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Phagocytic Roles of Glial Cells in Healthy and Diseased Brains

Yeon-Joo Jung and Won-Suk Chung*

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

Abstract

Glial cells are receiving much attention since they have been recognized as important regulators of many aspects of brain function and disease. Recent evidence has revealed that two different glial cells, astrocytes and microglia, control synapse elimination under normal and pathological conditions via phagocytosis. Astrocytes use the MEGF10 and MERTK phagocytic pathways, and microglia use the classical complement pathway to recognize and eliminate unwanted synapses. Notably, glial phagocytosis also contributes to the clearance of disease-specific protein aggregates, such as β-amyloid, huntingtin, and α-synuclein. Here we reivew recent findings showing that glial cells are active regulators in brain functions through phagocytosis and that changes in glial phagocytosis contribute to the pathogenesis of various neurodegenerative diseases. A better understanding of the cellular and molecular mechanisms of glial phagocytosis in healthy and diseased brains will greatly improve our current approach in treating these diseases.

Key Words: Phagocytosis, Astrocytes, Microglia, Synapse elimination, Neurodegenerative disease

INTRODUCTION

Glial cells are non-neuronal cells that are classified into three major cell types, astrocytes, oligodendrocytes, and microglia, which have different characteristics and functions in the central nervous system (CNS). Astrocytes play essential roles in the regulation of various brain functions. Astrocytes support neuronal survival and metabolism, control blood flow through vasodilation and vasoconstriction, and uptake neurotransmitters and ions at synaptic clefts (Araque *et al*., 1999, Sofroniew and Vinters, 2010; Hayakawa *et al*., 2016). Astrocytes also modulate synaptic formation, function and elimination at all stages of development and in adulthood (Allen and Barres, 2005; Stevens *et al*., 2007; Allen *et al*., 2012; Chung *et al*., 2013; Clarke and Barres, 2013; Lopez-Murcia *et al*., 2015; Lee *et al*., 2016; Yang *et al*., 2016a; Terni *et al*., 2017).

Phagocytosis is an essential element of the innate immune response, which functions as a defense mechanism against pathogens during infection and clearance mechanism for cellular debris produced during normal brain development and injuries (Fricker *et al*., 2012; Jones *et al*., 2013). Microglia in the CNS have been regarded as the only major phagocytes that mediate the elimination of synapses, apoptotic cells, neural debris, and pathogenic proteins (Tahara *et al*., 2006; Jana *et al*., 2008; Meyer-Luehmann *et al*., 2008; Wang *et al*., 2015; Pomilio *et al*., 2016). However, It has been recently shown that astrocytes also have a strong phagocytic capacity and participate in the elimination of synapses and neuronal debris from the brain (Chung *et al*., 2013; Bellesi *et al*., 2017). Glial phagocytosis may be directly associated with the prevalence of various neurodegenerative diseases because defects in the phagocytic function of glial cells could result in the accumulation of unwanted elements in the brain with an abnormal immune response. This review discusses recent findings on the phagocytic roles of glial cells in the regulation of normal brain function and speculates on their potential roles in diseased brains.

GLIAL CELLS ARE INVOLVED IN SYNAPSE ELIMINATION VIA PHAGOCYTOSIS

Neurons generate excess synapses during development. These excess synapses subsequently undergo selective elimination to achieve precise neural connectivity (Allen *et al*., 2012; Clarke and Barres, 2013; Lee *et al*., 2016). Synapse elimination events also persist in the mature nervous system

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***Corresponding Author**
E-mail: wonsuk.chung@kaist.ac.kr
Tel: +82-42-350-2624, Fax: +82-42-350-5624

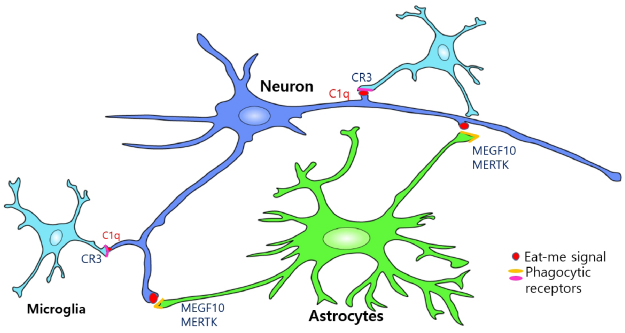

Fig. 1. Astrocytes and Microglia mediate synapse elimination by phagocytic pathways. Astrocytes (green) eliminate synapses from neurons (blue) by recognizing "eat-me" signals (red) presented in the unwanted synapses and phagocytosing them through MEGF10 and MERTK receptors (yellow). Astrocytes also mediate synapse elimination indirectly by inducing C1q expression in neurons (blue). C1qtagged synapses can be recognized and eliminated by complement component-3 receptor (C3R, magenta) in microglia (light blue).

via experience-dependent structural synaptic plasticity, but the number of elimination events may decline with age (Song *et al*., 2008; Chung and Barres, 2012; Lopez-Murcia *et al*., 2015; Cho *et al*., 2016; Lee *et al*., 2016; Terni *et al*., 2017). Therefore, synapse elimination is critical in the shaping of neural circuits during development and the regulation of synaptic plasticity in response to experience and memory. Certain neurodegenerative diseases, such as Alzheimer's disease (AD), are associated with profound synapse loss early in the disease state (Jana *et al*., 2008; Meyer-Luehmann *et al*., 2008; Pihlaja *et al*., 2008; Suh *et al*., 2013; Sollvander *et al*., 2016), which underscores the importance of understanding the molecular mechanisms of synapse loss.

The mechanisms of inappropriate synapse clearing during development are not known, but studies in mammals and flies have found that glial cells play central roles in this process. Glial cells are involved in all of the processes of developmental synapse elimination, including the proper recognition, engulfment, and degradation of synapses and neural debris (Allen and Barres, 2005; Chung and Barres, 2012; Diniz *et al*., 2014). Microglia in the mammalian CNS monitor and clear synapses during development via the complement pathway (Schafer *et al*., 2012). Our previous study demonstrated that astrocytes mediated synapse elimination via the MEGF10 (Multiple EGF-Like Domains 10) and MERTK (Mer proto-oncogene Tyrosine Kinase) phagocytic pathways (Chung *et al*., 2013) (Fig. 1).

MECHANISMS OF MICROGLIA-MEDIATED SYNAPSE ELIMINATION

Microglia are derived from the hematopoietic lineage and express typical pattern recognition receptors. Microglial processes interact with presynaptic boutons and dendritic spines in normal brains, and direct contacts have been observed using electron and two-photon microscopy (Nimmerjahn *et al*., 2005). Each microglial cell surveys several synapses simultaneously and quickly changes its motility in response to extracellular stimuli.

Notably, microglia play critical roles in shaping the neural circuit connectivity of developing and normal brains. Microglia prune synaptic connections by engulfing pre- and postsynaptic elements in the hippocampus and retinogeniculate system during postnatal development (Paolicelli and Gross, 2011; Schafer *et al*., 2012).

Unwanted developing synapses in the retinogeniculate system are tagged with complement protein C1q, which is the initiating protein of the classical complement cascade (Stevens *et al*., 2007). The binding of C1q and opsonization of unwanted synapses trigger a protease cascade, which leads to the deposition of the downstream complement protein C3 (Gasque, 2004). Deposited C3 directly activate C3 receptors on microglia, which trigger elimination via microglial phagocytosis (Stevens *et al*., 2007) (Fig. 1).

The relevant complement proteins are normally downregulated by adulthood in the brain, but recent studies have revealed that C1q is highly upregulated in aging brains (Stephan *et al*., 2013) and most neurodegenerative diseases (Hong *et al*., 2016), where it mediates abnormal synapse elimination (see the next section).

MECHANISMS OF ASTROCYTE-MEDIATED SYNAPSE ELIMINATION

Astrocytes are the most abundant cell type in the brain and constitute approximately 40% of brain cells. It has been estimated that one astrocyte ensheathes tens of thousands of synapses with its fine processes, which allows astrocytes to act as the first responders to any changes in synaptic activity, besides neurons. The fine astrocyte processes are highly dynamic and constantly modulate their association with synapses over the course of minutes. The degree of these dynamics are dependent on the physiological conditions. Notably, previous microarray studies of acutely isolated mouse brain astrocytes unexpectedly revealed that these cells express many components of evolutionarily conserved phagocytic pathways (Cahoy *et al*., 2008; Zhang *et al*., 2014). Two main pathways, the MEGF10 and MERTK pathways, were identified to begin serially but converge downstream in ways that remain poorly understood. Each pathway begins with a transmembrane signaling receptor that recognizes apoptotic cells via recognition of "eat-me" signals, such as phosphatidylserine (PS) in the outer leaflet of the target's plasma membrane or after the target has been coated by an opsonin. The first pathway includes MEGF10, which triggers the phagocytic process by engaging the intracellular protein GULP, engulfment adaptor PTB domain containing 1 (GULP1). ATP-binding cassette subfamily A member 1 (ABCA1: cholesterol efflux transporter) is also required, but the function of this protein in this pathway is poorly understood. *Megf10* exhibits a high homology with *draper* in flies and *ced-1* in worms, which play critical roles during phagocytosis of each organisms. The second phagocytic pathway is the MERTK pathway. MERTK works with the integrin pathway to regulate CRKII/DOCK180/Rac1 modules in controlling the rearrangement of the actin cytoskeleton upon phagocytosis. Several bridging molecules that recognize PS and interact with MERTK are GAS6, Protein S, and LGALS3, all of which are highly expressed and secreted by astrocytes.

Eliminating synapses via the MEGF10 and MERTK phagocytic pathways allows astrocytes to actively contribute to neural activity-dependent synapse pruning that mediates the

refinement of neural circuits in the developing mouse brain (Chung *et al*., 2013). Retinal ganglion cells in developing mice deficient in the *Megf10* and *Mertk* pathways exhibit failure in the normal refinement of connections and retain excess functional synapses with their primary targets, which are neurons in the dorsal lateral geniculate nucleus. This finding supports the active participation of astrocytes in the eliminating of live synapses rather than a simple removal of dead synaptic debris. Astrocytes also recognize and preferentially engulf weak synapses instead of strong synapses, and the presence of strong synapses is required to initiate this elimination process (Chung *et al*., 2013).

Microglia are traditionally thought to be the major glial cells that mediate synapse elimination, but astrocytes play a dominant role in eliminating synapses in the developing dorsal lateral geniculate nucleus. This role is partially due to the large number of astrocytes because astrocytes outnumber microglia 7~10-fold in developing brains. Astrocytes also continuously engulf excitatory and inhibitory synapses throughout the brain during adulthood, which suggests that astrocytes constantly remodel the synaptic architecture of our brains in response to our experiences.

GLIAL CELLS IN AGING AND NEURODEGENERATIVE DISEASES

Aging is the major risk factor for neurodegenerative diseases and cognitive decline. Synapse dynamics is the rate of synapse formation and elimination and is significantly altered by aging and changes in glial gene expression. Notably, C1q accumulates in aged brains approximately 300-fold greater compared to younger brains (Fraser *et al*., 2010; Depboylu *et al*., 2011; Stephan *et al*., 2013). This dramatic accumulation of C1q protein likely increases the vulnerability of brains to hyperactivation of the complement cascade, which can damage even healthy synapses via uncontrolled microglial phagocytosis. However, the triggers for the accumulation of C1q protein and microglial hyperactivation in healthy aging brains are not known. Microglia exhibit increased immune responses, including phagocytosis, following various stimuli and aging, but astrocytes appear to lose their phagocytic capacity during reactive astrogliosis (Hong *et al*., 2016; Liddelow *et al*., 2017), which is a common feature of astrocytes in aging brains.

Drosophila glial cells in aged brains also lose their phagocytic capacity because of the decreased translation of *draper*, which is a homolog of *Megf10* that astrocytes use for phagocytosing synapses (Tasdemir-Yilmaz and Freeman, 2014; Pearce *et al*., 2015; Purice *et al*., 2016). Restoration of the Draper levels rescue the phagocytic capacity of glial cells, which efficiently clear damaged axonal debris in aged brains to a similar extent as young brains. Taken together, these new findings suggest that aging may alter the phagocytic capacity of glial cells in the mammalian brain and lead to changes in brain and synaptic homeostasis, which may increase the vulnerability of aged brains to develop various neurodegenerative diseases.

GLIAL CELL PHAGOCYTOSIS IN ALZHEIMER'S DISEASE (AD)

The release of proinflammatory cytokines by reactive microglia and astrocytes surrounding β-amyloid (Aβ) plaques is one of the leading factor in chronic inflammatory responses in AD (Salminen *et al*. 2009; McGeer and McGeer, 2013). This neuroinflammation plays a critical role in the pathogenesis of AD via the induction of neuronal toxicity and cognitive decline (Akiyama *et al*., 2000; Lee *et al*., 2010). However, reactive gliosis may also be beneficial because reactive microglia and astrocytes are able to phagocytose and clear Aβ deposits (Lee *et al*., 2010). Microglia mediate the clearance of Aβ through receptor-mediated phagocytosis via the use of advance glycation end products (RAGE), toll-like receptors 2 (TLR2) and 4 (TLR4), scavenger receptor CD36, PS receptor and purine receptor P2Y6 (Noda and Suzumura, 2012; Jones *et al*., 2013). Microglial uptake of Aβ and its subsequent targeting to the endosome-lysosome pathway were examined in detail using active microglia phagocytosing of monomeric, oligomeric and fibrillar Aβ (Lee and Landreth, 2010). Ultrastructural studies also identified intra-cytoplasmic fragments of Aβ in AD microglia. Microglia are activated and recruited to Aβ deposits in brains that contain neurons that overexpress amyloid precursor protein (APP) (Tahara *et al*., 2006; Bolmont *et al*., 2008; Meyer-Luehmann, *et al*., 2008). Microglia constitutively express TLR2 (Olson and Miller, 2004; Hanke and Kielian, 2011), and TLRs play a role in Aβ-induced microglial activation (Chen *et al*., 2006; Tahara *et al*., 2006; Tang *et al*., 2007; Liu *et al*., 2012). Aβ-triggered inflammatory activation is reduced in TLR2-deficient microglia (Jana *et al*., 2008; Suh *et al*., 2013), and TLR2 deficiency reduces Aβ-triggered inflammatory activation in cultured microglia, which suggests a beneficial effect of TLR2 inhibition in AD pathogenesis (Liu *et al*., 2012).

Triggering receptors expressed by myeloid cells (TREMs) are surface receptors on microglia, and TREM mutations confer a dramatically elevated risk for AD and other neurodegenerative diseases (Cuyvers *et al*., 2014). Microglia highly express TREM2 (Painter *et al*., 2015), which is a key determinant of the CNS response to Aβ accumulation (Zhang *et al*., 2013; Matarin *et al*., 2015; Slkoe and Hardy, 2016). Deletion of the TREM2 allele in human APP (hAPP) transgenic mice decreased the number of microglia associated with Aβ deposits (Ulrich and Holtzman, 2016). Defective mTOR signaling in TREM2-deficient microglia is associated with a compensatory increase in autophagy *in vitro* and *in vivo* in AD models (Ulland *et al*., 2017). TREM2 detects damage associated with lipids, which enables microglia to sense Aβ accumulation and cell damage and supports microglia survival and Aβ-mediated reactive microgliosis (Wang *et al*., 2015). A recent study also described a novel microglia type associated with neurodegenerative disease (DAM) in AD model mice. Single cell analysis of DAM revealed that the DAM program was activated in a TREM2-independent and -dependent manner in the two-step activation processes, and disease-related gene expression changes were observed (Keren-Shaul *et al*., 2017).

AD is associated with profound synapse loss early in the disease state. A recent study demonstrated reactivation of the complement pathway that is downregulated after initial synaptic pruning periods in AD brains and mediates abnormal synapse pruning via microglial cells (Hong *et al*., 2016). Functional suppression of C1q prevents synapse loss during

disease progression, which supports the microglial cell mediation of synapse loss in AD brains. Reactive astrocytes also surround the sites of Aβ deposits in human and animal AD models and contribute to AD pathophysiology via the release of proinflammatory cytokines and activation of microglia-mediated cytotoxicity (Jana *et al*., 2008; Park *et al*., 2008; Lee *et al*., 2010; Fu *et al*., 2014; Painter *et al*., 2015; Yang *et al*., 2016b). However, astrocytes are also competent phagocytes, and their ability to engulf Aβ may be important in the identification of strategies to reduce Aβ accumulation in AD (Jones *et al*., 2013). Few studies have examined the phagocytic roles of astrocytes in Aβ clearance, but a decrease in Aβ levels was reported when astrocytes were added to brain sections prepared from a mouse model of AD (Wyss-coray *et al*., 2003). Astrocytes transplantations into the brains of transgenic AD mouse models containing mutated APP and PSEN1 (PS1), APPswe/PS1dE9, were found near Aβ deposit and internalized Aβ. Thus, astrocytes have a capacity of clearance of Aβ (Pihlaja *et al*., 2008). In addition, astrocytic LRP-1 is reported to mediate Aβ clearance and regulates Aβ metabolism both *in vitro* and *in vivo* models (Liu *et al*., 2017).

The phagocytic roles of astrocytes may also be important in maintaining brain homeostasis. A recent study found that the strongest genetic risk factor for AD, APOE4, suppressed astrocyte-mediated phagocytosis (Chung *et al*., 2016). By contrast, the protective APOE allele for AD, APOE2, significantly enhanced the phagocytic capacity of astrocytes *in vitro* and *in vivo*. C1q protein accumulation, which may represent the amount of senescent synapses with increased vulnerability to complement-mediated degeneration, was also significantly reduced in APOE2 knock-in (KI) animals, and C1q accumulation was significantly increased in APOE4 KI animals in the 18-month-old mouse hippocampus. Astrocytes constantly engulf synapses in adult brains, and the decreased C1q accumulation in aged APOE2 KI animals supports the hypothesis that maintaining the synaptic environment devoid of senescent synapses prevents aberrant immune activation/inflammation and neurodegeneration. By contrast, a decrease in the overall phagocytic capacity of astrocytes may lead to the accumulation of senescent synapses and their debris, which may be at least partially responsible for the enhanced vulnerability of the brain to AD via hyperactivation of the complement pathway (Chung *et al*., 2016).

GLIAL CELL PHAGOCYTOSIS IN HUNTINGTON'S DISEASE (HD)

HD is an autosomal dominant inherited neurodegenerative disorder. Patients suffering from HD exhibit specific symptoms, such as random involuntary movements as well as psychiatric and cognitive impairment (Jiang *et al*., 2016; Jansen *et al*., 2017). These symptoms are highly associated with neuronal dysfunction in the striatum and other brain regions (Ghoshi and Tabriz, 2015). HD is caused by an expanded polyglutamine repeat localized to the N-terminal region of the huntingtin protein, with intracellular accumulation and aggregation (Jiang *et al*., 2016). Astrocytes and microglia are activated in HD patients, as shown by GFAP and Iba1 upregulation (Jansen *et al*., 2016). Astrocytes with mutant huntingtin (mHTT) downregulate potassium channel Kir4.1, which leads to increased extracellular potassium concentrations and subsequent neuronal excitability (Tong *et al*., 2014; Sofroniew, 2015). The loss of the Kir4.1- and Glt-1 (Glutamate transporter 1)-mediated homeostatic functions of astrocytes cause defects in astrocytic glutamate and Ca^{2+} signaling, which contribute to the altered neuronal physiology in the striatum. Defects in the striatal circuit in HD patients are remedied by correcting key astrocyte homeostatic dysfunctions that precede overt astrogliosis and neurodegeneration (Jiang *et al*., 2016).

mHTT aggregation in drosophila neurons was transported to astrocytes via Draper. This phagocytic clearance of neuronal mHTT aggregation by glial cells may contribute to the spread of pathogenic protein aggregates in various neurodegenerative diseases (Pearce *et al*., 2015). Although there are a few studies reporting the presence of mHTT inclusions in astrocytes and oligodendrocytes (Shin *et al*., 2005; Tong *et al*., 2014; Huang *et al*., 2015; Jansen *et al*., 2016), precise molecular mechanisms of this phenomenon and its implications during the initation and progression of HD remain elusive.

GLIAL PHAGOCYTOSIS IN PARKINSON'S DISEASE (PD)

PD is a progressive neurological disorder that is characterized by the loss of dopaminergic neuron in substantia nigra pars compacta (SNpc), which results in tremor, bradykinesia, and muscle stiffness. α-synuclein is a major component of the intracellular protein deposits, called Lewy bodies, in specific regions of the brain stem, spinal cord, and cortex (Park *et al*., 2008; Lees *et al*., 2009; Phatnani *et al*., 2015). Previous studies have demonstrated that α-synuclein influenced microglia activation (Austin *et al*., 2006; Klegeris *et al*., 2008). Microglial cells treated with extracellular monomeric α-synuclein exhibited increased phagocytic activity *in vitro* in time- and dose-dependent manners (Park *et al*., 2008; Fu *et al*., 2014). By contrast, aggregated α-synuclein inhibited the phagocytic capacity of microglial cells by antagonizing monomeric-facilitated clearance and decreasing the basal microglial phagocytic capability (Park *et al*., 2008; Fu *et al*., 2014). A recent study reported that astrocyte-derived GDNF regulated midbrain microglial activation and exhibited a neuroprotective effect via inhibition of the degeneration of dopaminergic neurons in the nigrostriatal system in PD animal models (Rocha *et al*., 2012). Previous studies also demonstrated that various microglial receptors, including the C1q-mediated clearance pathway (Depboylu *et al*., 2011) and scavenger receptor class B (Michaelakakis *et al*., 2012), were involved in the endocytosis of α-synuclein. Several studies have demonstrated that microglia were more effective in endocytosing α-synuclein compared to astrocytes and neurons (Rojanathammanee *et al*., 2011; Fu *et al*., 2014), but whether microglial uptake of α-synuclein plays a beneficial or harmful response to the pathophysiology of PD is not clear.

GLIAL PHAGOCYTOSIS IN MOTOR NEURON DISEASES

Amyotrophic lateral sclerosis (ALS) is characterized by a progressive loss of motor neurons in the motor cortex, brainstem, and spinal cord (Hardiman *et al*., 2011; Radford, 2015). ALS exhibits rapid disease progression and leads to death

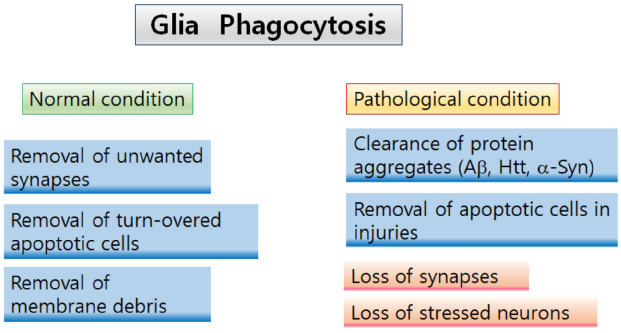

Fig. 2. Two aspects of glial phagocytosis Astrocyte- and microglia-mediated phagocytosis play various roles under different physiological conditions. In normal and healthy conditions, glial phagocytosis involves in regulation of neural circuit remodeling by eliminating unwanted synapses and neurites. Furthermore, glia mediates removal of apoptotic cells and neuronal debris in maintaining brain homeostasis. In pathological conditions, astrocytes and microglia phagocytose protein aggregates, such as A, Htt, and α-Syn to clear accumulated proteins in the brains with neurodegenerative diseases. However, hyperactivation of glial phagocytosis can play deleterious roles by phagocytosing and eliminating intact synapses and stressed neurons, contributing to the initiation and progression of neurodegeneration and cognitive declines.

within 2-3 years of symptom onset (Lasiene and Yamanaka, 2011). Astrocytes and microglia are critical regulators via the removal of damaged motor neurons and mutant SOD1 in ALS pathology. Astrocytes become activated and release increased levels of cytokines in ALS, including TNF-α, IFN-γ, and IL-1β (Philips and Robberecht, 2011). Astrocytic protein inclusions containing mSOD1 are an early feature of the disease in the mSOD1 mouse model. Selective expression of mSOD1 in astrocytes alone failed to provoke an ALS phenotype (Gong and Elliott, 2000), but the silencing of mSOD1 expression in astrocytes significantly slowed disease progression in the $SOD1^{G37R}$ mouse model (Yamanaka *et al*., 2008), without affecting the level of astrogliosis. Previous studies have demonstrated that normal motor neurons develop features of ALS pathology when surrounded by mSOD1-expressing glial cells in chimeric mice models (Clement *et al*., 2003). Benkler *et al*. (2013) demonstrated that the reduced glutamatergic response of astrocytes in $SOD1^{G93A}$ mouse models may lead to disruption of glutamate homeostasis and accumulative CNS damage, which facilitate motor neuron degeneration.

Microglia are also a component of ALS pathology. Activated microglia are widely detected in the brains of living ALS patients using positron emission tomography (Turner *et al*., 2004; Lasiene and Yamakata, 2011). Activation of microglia results in the elevation of proinflammatory cytokines in mutant SOD1 mice. Mutant SOD1-expressing microglia release higher levels of TNF-α and IL-6 compared to wild-type microglia following LPS exposure (Weydt *et al*., 2004). Dysfunctional microglial phagocytosis is also related to ALS risk. Profilin 1 is a regulator of actin dynamics for phagocytosis (Kim *et al*., 2012; Radford *et al*., 2015). Profilin 1 mRNA expression level is upregulated in activated microglia, which leads to changes in cell morphology and phagocytic capacity (Dong *et al*., 2004). Mutations of profilin 1 were identified in familial ALS and are essential in the upregulation of the phagocytosis of microglia (Radford *et al*., 2015).

CONCLUSIONS AND PERSPECTIVES

We have described evidence that glial cells are active regulators in brain functions via phagocytosis. This evidence raises the intriguing possibility that the phagocytic activity of astrocytes and microglia regulate normal synaptic function and that changes in glial phagocytosis contribute to the pathogenesis of neurological disorders (Fig. 2).

Reactive gliosis and neuroinflammation were previously considered secondary events caused by the degeneration of neurons. However, recent findings demonstrated that microglia and astrocytes contributed to the initiation of neurodegenerative diseases, including AD, HD, PD and ALS. Astrocytes and microglia are closely associated with each other at the sites of pathogenic regions, and these two cell types may crosstalk and induce reciprocal activation. For example, astrocytes regulate the morphology, differentiation, and activation state of microglia (Streit *et al*., 1999; Rocha *et al*., 2012). Reactive microglial cells during LPS-induced systemic inflammation produce TNF-α, C1q and IL-1α, which activate astrocytes and induce the expression of neurotoxins from reactive astrocytes (Liddelow *et al*., 2017).

Glial cell phagocytosis may play beneficial or deleterious roles in the brain when they are dysregulated. The phagocytic capacity of astrocytes and microglia may be necessary for the clearing of unnecessary synapses, synaptic debris and extracellular protein aggregates, thus maintaining brain homeostasis and preventing aberrant immune responses. In contrast, hyperactivation of phagocytic pathways, such as classical complement cascades and MERTK phagocytic pathways, can induce damage to live synapses during AD (Savage *et al*., 2015) and neurons during stroke (Neher *et al*., 2013), respectively.

Further studies would be necessary to reveal the exact molecular and cellular players of the phagocytic events in specific disease settings. This knowledge is crucial to the development of relevant therapeutics targets and strategies for each specific disease.

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