for future investigation is whether the normal developmental pruning pathway becomes aberrantly upregulated to mediate synapse loss by microglia in early stages of AD and other NDDs [51]. Indeed, emerging data suggest complement may be a key mechanism underlying synaptic vulnerability in early stages of AD [55].

Another immune-related pathway that warrants further studies on synaptic health in the AD brain is TREM2, which is expressed on myeloid cells and whose missense mutations lead to a significant risk of developing AD [6,7]. Recent exciting studies have extensively focused on whether TREM2 may affect Aβ plaque pathogenesis; TREM2 appears to affect the

number of plaque-associated microglia but its overall impact on plaque burden seems to depend on age and on which mouse model was utilized [56,57•,58•,59••]. TREM2 has been shown to impact inflammatory signals as well as phagocytosis in the peripheral immune system [60]; however, the normal function of TREM2 in the CNS remains poorly understood. Interestingly, loss-of-function mutations in *TREM2* or *DAP12*, an adaptor protein for TREM2 signaling, underlie the Nasu–Hakola disease, in which patients display progressive presenile dementia [61,62], and mice expressing mutations in *DAP12* display impaired synaptic maturation [63]. These findings suggest that TREM2 may play an important role for maturation and maintenance of synaptic function and connectivity. Interestingly, TREM2 has recently been suggested to interact with certain lipids to promote microglial survival [59••]. This is particularly intriguing given that certain lipid structures on neuronal membranes have been implicated to bind Aβ oligomers to potentially mediate downstream synaptic dysfunction [64]. Collectively, these findings raise the possibility that TREM2 could play a role in early synaptic dysfunction in AD and the need to better understand the biology of TREM2 in the context of both the healthy and diseased CNS.

Role of microglia in region-specific network dysfunction

Region-specific loss of synaptic integrity and aberrant neuronal networks have been linked to many developmental and neurodegenerative diseases [65–68]. In AD, elaborate *in vivo* tools including structural and functional magnetic resonance imaging, positron emission tomography (PET) and microdialysis in humans and animal models have revealed an intricate relationship among cognitive status, neural or astroglial activity, Aβ dynamics, and CNS structural changes [69–74]. Interestingly, areas of the human brain that develop the most Aβ deposits may also have among the highest basal rates of metabolic and neural activity [71,73,75]. This is particularly intriguing as neuronal activity has been shown to regulate Aβ production [76,77] and also to modulate microglia–synapse interactions [28,29] and microglial elimination of synapses in the developing brain [31]. We propose a model in which aberrant neuronal activity through genetic or other causes may trigger local activation of microglia and other immune-related molecules and thus contribute to region-specific vulnerability in AD and potentially other NDDs.

Conclusion

Emerging evidence implicates microglia as key participants in the wiring of the developing brain. Given the intricate relationship between synaptic/neuronal activity, cognition and microglial function in development, it is a salient time for AD research to focus on understanding whether, and how, microglia contribute to synaptic loss and impairment with attendant behavioral outcomes. If so, microglia and relevant immune-related pathways may act as early therapeutic targets in AD and potentially other NDDs involving synaptic dysfunction and memory decline.

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• of special interest

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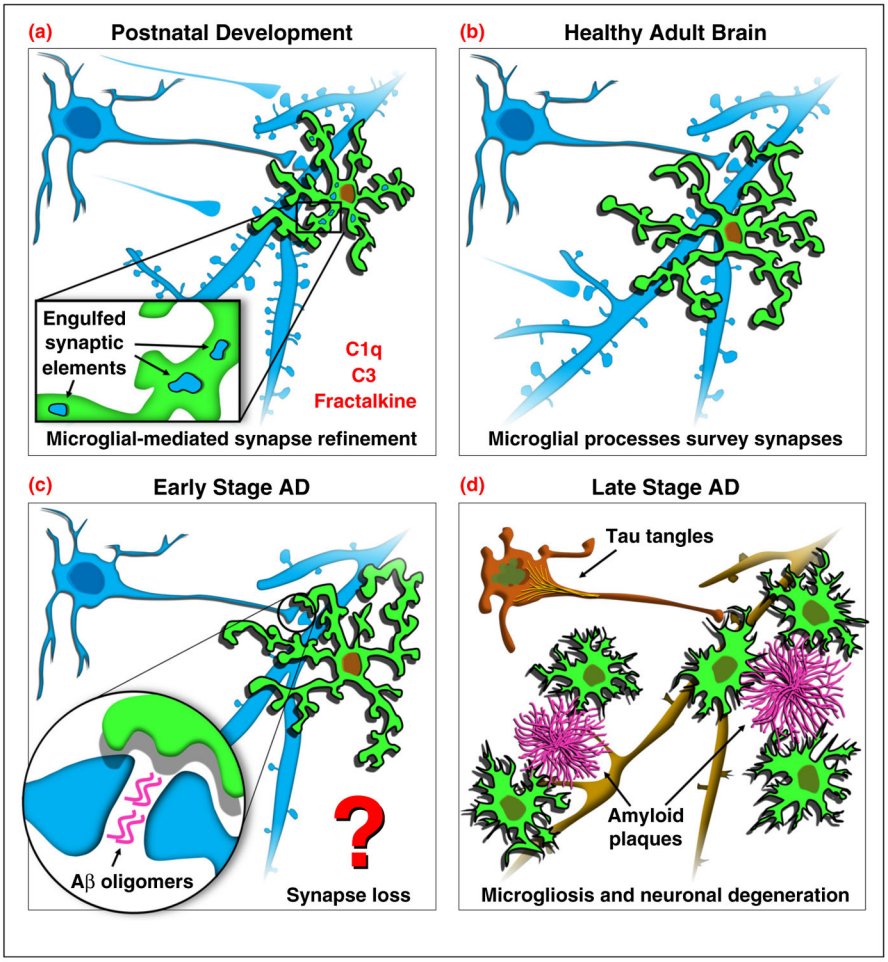

Figure 1.

Potential roles of microglia in the healthy and diseased brain. **(a)** During early postnatal development, microglia (illustrated in green) help refine excessive synaptic connections (illustrated in blue). Insert highlights microglial engulfment of synaptic elements. Microglia-related proteins including complement proteins and fractalkine have been suggested to mediate this process. **(b)** In the healthy adult brain, microglial processes are dynamic and continuously survey surrounding synapses. **(c)** Synapse loss is an early hallmark of AD pathology, thought to be initiated by Aβ oligomers. What microglia do in this early stage of AD remains poorly understood. **(d)** Late stage AD is characterized by the presence of extracellular plaque deposition, intraneuronal tangle formation and neurodegeneration, often accompanied by significant levels of microgliosis and neuroinflammation. Recent research has given insight into how microglia may impact plaque and plaque-related neuropathology in late stage AD; however, what microglia do in early AD, in particular, how their dynamic interactions with synapses are impacted, is unknown. An intriguing hypothesis is whether microglia and immune-related molecules that mediate synaptic engulfment in the developing brain (a) may be aberrantly reactivated in the AD brain (c) to mediate synapse loss and degeneration.