Neurosci. Bull. October, 2019, 35(5):921–933
https://doi.org/10.1007/s12264-019-00388-3

www.neurosci.cn
www.springer.com/12264

REVIEW

Dual Functions of Microglia in Ischemic Stroke

Chuan Qin[1] · Luo-Qi Zhou[1] · Xiao-Tong Ma[1] · Zi-Wei Hu[1] · Sheng Yang[1] · Man Chen[1] · Dale B. Bosco[2] · Long-Jun Wu[2] · Dai-Shi Tian[1]

Received: 1 November 2018 / Accepted: 30 December 2018 / Published online: 6 May 2019

Abstract Ischemic stroke is a leading cause of morbidity and mortality worldwide. Resident microglia are the principal immune cells of the brain, and the first to respond to the pathophysiological changes induced by ischemic stroke. Traditionally, it has been thought that microglial activation is deleterious in ischemic stroke, and therapies to suppress it have been intensively explored. However, increasing evidence suggests that microglial activation is also critical for neurogenesis, angiogenesis, and synaptic remodeling, thereby promoting functional recovery after cerebral ischemia. Here, we comprehensively review the dual role of microglia during the different phases of ischemic stroke, and the possible mechanisms controlling the post-ischemic activity of microglia. In addition, we discuss the dynamic interactions between microglia and other cells, such as neurons, astrocytes, oligodendrocytes, and endothelial cells within the brain parenchyma and the neurovascular unit.

Keywords Microglia · Inflammation · Ischemic stroke · Signaling pathways

✉ Long-Jun Wu
wu.longjun@mayo.edu

✉ Dai-Shi Tian
tiands@tjh.tjmu.edu.cn

[1] Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

[2] Department of Neurology, Mayo Clinic, Rochester, MN 55905, USA

Introduction

Stroke is a major issue in terms of increasing incidence and morbidity and mortality worldwide. Microglia are the resident immune cells and the first line of defense against injury in the central nervous system(CNS) [1]. Almost immediately after the onset of brain ischemia, microglia are activated and can have both beneficial and detrimental effects during all stages of ischemic stroke [2]. For instance, after ischemic injury, microglia rapidly migrate towards the lesion site and exacerbate tissue injury by producing inflammatory cytokines and cytotoxic substances, while also contributing to tissue repair and remodeling by clearing up debris and producing anti-inflammatory cytokines and growth factors [3, 4]. This dual role of microglia is associated with their functional state under different cellular contexts and pathological stages after ischemia [3]. While the detrimental effects of pro-inflammatory signals released by classically activated microglia occur in the early stages of stroke, the role of adaptive microglia is still controversial[1]. Microglia are highly plastic cells and their activation is a complicated process that can be affected by many substances and surrounding cells such as neurons, astrocytes, oligodendrocytes, and endothelial cells [5, 6]. Emerging studies have focused on regulatory mechanisms that underlie the activation of microglia and have been aimed at redirecting microglia from a detrimental to a neuroprotective phenotype [7, 8]. Microglial activation during different pathological stages of ischemic stroke, different molecular pathways and mediators that regulate microglial activation, dynamic interactions between microglia and surrounding cells, and the underlying therapeutic targets are reviewed.

Activation of Microglia Following Ischemic Stroke

Microglia classically exhibit a ramified morphology characterized by a small soma and fine processes under physiological circumstances; this is often referred to as the "resting microglia" state. Disruption of brain homeostasis contributes to microglial activation, which is observed as morphological changes like process thickening and hypertrophy of the cell body, with coinciding up-regulation of a variety of cell surface markers, including cluster of differentiation (CD) 45, major histocompatibility complex II, and CD68 [9]. It has been reported that inflammation plays a critical role in the pathogenesis of ischemic stroke [10]. Following ischemia, inflammation is initiated along with several agents including reactive oxygen species (ROS), necrotic cells, and impaired tissues, which in turn activate inflammatory cells including microglia [11].

During the pathological stages of ischemic stroke, endogenous microglia and recruited macrophages are activated and have functionally distinct phenotypes (Fig. 1). M1-type (classical) microglia produce pro-inflammatory mediators including tumor necrosis factor α (TNF-α), interleukin (IL)-1β, interferon-γ (IFN-γ), IL-6, inducible nitric oxide synthase (iNOS), and proteolytic enzymes (MMP9, MMP3) [12]. Conversely, M2-type (alternative) microglia are characterized by the production of IL-10, transforming growth factor β (TGF-β), insulin-like growth factor, and vascular endothelial growth factor (VEGF), which are pro-angiogenic and anti-inflammatory [13]. It is worth noting that this binary classification is an over-simplification, as there exist numerous overlapping functional states of microglia. Microglia can either promote injury or facilitate repair, depending on the activation signals they receive [3, 14]. The concept of microglial heterogeneity during pathology is relatively new [15]. For instance, M2a-like microglia, which can stimulate tissue repair, immunity against parasites, and growth, can be induced by IL-4 or IL-13. This phenotype is identified by upregulated arginase 1,CD206, and chitinase 3-like 3 (Ym-1) [15]. M2b is an intermediate phenotype, possessing the traits both of inflammatory and restorative microglia. When encountering glucocorticoids, IL-10, or apoptotic cells, the M2c phenotype can appear, which is associated with tissue remodeling after inflammation subsides. Up-regulation of CD206, TGF-β, and CD163 can be seen in this phenotype [15, 16].

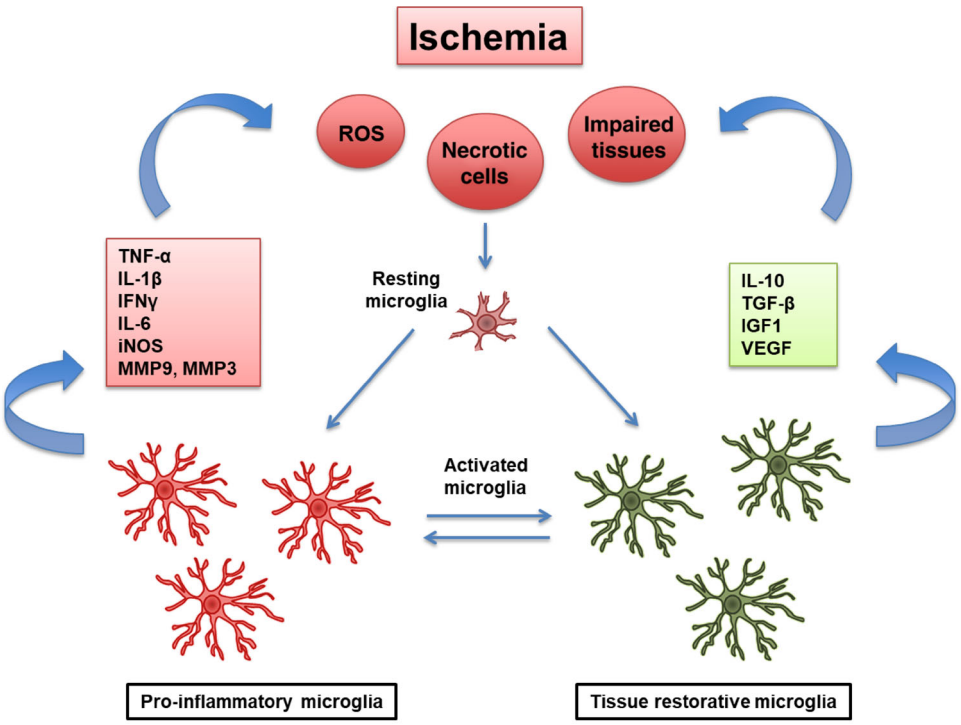

Fig. 1 In ischemic stroke, resting microglia are activated and polarized into functionally distinct phenotypes that range between two extremes. Classical microglia produce pro-inflammatory mediators including tumor necrosis factor α (TNF-α), interleukin 1 beta (IL-1β), interferon-γ (IFNγ), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and proteolytic enzymes (MMP9, MMP3), identified as pro-inflammatory. Alternative microglia are characterized by the production of IL-10, transforming growth factor β (TGF-β), insulin-like growth factor (IGF1) and vascular endothelial growth factor (VEGF), which are pro-angiogenic and anti-inflammatory. Alternative microglia are associated with tissue repair and remodeling, immunity against parasites, and growth stimulation.

Clinical studies of ischemic stroke have revealed that microglial activation can be detected during the acute, sub-acute, and chronic phases of ischemic stroke [17]. Consistently, in experimental animal models of ischemic stroke, the microglial activation status changes at different stages of ischemic stroke and correlates with the severity of ischemia [18, 19]. We next review how microglia respond at different stages of ischemic stroke.

Microglial Activation During the Acute Phase of Experimental Ischemic Stroke

Activated microglia can be observed in different brain regions and phases of ischemia. During the acute phase of ischemic stroke, neurons in the infarct core region suffer from deprivation of oxygen and glucose. Microglial processes respond to neuronal hyperactivity within a few minutes [20, 21]. Thirty minutes after the onset of permanent middle cerebral artery occlusion (MCAO), activated microglia are detected in the boundary zone of the ischemic lesion. However, no TUNEL-positive cells are detected till 6h after MCAO, which agrees with the clinically-reported reversible phase [22]. It has also been reported that microglia marked by ionized Ca^{2+}-binding adapter molecule 1 rapidly appear in the peri-ischemic area 3.5 h after reperfusion in the rat transient MCAO model and peak at 7 days [23]. However, studies of cerebellar cortical stroke in a mouse photothrombotic stroke model have shown that neurons in the core lesion die after 2 h, and this is accompanied by the activation of astrocytes and microglia. Under these conditions, activated microglia persist in the residual infarct region for up to 30 days [24].

Microglial Activation During the Sub-acute Phase of Experimental Ischemic Stroke

During the sub-acute phase, the ischemic lesion expands following a transient ischemic stroke and is associated with edema and the infiltration of peripheral leukocytes. The response of microglia during this sub-acute phase can be characterized by changes in morphology and polarization in the peri-infarct region [25]. Activated microglia are found in the ischemic core and the boundary zone at 24 h and further increase in the ischemic core at 72 h [26]. Amoeboid-like microglia are present in the ischemic lesion between 2 and 10 h of reperfusion. Round and amoeboid cells become predominant in the core lesion and mingle with highly-ramified microglia to the boundary at 22 h of reperfusion [27].

Microglial Activation During the Chronic Phase of Experimental Ischemic Stroke

During the chronic phase, activated microglia are located in the peri-infarct region and distal areas. The number of amoeboid-like microglia is enriched in the core area between 3 and 7 days after stroke [28]. A recent study reported dynamic progression of microglial activation after photothrombotic stroke. First, amoeboid-like activated microglia are observed in the infarct core but close to the inner boundary within 24 h after photothrombosis, with more amoeboid cells infiltrating into the core by day 4. By day 7, amoeboid cells occur throughout the entire lesion. Interestingly, at 28 and 60 days post-lesion, only a few amoeboid cells are found, and the microglial reaction weakens [29]. In a rat photothrombotic stroke model, multiple infarct subregions have been defined as the hypocellular infarct core, outer infarct core, infarct margin, demarcation zone, and peri-infarct zone according to the degree of neuroinflammation. Eight days post-injury, microglial sub-phenotypes overlap in a comparatively thin belt around the necrotic infarct core. The phosphoglycoprotein osteopontin promotes neural survival after ischemic stroke and shifts inflammatory microglia towards a more neutral phenotype [30]. Consistent with this, in a white-matter ischemia model induced by chronic hypoperfusion by bilateral carotid artery stenosis, microglia are largely activated 3 days post-injury, accumulate within 10 days, and decline after 1 month. Cerebral hypoperfusion induces microglial activation, production of associated pro-inflammatory cytokines, and priming of microglial polarization toward the M1 phenotype, while the immune modulator fingolimod attenuates microglia-mediated neuroinflammation after white-matter ischemia and promotes oligodendrocytogenesis by shifting microglia toward M2 polarization [31].

Molecular Mechanisms Underlying Microglial Activation Following Ischemic Stroke

Since the activation of microglia is critical to the pathogenesis, it is of great importance to look into the innate mechanisms and environmental cues of microglial activation following ischemic stroke. This provides a better understanding of the relationship between microglia and ischemic stroke and the molecules that can be targeted for stroke treatment.

Damage-Associated Molecular Pattern Molecules (Damps) and Microglial Activation

DAMPs are molecules passively released from dying cells that facilitate the activation of microglia [32]. In general, DAMPs can be divided into pathogen-associated molecular patterns (PAMPs) and alarmins. Both proteins like high-mobility group box 1 and heat-shock proteins, and non-protein alarmins like adenosine triphosphate (ATP) are included. Released by necrotic cells after brain ischemia, DAMPs take part in the early reactions after stroke onset; they activate microglia and have both neuroprotective and inflammatory functions [1]. While their functions still need to be further distinguished, all DAMPs may serve as potential intervention targets for the treatment of ischemic stroke.

Toll-Like Receptors (Tlrs) and Microglial Activation

TLRs are an innate immune receptor family that play critical roles in the neuroinflammation process induced by stroke [33]. A variety of TLRs, including TLR2, TLR3, TLR4, TLR7, and TLR9, have been implicated in stroke outcomes [34]. It has been reported that TLR2, TLR4, and TLR9 expression is increased after cerebral ischemic injury [35, 36]. TLR2 deficiency in mice can alleviate the symptoms of ischemic stroke [37]. An increase of TLR4 expression leads to activation of the NF-κB pathway, release of the pro-inflammatory cytokines TNF-α and IL-6, and exacerbation of neuronal damage and apoptosis [35]. Collectively, this indicates that TLRs are tightly connected to microglial activation in ischemic stroke.

Notch Signaling and Microglial Activation

The Notch signaling pathway can be activated by inflammatory cytokines, and subsequently regulates adaptive and innate immune responses [38]. Recent studies have shown that up-regulation of Notch signaling greatly contributes to the activation of microglia after ischemic stroke. For instance, negative regulators of Notch, such as DAPT (N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester)7 and DBZ ((S,S)-2-[2-(3,5-difluorophenyl)acetylamino]-N-(5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)propionamide), suppress microglial activation and alleviate the symptoms of ischemic stroke [39–41]. Following hypoxia, inhibition of the Notch signaling pathway reduces NF-kB/p65 expression and translocation through suppression of the TLR4/MyD88/TRAF6 pathways. These results suggest a potential explanation for the relationship between Notch and microglial activation in ischemic stroke [42].

Other Signaling Pathways and Microglial Activation

Other signaling factors have been reported to be involved in microglial activation including the Hv1 proton channel [43, 44], the Na^+/H^+ exchanger [45], TGF-β1/Smad3(-mothers against decapentaplegic homolog 3) [46], zinc finger E-box binding homeobox 1 (ZEB1) [47], signal transducer and activator of transcription 3 (STAT3) [31, 48, 49], interferon regulatory factor 2 binding protein 2 (IRF2BP2) [50], the cystathionine β-synthase–hydrogen sulfide–AMP-activated protein kinase cascade [51], and peroxisome proliferator-activated receptor α/γ dual signaling [52]. However, treatments targeting specific pathways may be insufficient, due to the fact that a plethora of signaling events underlie microglial activity during ischemic pathology, making it difficult to completely abolish microglial activation. In addition, a number of signaling pathways yet to be characterized exist. Thus, further investigations are needed to fully understand the mechanisms associated with microglial activation during ischemic stroke.

Functions of Microglia During Ischemic Stroke

Microglia and Blood-Brain-Barrier Disruption

The blood-brain-barrier (BBB) is composed of a continuous layer of endothelial cells connected by tight junctions (TJs), which help to form a selective physical barrier and maintain brain homeostasis [53]. It has been shown that the BBB is highly disrupted during cerebral ischemia and reperfusion, in which neurovascular inflammation, characterized by up-regulation of inflammatory mediators and proteases from endothelial and immune cells, plays a significant role [54]. BBB disruption may also be strongly associated with the activation state of microglia. It has been shown that following a stroke, BBB integrity is greatly diminished by inflammatory microglia *via* up-regulation of pro-inflammatory cytokines including IL-1β, TNF-α, and IL-6. Disrupted BBB integrity is also associated with increased paracellular permeability, possibly through altered cytoskeletal organization, TJ expression, and MMP production [55]. It has also been reported that under ischemic stroke conditions, blood vessels become permissive to blood serum components, which leak into the tissue and thus promote microglial recruitment [56]. Conversely, the pro-angiogenic microglia may enhance BBB integrity after ischemic stroke, presumably *via* stimulation of TJ expression [57].

Microglia and Neurogenesis

The constant process of neurogenesis contributes to the generation of new neurons over an individual's lifetime [58]. Several studies have revealed that neurogenesis is increased following ischemic stroke both in rodents and primates [59–61]. Under pathological conditions such as ischemic stroke, microglia can function as double-edged swords with respect to neurogenesis [62]. It has been shown that in the ipsilateral sub-ventricular zone (SVZ), activated microglia with ramified or intermediate morphology are associated with neuroblast migration after stroke, while amoeboid microglia in the peri-infarct area may be detrimental to neurogenesis [62]. However, other studies challenge this idea and show that the number and migration of neuroblasts are not affected by microglia [63]. It has also been shown that pro-angiogenic microglia may enhance neural proliferation and differentiation following ischemic stroke, possibly *via* up-regulation of TGF-α [64]. Elevated numbers of IGF-1-expressing microglia have been found in the SVZ in the chronic phase of stroke. The long-term accumulation of microglia with this pro-neurogenic phenotype in the SVZ implies a supportive role for these cells after stroke [62]. Understanding the relationship between microglia and neurogenesis could shed a new light on the role of microglia in CNS repair after ischemic stroke.

Microglia and Angiogenesis

Emerging evidence suggests that microglia can influence the formation of new blood vessels, a process called angiogenesis [65]. In mice deficient in macrophage colony-stimulating factor, it has been reported that the complexity of developing retinal vasculature is reduced. This coincides with the elimination of microglia, suggesting a potential role for microglia in angiogenesis [66]. During ischemic stroke, microglia are closely associated with blood vessels and form perivascular clusters and phagocytic structures. The clustering of microglia around the vasculature results in vessel disintegration along with the upregulation of phagocytic CD68 expression in the penumbra [56]. Subsequently, VEGF, a molecule known to promote angiogenesis, is released by microglia, suggesting that microglia promote the reconstruction of cerebral blood vessel following ischemic stroke [67, 68]. Therefore, microglia may serve a dual function in ischemic stroke, based on the stage.

Microglia and Synaptic Remodeling

Synaptic remodeling is correlated with rehabilitation and the outcome of ischemic stroke [69]. It has been reported that the onset of ischemic stroke can lead to synaptic dysfunction [70]. Recent studies have shown that microglia participate in synaptic remodeling, which plays an important role in promoting neural circuit refinement. For instance, microglia promote synaptic pruning *via* CX3C chemokine receptor 1 (CX3CR1) or complement during brain development [71, 72]. In addition, microglia promote spine formation and synaptic maturation [73, 74]. Interestingly, neuronal activity and synaptic function is also modulated by the state of microglia. It has been shown that long-term synaptic depression is triggered by microglial CR3 activation following inflammatory stimulus *via* NADPH oxidase, which is one of the main mediators of neurotoxicity in stroke [75]. Taken together, microglia could play a critical role in synaptic remodeling and thus modulate the function of neural circuit following ischemic injury.

Microglial Crosstalk with Other Cells During Ischemic Stroke

Ischemia affects microglia and all other cells in the brain parenchyma as well as the neurovascular unit (Fig. 2). Dynamic interactions between microglia and neurons, microglia and astrocytes, microglia and oligodendrocyte, and microglia and endothelial cells can tip the balance between the acute injury and post-stroke recovery [76].

Microglia and Neurons

Following ischemic stroke, neuronal damage initiates early activation processes in microglia to engulf cellular debris and regulate neuronal function *via* various neurotransmitters and modulators, such as glutamate, fractalkine,Lipocalin-2 (LCN2), Triggering receptor expressed on myeloid cells-2 (TREM2), and others [44, 77, 78]. Activated microglia have beneficial functions essential for neuron survival [62]. Indeed, ablation of microglia results in a significant increase in infarct size and is associated with elevated neuronal apoptosis [79]. Microglia-derived IGF-1 has also been identified as a trophic factor involved in the maintenance of neuronal survival. However, activated microglia may not always be beneficial. Mounting evidence indicates that uncontrolled microglial activation can give rise to progressive neurotoxic consequences by the excess production of a large array of cytotoxic factors such as superoxide [43, 80, 81], nitric oxide (NO) [82], and TNF-α [83].

Involvement of Glutamate in Microglia and Neuron Crosstalk

Neuronal N-methyl-D-aspartate receptors trigger ATP release which can activate the microglial P2Y12 receptor

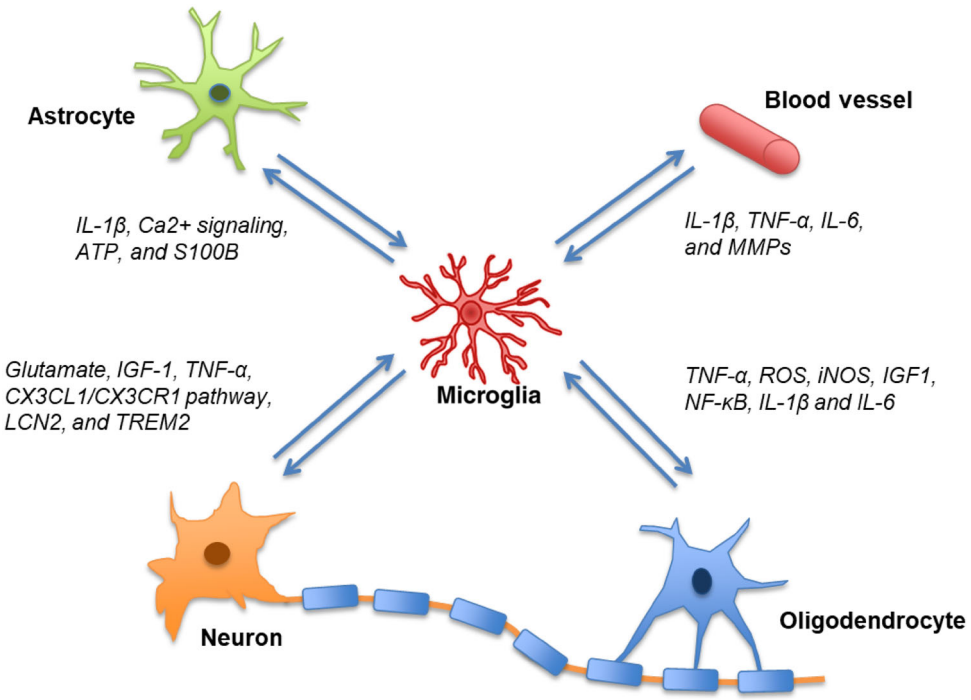

Fig. 2 Dynamic interactions between microglia and all cells within the neurovascular unit underlie the balance between acute injury and post-stroke recovery. After ischemic stroke, neuronal damage initiates an early activation processes of microglia. Activated microglia, in turn, have beneficial functions essential for neuron survival, and give rise to progressive neurotoxic consequences by the excess production of a large array of cytotoxic factors at the same time. Microglia are active earlier than astrocytes, which promote astrocyte activation. Astrocyte in turn further activate distant microglia and restrict microglial activity, with increasing secretion of characteristic inflammatory cytokines. In addition, microglial activation is an important course for damaging oligodendrocyte precursor cells/oligodendrocytes in ischemic white-matter injury. Disruption of the blood-brain barrier attracts and activates distant microglia, which in turn migrate towards the disrupted blood vessels and further start to phagocytize the damaged vessels. Activated microglia express pro-inflammatory cytokines extremely highly, which provokes the rapid disintegration of blood vessels, but also regulate primary brain endothelial cell proliferation and promote angiogenesis in a biphasic manner.

and subsequently induce microglial process extension and inflammatory responses [20, 21, 84]. Interestingly, glutamate released by activated microglia either from the connexin 32 hemi-channel or reversed glutamate transporters induces excito-neurotoxicity and may contribute to neuronal damage. Consistently, the neuronal damage induced by activated microglia is almost completely inhibited by using a glutaminase inhibitor or hemichannel blockers to diminish microglial glutamate release [85].

Involvement of the CX3CL1/CX3CR1 Pathway in Microglia and Neuron Crosstalk

The chemokine fractalkine, also known as CX3CL1, is secreted byneurons. CX3CR1 is exclusively expressed on microglia and has been implicated in microglial chemotaxis, microglia-mediated neurotoxicity [86], and microglial activation [87]. Studies have shown that microglia fail to acquire an amoeboid morphology after the administration of CX3CL1 toCX3CR1-knockout mice, demonstrating that CX3CL1/CX3CR1 plays a critical part in microglial activation [88]. Early studies also showed that deficiency of CX3CR1 in microglia improves outcomes following ischemic brain injury, including smaller infarct volume, depressed neuronal apoptosis, and reduced ROS levels [89–91]. Soluble CX3CL1 secreted by glutamate-damaged neurons provides both phagocytic and neuroprotective signals to promote the microglial clearance of neuronal debris through the release of milk fat globule-EGF factor 8 [92].

Involvement of LCN2 in Microglia and Neuron Crosstalk

LCN2 is widely expressed in multiple tissues, and its receptors are located in microglia, astrocytes, epithelial cells, and neurons [93]. Originally, LCN2 was found to be secreted by active microglia and acted in an autocrine manner to sensitize microglia to self-regulatory apoptosis and to endow them with an amoeboid form [93]. Interestingly, a recent study found that injured neurons release LCN2 as a ‘help-me’ signal to activate microglia into a pro-recovery phenotype [94]. LCN2 is also upregulated within neurons in rat focal cerebral ischemia. Under stimulation byLCN2 *in vitro*, primary microglia are

activated, with enhanced expression of IL-10 and phagocytotic activity. Meanwhile, LCN2-activated microglia upregulate the neuronal expression of synaptic proteins, such as synaptophysin, postsynaptic density protein 95 and synaptotagmin, and thus protect neurons against oxygen and glucose deprivation [94].

Involvement of TREM2 in Microglia and Neuron Crosstalk

TREM2, an innate immune receptor of the TREM family, is exclusively expressed by microglia in the brain and thought to phagocytose injured brain cells. TREM2 does not have an intrinsic signaling motif and its stimulation induces phosphorylation of the membrane adaptor protein DAP12 and extracellular signal-regulated kinase, reorganization of the cytoskeleton, and increased phagocytosis. It has also been demonstrated that TREM2-deficient microglia show increased gene transcription of TNF-α and nitric oxide synthase (NOS). Overexpression of TREM2 in microglia results in increased phagocytosis of apoptotic neurons and decreased gene transcription of TNF-α, IL-1β, and NOS [95]. Recent studies have explored microglial TREM2 function in both *in vitro* and *in vivo* stroke models [96]. The number of activated microglia and microglial phagocytosis of injured neurons are lower in TREM2-deficientmice than in wild-type mice after MCAO and reperfusion. Consistently, TREM2-deficientmice also have worse neurological recovery [97]. Therefore, these results imply that microglial TREM2 signaling is involved in the clearance of dead neurons and suppresses the production of pro-inflammatory cytokines to maintain the local immunosuppressive microenvironment in the CNS.

Microglia and Astrocytes

Both microglia and astrocytes are active participants in various pathological conditions such as stroke, chronic pain, and neurodegenerative disorders. Microglia respond to injury earlier than astrocytes, and can promote astrocyte activation. Astrocytes in turn further activate distant microglia, increasing the secretion of characteristic inflammatory cytokines. Mediators contributing to microglia-astrocyte crosstalk include IL-1β, Ca^{2+} signaling, ATP, and S100 Ca^{2+}-binding protein B (S100B) [98–100].

Involvement of IL-1β in Microglia and Astrocyte Crosstalk

Among the various cytokines, IL-1β has been reported to be mainly produced by microglia [101]. Exogenous administration of IL-1β promotes astrocytic activation, which leads to astrogliosis [102]. Consistently, astrocytic activation is delayed in mice lacking IL-1β receptors [103]. Apart from stimulating astrocytic activation, IL-1β also mediates neurotoxicity of activated astrocytes. It has been reported that large amounts of NO are released from primary human astrocytes, and this is completely blocked by IL-1β receptor agonist protein [104]. Moreover, IL-1β dose-dependently inhibits astrocytic glutamate uptake, resulting in the accumulation of glutamate and subsequent neuronal excitotoxicity [105].

Involvement of Ca^{2+} Signaling and ATP in Microglia and Astrocyte Crosstalk

One of the notable characteristics of ischemia is the elevated level of intracellular Ca^{2+} in astrocytes. Interestingly, astrocytic Ca^{2+} wavespropagate to neighboring microglia [106]. It has been reported that, except for microglia located in the penumbra, microglia far from the infarct core are also activated, even though the tissue shows no sign of damage [107]. Astrocytic Ca^{2+} signaling, in particular their ability to propagate long-distance Ca^{2+} waves, may trigger the activation of distant microglia [108]. This is important, especially for delaying the expansion of infarct volume in focal ischemia [109]. Blocking purinergic receptors with an antagonist abolishes this spread [110]. Microglia express a variety of purinergic receptors including P2X4, P2X7, P2Y6, and P2Y12, so it is possible that ATP is the messenger in astrocyte-to-microglia communication under stroke conditions [111].

Involvement of S100B in Microglia and Astrocyte Crosstalk

S100B belongs to the large S100superfamily, is mainly expressed by astrocytes in the brain, and plays crucial roles in cell proliferation, differentiation, apoptosis, signal transduction, and metabolism [112]. The level of serum S100B is elevated in patients who suffer an ischemic stroke, making it a novel diagnostic biomarker [112]. Astrocytes can release S100B and augment this release when stimulated by several factors, including TNF- [113]. Under physiological conditions, S100B functions as a neurotrophic factor, counteracting the stimulatory effect of neurotoxins on microglia [114] and facilitating glutamate uptake [115]. Under pathological conditions, like stroke, at high concentrations S100B binds to Receptor for Advanced Glycation End products, which may regulate microglial activation over the course of brain damage [116]. Thus, secretory S100B is a possible mediator of astrocyte-microglia crosstalk in ischemic stroke.

Microglia and Oligodendrocytes

Several mechanisms, including oxidative stress, excitatory amino-acids, trophic factor deprivation, and activation of apoptotic pathways, have been shown to initiate

oligodendrocyte injury under ischemic conditions [117]. Among them, microglial activation is thought to be an important cause of damage to oligodendrocyte precursor cells/oligodendrocytes during ischemic white-matter injury [31]. However, other studies have suggested that whether the interaction between activated microglia and oligodendrocytes is deleterious or beneficial may depend on the developmental stage of the cells. Activated microglia have been found to be harmful to oligodendrocyte pro-genitor cells but increase the survival of mature oligodendro-cytes [118].

Involvement of TNF-α and Inflammatory Cytokines in the Microglia and Oligodendrocyte Crosstalk

Early studies showed that once microglia are activated, they produce TNF-α to kill oligodendrocytes, and this effect is enhanced in the presence of complements [119]. In addition, activated microglia produce TNF-α and IL-1β, which are associated with periventricular white-matter injury in hypoxic neonatal brain [120]. Tissue inhibitor of metalloproteinase3 (TIMP-3) blocks the separation of various members of the TNF death receptor families, including TNFR-1, by inhibiting TNF-α converting enzyme and stabilizing the death receptor on the surface of oligodendrocytes. Conversely, TIMP-3 also enhances the inflammatory response and release of inflammatory mediators by microglia, including TNF-α and MMP-3, which promote oligodendrocyte apoptosis [121]. Inflammatory cytokines derived from activated microglia, such as IL-1β and IL-6, are also important to the survival, differentiation, and maturation of oligodendrocytes [122, 123]. However, microglial activation induced by IL-4 shows bias towards oligodendrogenesis [124].

Involvement of Oxidative Stress in Microglia and Oligodendrocyte Crosstalk

One source of ROS production is from the multi-subunit phagocyte NADPH oxidase under stroke conditions. Oxidative stress occurs when the balance between the formation of ROS and the ability of cells to defend against them is disrupted. ROS produced by microglia has detrimental effects on both neurons and oligodendrocytes and has been implicated in causing damage to myelin sheaths [125]. Activated microglia are also known to release ROS and reactive nitrogen species, which plays a crucial role in the development of white-matter lesions [126].

Involvement of iNOS in Microglia and Oligodendrocyte Crosstalk

During the acute stage of transient focal cerebral ischemia, the upregulation of iNOS in Iba1-positive microglia plays a pivotal role in the damage to neurons and oligodendrocytes in the ipsilateral hippocampal CA1 region [127]. Excessive production of NO from microglial iNOS is toxic to oligodendrocytes [128].

Microglia and Endothelial Cells

The brain parenchyma is protected by the BBB, which is formed by a continuous layer of endothelial cells, pericytes, astrocytes, microglia, and the surrounding basement membrane. Using time-lapse two-photon microscopy *in vivo*, it has been shown that microglia become activated and start to expand cellular protrusions towards adjacent blood vessels soon after disruption of the BBB [129]. These activated perivascular microglia start to engulf endothelial cells, which may be mediated by the CX3CR1 receptor [130, 131]. In the meantime, activated microglia highly express metalloproteins, ROS, VEGF, and pro-inflammatory cytokines [132, 133]. Upregulation of VEGF, a pro-angiogenic molecule, increases in the penumbra and induces quiescent vessels to sprout, which provokes a rapid disintegration of blood vessels [132]. This subsequently leads to leakage of serum components and triggers microglial phagocytosis. These serum proteins attract and activate distant microglia, which migrate towards the disrupted blood vessels and further start to phagocytize the damaged vessel [130]. The increasingly damaged blood vessels together with the upregulation of endothelial ICAM-1, P-selectin, and VCAM, allow for the extravasation of leukocytes and amplifying the inflammation process, a vicious cycle under stroke conditions.

It is well established that angiogenesis occurs following cerebral ischemia [134]. Interestingly, studies of ischemic tissue have demonstrated a strong association between new vessel formation and microglial recruitment and activation [65, 135]. During the acute and sub-acute phases of stroke, anti-inflammatory microglia are predominantly localized in the ischemic area, and promote angiogenesis *via* secretion of TGF-β in the potentially salvageable penumbra [136]. Endothelial cell proliferation is a fundamental early step in the angiogenic process. Microglia regulate primary brain endothelial cell proliferation in a biphasic manner. Conditioned medium from resting microglia inhibits endothelial cell proliferation, while conditioned medium from activated microglia promotes it [57]. Moreover, endothelial cell proliferation is inhibited by TGF-β, but promoted by TNF-α. In addition, after the administration of M2-like microglia, remodeling factors such as VEGF, TGF-β, and

MMP-9 are secreted in the brain parenchyma, resulting in angiogenesis [137].

Microglia as a Therapeutic Target for Ischemic Stroke

A large variety of receptors are present in microglia. Their activation by immune signals, neurotransmitters, neuropeptides, and metabolites drives microglia toward different phenotypes and functions [7]. Activated microglia with different morphology and phenotypes are important for the stroke-induced neuroinflammatory response. Although activated microglia are thought to be beneficial in clearing cellular debris, their excessive activation may hinder brain repair. Therapeutic strategies targeting microglial activation have shown promise [8, 138, 139]. Minocycline and other inhibitors of microglial activation have shown possible beneficial effects. Improved neurogenesis and neurological function has been reported with reduced microglial activation in an MCAO model treated with minocycline [140, 141]. Inhibitors targeting the TLR4 pathway, DAMP pathway, and inflammasomes such as NLRP1 and NLRP3, have also shown possible protective effects with smaller infarct volumes and better neurological scores [34].

It is generally considered that pro-inflammatory microglia are harmful, while tissue restorative microglia are beneficial in stroke. Thus, shifting the phenotypic balance towards a restorative phenotype could be a novel therapeutic intervention for stroke. Several drugs promoting this phenotype transition have shown protective effects in stroke [7, 8, 31]. Previous studies have demonstrated that intravascular administration of beneficial microglia improves the functional outcome after ischemic stroke. Moreover, subcutaneous or intraventricular administration of IL-4 can induce the proposed phenotypic shift and improves the functional outcome after ischemic stroke [142]. Microglial phenotype correlates with the balance of cAMP-response element binding protein (CREB) *versus* NF-κB. Increasing the ratio of CREB to NF-κB contributes to a selective activation of restorative microglia [143]. Finally, targeting cell metabolism has emerged as a new potential therapeutic approach to redirect microglia from detrimental to pro-regenerative, and has been demonstrated to dampen excessive microglial activation after stroke [7].

Conclusion

Microglial cells are traditionally considered to be the resident immune cells of the CNS. Recent studies have shown that microglia constantly scan CNS tissue and maintain homeostasis in the brain environment [129, 144]. However, once disturbed, microglia are activated and migrate to injury sites [145]. Activated microglia play essential roles in BBB disruption, neurogenesis, angiogenesis, and synaptic remodeling [55, 62, 66, 71]. Yet not all activation is the same; gene expression is often determined by the signals microglia encounter [146]. They dynamically interact with, and are influenced by, other cell types such as neurons, astrocytes, oligodendrocytes, and endothelial cells during the different stages of ischemic stroke [76]. Microglial polarization seems to be a key factor in ischemic stroke. Therefore, methods that promote restorative microglia are of interest [8]. Several drugs have been discovered and have considerable effects. However, controversy still surrounds the modulation of microglial function in ischemic stroke. Thus, more work is needed before the targeting of microglia finds clinical application.

Acknowledgements This review was supported by the National Natural Science Foundation of China (81571132, 81873743, and 81801223), Fundamental Research Funds for the Central Universities, China (2017KFYXJJ107 and 2017KFYXJJ124), and the National Institutes of Health, USA (R01NS088627).

References

1. Gulke E, Gelderblom M, Magnus T. Danger signals in stroke and their role on microglia activation after ischemia. Ther Adv Neurol Disord 2018, 11: 1756286418774254.
2. Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, Zheng P, *et al.* Microglial and macrophage polarization-new prospects for brain repair. Nat Rev Neurol 2015, 11: 56–64.
3. Ma Y, Wang J, Wang Y, Yang GY. The biphasic function of microglia in ischemic stroke. Prog Neurobiol 2017, 157: 247–272.
4. Wen YD, Zhang HL, Qin ZH. Inflammatory mechanism in ischemic neuronal injury. Neurosci Bull 2006, 22: 171–182.
5. Chen G, Luo X, Qadri MY, Berta T, Ji RR. Sex-dependent glial signaling in pathological pain: distinct roles of spinal microglia and astrocytes. Neurosci Bull 2018, 34: 98–108.
6. Fang X, Sun D, Wang Z, Yu Z, Liu W, Pu Y, *et al.* MiR-30a positively regulates the inflammatory response of microglia in experimental autoimmune encephalomyelitis. Neurosci Bull 2017, 33: 603–615.
7. Fumagalli M, Lombardi M, Gressens P, Verderio C. How to reprogram microglia toward beneficial functions. Glia 2018.
8. Wang J, Xing H, Wan L, Jiang X, Wang C, Wu Y. Treatment targets for M2 microglia polarization in ischemic stroke. Biomed Pharmacother 2018, 105: 518–525.
9. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Ann Rev Immunol 2009, 27: 119–145.
10. Chamorro A, Hallenbeck J. The harms and benefits of inflammatory and immune responses in vascular disease. Stroke 2006, 37: 291–293.
11. Amantea D, Nappi G, Bernardi G, Bagetta G, Corasaniti MT. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. Febs Journal 2009, 276: 13–26.

12. Yenari MA, Kauppinen TM, Swanson RA. Microglial activation in stroke: therapeutic targets. Neurotherapeutics 2010, 7: 378–391.
13. Ponomarev ED, Veremeyko T, Weiner HL. MicroRNAs are universal regulators of differentiation, activation, and polarization of microglia and macrophages in normal and diseased CNS. Glia 2013, 61: 91–103.
14. Al Mamun A, Chauhan A, Yu H, Xu Y, Sharmeen R, Liu F. Interferon regulatory factor 4/5 signaling impacts on microglial activation after ischemic stroke in mice. Eur J Neurosci 2018, 47: 140–149.
15. Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 2009, 4: 399–418.
16. Chhor V, Le Charpentier T, Lebon S, Ore MV, Celador IL, Josserand J, *et al.* Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia in vitro. Brain Behav Immun 2013, 32: 70–85.
17. Gulyas B, Toth M, Schain M, Airaksinen A, Vas A, Kostulas K, *et al.* Evolution of microglial activation in ischaemic core and pen-infarct regions after stroke: A PET study with the TSPO molecular imaging biomarker C-11 vinpocetine. J Neurol Sci 2012, 320: 110–117.
18. Morrison HW, Filosa JA. A quantitative spatiotemporal analysis of microglia morphology during ischemic stroke and reperfusion. J Neuroinflammation 2013, 10.
19. Yan T, Chopp M, Chen J. Experimental animal models and inflammatory cellular changes in cerebral ischemic and hemorrhagic stroke. Neurosci Bull 2015, 31: 717–734.
20. Eyo UB, Gu N, De S, Dong H, Richardson JR, Wu LJ. Modulation of microglial process convergence toward neuronal dendrites by extracellular calcium. J Neurosci 2015, 35: 2417–2422.
21. Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ. Neuronal hyperactivity recruits microglial processes *via* neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. J Neurosci 2014, 34: 10528–10540.
22. Rupalla K, Allegrini PR, Sauer D, Wiessner C. Time course of microglia activation and apoptosis in various brain regions after permanent focal cerebral ischemia in mice. Acta Neuropathol 1998, 96: 172–178.
23. Ito D, Tanaka K, Suzuki S, Dembo T, Fukuuchi Y. Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. Stroke 2001, 32: 1208–1215.
24. Gorlamandala N, Parmar J, Craig AJ, Power JM, Moorhouse AJ, Krishnan AV, *et al.* Focal ischaemic infarcts expand faster in cerebellar cortex than cerebral cortex in a mouse photothrombotic stroke model. Transl Stroke Res 2018, 9: 643–653.
25. Neher JJ, Emmrich JV, Fricker M, Mander PK, Thery C, Brown GC. Phagocytosis executes delayed neuronal death after focal brain ischemia. Proc Natl Acad Sci U S A 2013, 110: E4098–E4107.
26. Shi QJ, Wang H, Liu ZX, Fang SH, Song XM, Lu YB, *et al.* HAMI 3379, a CysLT2R antagonist, dose- and time-dependently attenuates brain injury and inhibits microglial inflammation after focal cerebral ischemia in rats. Neuroscience 2015, 291: 53–69.
27. Zhang Z, Chopp M, Powers C. Temporal profile of microglial response following transient (2h) middle cerebral artery occlusion. Brain Research 1997, 744: 189–198.
28. Perego C, Fumagalli S, De Simoni MG. Temporal pattern of expression and colocalization of microglia/macrophage phenotype markers following brain ischemic injury in mice. J Neuroinflammation 2011, 8: 174.
29. Nowicka D, Rogozinska K, Aleksy M, Witte OW, Skangiel-Kramska J. Spatiotemporal dynamics of astroglial and microglial responses after photothrombotic stroke in the rat brain. Acta Neurobiol Exp (Wars) 2008, 68: 155–168.
30. Ladwig A, Walter HL, Hucklenbroich J, Willuweit A, Langen KJ, Fink GR, *et al.* Osteopontin augments M2 microglia response and separates M1- and M2-polarized microglial activation in permanent focal cerebral ischemia. Mediators Inflamm 2017, 2017: 7189421.
31. Qin C, Fan WH, Liu Q, Shang K, Murugan M, Wu LJ, *et al.* Fingolimod protects against ischemic white matter damage by modulating microglia toward M2 polarization *via* STAT3 pathway. Stroke 2017, 48: 3336–3346.
32. Huang J, Xie Y, Sun X, Zeh HJ, III, Kang R, Lotze MT, *et al.* DAMPs, ageing, and cancer: The 'DAMP Hypothesis'. Ageing Res Rev 2015, 24: 3–16.
33. Fang H, Wang PF, Zhou Y, Wang YC, Yang QW. Toll-like receptor 4 signaling in intracerebral hemorrhage-induced inflammation and injury. J Neuroinflammation 2013, 10: 27.
34. Xiong XY, Liu L, Yang QW. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. Prog Neurobiol 2016, 142: 23–44.
35. Zhao SC, Ma LS, Chu ZH, Xu H, Wu WQ, Liu F. Regulation of microglial activation in stroke. Acta Pharmacol Sin 2017, 38: 445–458.
36. Ji Y, Zhou Y, Pan J, Li X, Wang H, Wang Y. Temporal pattern of Toll-like receptor 9 upregulation in neurons and glial cells following cerebral ischemia reperfusion in mice. Int J Neurosci 2016, 126: 269–277.
37. Bohacek I, Cordeau P, Lalancette-Hebert M, Gorup D, Weng YC, Gajovic S, *et al.* Toll-like receptor 2 deficiency leads to delayed exacerbation of ischemic injury. J Neuroinflammation 2012, 9: 17.
38. Ando K, Kanazawa S, Tetsuka T, Ohta S, Jiang X, Tada T, *et al.* Induction of Notch signaling by tumor necrosis factor in rheumatoid synovial fibroblasts. Oncogene 2003, 22: 7796–7803.
39. Wei Z, Chigurupati S, Arumugam TV, Jo DG, Li H, Chan SL. Notch activation enhances the microglia-mediated inflammatory response associated with focal cerebral ischemia. Stroke 2011, 42: 2589–2594.
40. Yuan Y, Rangarajan P, Kan EM, Wu Y, Wu C, Ling EA. Scutellarin regulates the Notch pathway and affects the migration and morphological transformation of activated microglia in experimentally induced cerebral ischemia in rats and in activated BV-2 microglia. J Neuroinflammation 2015, 12.
41. Arumugam TV, Chan SL, Jo DG, Yilmaz G, Tang SC, Cheng AW, *et al.* Gamma secretase-mediated notch signaling worsens brain damage and functional outcome in ischemic stroke. Nat Med 2006, 12: 621–623.
42. Yao L, Kan EM, Kaur C, Dheen ST, Hao A, Lu J, *et al.* Notch-1 signaling regulates microglia activation via NF-kappa B pathway after hypoxic exposure *in vivo* and *in vitro*. PLoS One 2013, 8.
43. Wu LJ, Wu G, Akhavan Sharif MR, Baker A, Jia Y, Fahey FH, *et al.* The voltage-gated proton channel Hv1 enhances brain damage from ischemic stroke. Nat Neurosci 2012, 15: 565–573.
44. Wu LJ. Voltage-gated proton channel HV1 in microglia. Neuroscientist 2014, 20: 599–609.
45. Song S, Wang S, Pigott VM, Jiang T, Foley LM, Mishra A, *et al.* Selective role of Na(+) /H(+) exchanger in Cx3cr1(+) microglial activation, white matter demyelination, and post-stroke function recovery. Glia 2018, 66: 2279–2298.
46. Yu Y, Li J, Zhou H, Xiong Y, Wen Y, Li H. Functional importance of the TGF-beta1/Smad3 signaling pathway in

oxygen-glucose-deprived (OGD) microglia and rats with cerebral ischemia. Int J Biol Macromol 2018, 116: 537–544.
47. Li D, Lang W, Zhou C, Wu C, Zhang F, Liu Q, *et al.* Upregulation of microglial ZEB1 ameliorates brain damage after acute ischemic stroke. Cell Rep 2018, 22: 3574–3586.
48. Chen S, Dong Z, Cheng M, Zhao Y, Wang M, Sai N, *et al.* Homocysteine exaggerates microglia activation and neuroinflammation through microglia localized STAT3 overactivation following ischemic stroke. J Neuroinflammation 2017, 14: 187.
49. Liang K, Zhu L, Tan J, Shi W, He Q, Yu B. Identification of autophagy signaling network that contributes to stroke in the ischemic rodent brain via gene expression. Neurosci Bull 2015, 31: 480–490.
50. Cruz SA, Hari A, Qin Z, Couture P, Huang H, Lagace DC, *et al.* Loss of IRF2BP2 in microglia increases inflammation and functional deficits after focal ischemic brain injury. Front Cell Neurosci 2017, 11: 201.
51. Zhang M, Wu X, Xu Y, He M, Yang J, Li J, *et al.* The cystathionine beta-synthase/hydrogen sulfide pathway contributes to microglia-mediated neuroinflammation following cerebral ischemia. Brain Behav Immun 2017, 66: 332–346.
52. Li Y, Xu L, Zeng K, Xu Z, Suo D, Peng L, *et al.* Propane-2-sulfonic acid octadec-9-enyl-amide, a novel PPARalpha/gamma dual agonist, protects against ischemia-induced brain damage in mice by inhibiting inflammatory responses. Brain Behav Immun 2017, 66: 289–301.
53. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 2008, 57: 178–201.
54. del Zoppo GJ. Inflammation and the neurovascular unit in the setting of focal cerebral ischemia. Neuroscience 2009, 158: 972–982.
55. Pan W, Kastin AJ. Tumor necrosis factor and stroke: Role of the blood-brain barrier. Prog Neurobiol 2007, 83: 363–374.
56. Jolivel V, Bicker F, Binamé F, Ploen R, Keller S, Gollan R, *et al.* Perivascular microglia promote blood vessel disintegration in the ischemic penumbra. Acta Neuropathol 2015, 129: 279–295.
57. Welser JV, Li L, Milner R. Microglial activation state exerts a biphasic influence on brain endothelial cell proliferation by regulating the balance of TNF and TGF-beta1. J Neuroinflammation 2010, 7: 89.
58. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008, 132: 645–660.
59. Tsai YW, Yang YR, Wang PS, Wang RY. Intermittent hypoxia after transient focal ischemia induces hippocampal neurogenesis and c-Fos expression and reverses spatial memory deficits in rats. PLoS One 2011, 6: 9.
60. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 2002, 8: 963–970.
61. Tonchev AB. Brain ischemia, neurogenesis, and neurotrophic receptor expression in primates. Archives Italiennes De Biologie 2011, 149: 225–231.
62. Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J, *et al.* Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. Glia 2009, 57: 835–849.
63. Heldmann U, Mine Y, Kokaia Z, Ekdahl CT, Lindvall O. Selective depletion of Mac-1-expressing microglia in rat subventricular zone does not alter neurogenic response early after stroke. Exp Neurol 2011, 229: 391–398.
64. Choi JY, Kim JY, Kim JY, Park J, Lee WT, Lee JE. M2 Phenotype microglia-derived cytokine stimulates proliferation and neuronal differentiation of endogenous stem cells in ischemic brain. Exp Neurobiol 2017, 26: 33–41.
65. Zhao X, Eyo UB, Murugan M, Wu LJ. Microglial interactions with the neurovascular system in physiology and pathology. Dev Neurobiol 2018, 78: 604–617.
66. Kubota Y, Takubo K, Shimizu T, Ohno H, Kishi K, Shibuya M, *et al.* M-CSF inhibition selectively targets pathological angiogenesis and lymphangiogenesis. J Exp Med 2009, 206: 1089–1102.
67. Zhang ZG, Zhang L, Jiang Q, Zhang RL, Davies K, Powers C, *et al.* VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest 2000, 106: 829–838.
68. Xie L, Mao X, Jin K, Greenberg DA. Vascular endothelial growth factor-B expression in postischemic rat brain. Vasc Cell 2013, 5: 8.
69. Nie J, Yang X. Modulation of synaptic plasticity by exercise training as a basis for ischemic stroke rehabilitation. Cell Mol Neurobiol 2017, 37: 5–16.
70. Huerta PT, Volpe BT. Transcranial magnetic stimulation, synaptic plasticity and network oscillations. J Neuroeng Rehabil 2009, 6: 7.
71. Lauro C, Catalano M, Trettel F, Limatola C, Sci NYA. Fractalkine in the nervous system: neuroprotective or neurotoxic molecule? Neuroimmunomodulation Health Dis 2015, 1351: 141–148.
72. Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B. Microglia: dynamic mediators of synapse development and plasticity. Trends Immunol 2015, 36: 605–613.
73. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, 3rd, Lafaille JJ, *et al.* Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 2013, 155: 1596–1609.
74. Miyamoto A, Wake H, Ishikawa AW, Eto K, Shibata K, Murakoshi H, *et al.* Microglia contact induces synapse formation in developing somatosensory cortex. Nat Commun 2016, 7: 12540.
75. Zhang J, Malik A, Choi HB, Ko RWY, Dissing-Olesen L, MacVicar BA. Microglial CR3 Activation Triggers Long-Term Synaptic Depression in the Hippocampus via NADPH Oxidase. Neuron 2014, 82: 195–207.
76. Maki T, Hayakawa K, Pham LD, Xing C, Lo EH, Arai K. Biphasic mechanisms of neurovascular unit injury and protection in CNS diseases. CNS Neurol Disord Drug Targets 2013, 12: 302–315.
77. Tian DS, Li CY, Qin C, Murugan M, Wu LJ, Liu JL. Deficiency in the voltage-gated proton channel Hv1 increases M2 polarization of microglia and attenuates brain damage from photothrombotic ischemic stroke. J Neurochem 2016, 139: 96–105.
78. Tikamdas R, Singhal S, Zhang P, Smith JA, Krause EG, Stevens SM, Jr., *et al.* Ischemia-responsive protein 94 is a key mediator of ischemic neuronal injury-induced microglial activation. J Neurochem 2017, 142: 908–919.
79. Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 2007, 27: 2596–2605.
80. Wu LJ. Microglial voltage-gated proton channel Hv1 in ischemic stroke. Transl Stroke Res 2014, 5: 99–108.
81. Liu J, Tian D, Murugan M, Eyo UB, Dreyfus CF, Wang W, *et al.* Microglial Hv1 proton channel promotes cuprizone-induced demyelination through oxidative damage. J Neurochem 2015, 135: 347–356.
82. Moss DW, Bates TE. Activation of murine microglial cell lines by lipopolysaccharide and interferon-gamma causes NO-mediated decreases in mitochondrial and cellular function. Eur J Neurosci 2001, 13: 529–538.

83. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007, 8: 57–69.
84. Eyo UB, Bispo A, Liu J, Sabu S, Wu R, DiBona VL, *et al.* The GluN2A subunit regulates neuronal NMDA receptor-induced microglia-neuron physical interactions. Sci Rep 2018, 8: 828.
85. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, *et al.* Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. J Biol Chem 2006, 281: 21362–21368.
86. Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, *et al.* Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 2006, 9: 917–924.
87. Mizuno T, Kawanokuchi J, Numata K, Suzumura A. Production and neuroprotective functions of fractalkine in the central nervous system. Brain Res 2003, 979: 65–70.
88. Liang KJ, Lee JE, Wang YD, Ma W, Fontainhas AM, Fariss RN, *et al.* Regulation of dynamic behavior of retinal microglia by CX3CR1 signaling. Invest Ophthalmol Vis Sci 2009, 50: 4444–4451.
89. Tang Z, Gan Y, Liu Q, Yin JX, Liu Q, Shi J, *et al.* CX3CR1 deficiency suppresses activation and neurotoxicity of microglia/macrophage in experimental ischemic stroke. J Neuroinflammation 2014, 11: 26.
90. Denes A, Ferenczi S, Halasz J, Kornyei Z, Kovacs KJ. Role of CX3CR1 (fractalkine receptor) in brain damage and inflammation induced by focal cerebral ischemia in mouse. J Cereb Blood Flow Metab 2008, 28: 1707–1721.
91. Fumagalli S, Perego C, Ortolano F, De Simoni MG. CX3CR1 deficiency induces an early protective inflammatory environment in ischemic mice. Glia 2013, 61: 827–842.
92. Noda M, Doi Y, Liang J, Kawanokuchi J, Sonobe Y, Takeuchi H, *et al.* Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression. J Biol Chem 2016, 291: 14388.
93. Lee S, Lee J, Kim S, Park JY, Lee WH, Mori K, *et al.* A dual role of lipocalin 2 in the apoptosis and deramification of activated microglia. J Immunol 2007, 179: 3231–3241.
94. Xing C, Wang X, Cheng C, Montaner J, Mandeville E, Leung W, *et al.* Neuronal production of lipocalin-2 as a help-me signal for glial activation. Stroke 2014, 45: 2085–2092.
95. Takahashi K, Rochford CD, Neumann H. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 2005, 201: 647–657.
96. Wu R, Li X, Xu P, Huang L, Cheng J, Huang X, *et al.* TREM2 protects against cerebral ischemia/reperfusion injury. Mol Brain 2017, 10: 20.
97. Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, *et al.* Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. J Neurosci 2015, 35: 3384–3396.
98. Liu W, Tang Y, Feng J. Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. Life Sci 2011, 89: 141–146.
99. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, *et al.* A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell 2009, 137: 47–59.
100. Vallejo R, Tilley DM, Vogel L, Benyamin R. The role of glia and the immune system in the development and maintenance of neuropathic pain. Pain Pract 2010, 10: 167–184.
101. Griffin WS. Inflammation and neurodegenerative diseases. Am J Clin Nutr 2006, 83: 470S–474S.
102. John GR, Chen L, Rivieccio MA, Melendez-Vasquez CV, Hartley A, Brosnan CF. Interleukin-1beta induces a reactive astroglial phenotype via deactivation of the Rho GTPase-Rock axis. J Neurosci 2004, 24: 2837–2845.
103. Herx LM, Rivest S, Yong VW. Central nervous system-initiated inflammation and neurotrophism in trauma: IL-1 beta is required for the production of ciliary neurotrophic factor. J Immunol 2000, 165: 2232–2239.
104. Chao CC, Hu S, Sheng WS, Bu D, Bukrinsky MI, Peterson PK. Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. Glia 1996, 16: 276–284.
105. Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC. Cytokine effects on glutamate uptake by human astrocytes. Neuroimmunomodulation 2000, 7: 153–159.
106. Schipke CG, Boucsein C, Ohlemeyer C, Kirchhoff F, Kettenmann H. Astrocyte Ca^{2+} waves trigger responses in microglial cells in brain slices. Faseb J 2002, 16: 255–257.
107. Lehrmann E, Christensen T, Zimmer J, Diemer NH, Finsen B. Microglial and macrophage reactions mark progressive changes and define the penumbra in the rat neocortex and striatum after transient middle cerebral artery occlusion. J Comp Neurol 1997, 386: 461–476.
108. Nedergaard M, Dirnagl U. Role of glial cells in cerebral ischemia. Glia 2005, 50: 281–286.
109. Xie M, Yi C, Luo X, Xu S, Yu Z, Tang Y, *et al.* Glial gap junctional communication involvement in hippocampal damage after middle cerebral artery occlusion. Ann Neurol 2011, 70: 121–132.
110. Zanotti S, Charles A. Extracellular calcium sensing by glial cells: low extracellular calcium induces intracellular calcium release and intercellular signaling. J Neurochem 1997, 69: 594–602.
111. Shigemoto-Mogami Y, Koizumi S, Tsuda M, Ohsawa K, Kohsaka S, Inoue K. Mechanisms underlying extracellular ATP-evoked interleukin-6 release in mouse microglial cell line, MG-5. J Neurochem 2001, 78: 1339–1349.
112. Lu YL, Wang R, Huang HT, Qin HM, Liu CH, Xiang Y, *et al.* Association of S100B polymorphisms and serum S100B with risk of ischemic stroke in a Chinese population. Sci Rep 2018, 8: 971.
113. Edwards MM, Robinson SR. TNF alpha affects the expression of GFAP and S100B: implications for Alzheimer's disease. J Neural Transm (Vienna) 2006, 113: 1709–1715.
114. Reali C, Scintu F, Pillai R, Donato R, Michetti F, Sogos V. S100b counteracts effects of the neurotoxicant trimethyltin on astrocytes and microglia. J Neurosci Res 2005, 81: 677–686.
115. Tramontina F, Tramontina AC, Souza DF, Leite MC, Gottfried C, Souza DO, *et al.* Glutamate uptake is stimulated by extracellular S100B in hippocampal astrocytes. Cell Mol Neurobiol 2006, 26: 81–86.
116. Blais V, Rivest S. Effects of TNF-alpha and IFN-gamma on nitric oxide-induced neurotoxicity in the mouse brain. J Immunol 2004, 172: 7043–7052.
117. Matute C, Domercq M, Perez-Samartin A, Ransom BR. Protecting white matter from stroke injury. Stroke 2013, 44: 1204–1211.
118. Miller BA, Crum JM, Tovar CA, Ferguson AR, Bresnahan JC, Beattie MS. Developmental stage of oligodendrocytes determines their response to activated microglia in vitro. J Neuroinflammation 2007, 4: 28.
119. Zajicek JP, Wing M, Scolding NJ, Compston DA. Interactions between oligodendrocytes and microglia. A major role for complement and tumour necrosis factor in oligodendrocyte adherence and killing. Brain 1992, 115 (Pt 6): 1611–1631.
120. Kaur C, Ling EA. Periventricular white matter damage in the hypoxic neonatal brain: role of microglial cells. Prog Neurobiol 2009, 87: 264–280.

121. Yang Y, Jalal FY, Thompson JF, Walker EJ, Candelario-Jalil E, Li L, *et al.* Tissue inhibitor of metalloproteinases-3 mediates the death of immature oligodendrocytes via TNF-alpha/TACE in focal cerebral ischemia in mice. J Neuroinflammation 2011, 8: 108.
122. Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K. Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. J Neurosci 2014, 34: 2231–2243.
123. O'Kusky J, Ye P. Neurodevelopmental effects of insulin-like growth factor signaling. Front Neuroendocrinol 2012, 33: 230–251.
124. Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, *et al.* Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 2006, 31: 149–160.
125. van der Goes A, Brouwer J, Hoekstra K, Roos D, van den Berg TK, Dijkstra CD. Reactive oxygen species are required for the phagocytosis of myelin by macrophages. J Neuroimmunol 1998, 92: 67–75.
126. Haynes RL, Baud O, Li J, Kinney HC, Volpe JJ, Folkerth DR. Oxidative and nitrative injury in periventricular leukomalacia: a review. Brain Pathol 2005, 15: 225–233.
127. Uchida H, Fujita Y, Matsueda M, Umeda M, Matsuda S, Kato H, *et al.* Damage to neurons and oligodendrocytes in the hippocampal CA1 sector after transient focal ischemia in rats. Cell Mol Neurobiol 2010, 30: 1125–1134.
128. Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE. Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. J Immunol 1993, 151: 2132–2141.
129. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005, 308: 1314–1318.
130. Jolivel V, Bicker F, Biname F, Ploen R, Keller S, Gollan R, *et al.* Perivascular microglia promote blood vessel disintegration in the ischemic penumbra. Acta Neuropathol 2015, 129: 279–295.
131. Lou N, Takano T, Pei Y, Xavier AL, Goldman SA, Nedergaard M. Purinergic receptor P2RY12-dependent microglial closure of the injured blood-brain barrier. Proc Natl Acad Sci U S A 2016, 113: 1074–1079.
132. Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, *et al.* VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest 2000, 106: 829–838.
133. Kunsch C, Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. Circ Res 1999, 85: 753–766.
134. Hayashi T, Noshita N, Sugawara T, Chan PH. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. J Cereb Blood Flow Metab 2003, 23: 166–180.
135. Kuo NT, Benhayon D, Przybylski RJ, Martin RJ, LaManna JC. Prolonged hypoxia increases vascular endothelial growth factor mRNA and protein in adult mouse brain. J Appl Physiol (1985) 1999, 86: 260–264.
136. Li Y, Liu DX, Li MY, Qin XX, Fang WG, Zhao WD, *et al.* Ephrin-A3 and ephrin-A4 contribute to microglia-induced angiogenesis in brain endothelial cells. Anat Rec (Hoboken) 2014, 297: 1908–1918.
137. Kanazawa M, Miura M, Toriyabe M, Koyama M, Hatakeyama M, Ishikawa M, *et al.* Microglia preconditioned by oxygen-glucose deprivation promote functional recovery in ischemic rats. Sci Rep 2017, 7: 42582.
138. Jiang M, Liu X, Zhang D, Wang Y, Hu X, Xu F, *et al.* Celastrol treatment protects against acute ischemic stroke-induced brain injury by promoting an IL-33/ST2 axis-mediated microglia/macrophage M2 polarization. J Neuroinflammation 2018, 15: 78.
139. He Y, Ma X, Li D, Hao J. Thiamet G mediates neuroprotection in experimental stroke by modulating microglia/macrophage polarization and inhibiting NF-kappaB p65 signaling. J Cereb Blood Flow Metab 2017, 37: 2938–2951.
140. Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, *et al.* Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis 2013, 4: e525.
141. Yang Y, Salayandia VM, Thompson JF, Yang LY, Estrada EY, Yang Y. Attenuation of acute stroke injury in rat brain by minocycline promotes blood-brain barrier remodeling and alternative microglia/macrophage activation during recovery. J Neuroinflammation 2015, 12: 26.
142. Pepe G, Calderazzi G, De Maglie M, Villa AM, Vegeto E. Heterogeneous induction of microglia M2a phenotype by central administration of interleukin-4. J Neuroinflammation 2014, 11: 211.
143. Xia CY, Zhang S, Gao Y, Wang ZZ, Chen NH. Selective modulation of microglia polarization to M2 phenotype for stroke treatment. Int Immunopharmacol 2015, 25: 377–382.
144. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. J Neurosci 2009, 29: 3974–3980.
145. Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. The role of microglia in the healthy brain. J Neurosci 2011, 31: 16064–16069.
146. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. Physiol Rev 2011, 91: 461–553.