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Review Article

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Inflammation after Ischemic Stroke: The Role of Leukocytes and Glial Cells

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The immune response after stroke is known to play a major role in ischemic brain pathobiology. The inflammatory signals released by immune mediators activated by brain injury sets off a complex series of biochemical and molecular events which have been increasingly recognized as a key contributor to neuronal cell death. The primary immune mediators involved are glial cells and infiltrating leukocytes, including neutrophils, monocytes and lymphocyte. After ischemic stroke, activation of glial cells and subsequent release of pro- and anti-inflammatory signals are important for modulating both neuronal cell damage and wound healing. Infiltrated leukocytes release inflammatory mediators into the site of the lesion, thereby exacerbating brain injury. This review describes how the roles of glial cells and circulating leukocytes are a double-edged sword for neuroinflammation by focusing on their detrimental and protective effects in ischemic stroke. Here, we will focus on underlying characterize of glial cells and leukocytes under inflammation after ischemic stroke.

Key words: ischemic stroke, inflammation, glial cells, leukocytes

INTRODUCTION

The inflammatory response following acute ischemic stroke is a well-known and widely studied phenomenon. Pathological features of ischemia such as necrotic cells, cell death debris, and increased reactive oxygen species (ROS) can induce neuro-inflammatory by activating resident microglia and astrocytes as well as attracting infiltrating leukocytes from circulating blood. The recruitment of both brain and peripheral immune cell types in post-ischemic tissue can accelerate and expand an infarct initiated by ischemic insult.

The first-line responders to central nervous system (CNS) injury are microglia and astrocytes. Microglia are the resident macrophages of the brain and a key modulator of immunologic responses after ischemic stroke [1, 2]. Microglia constitute 15% of the total glial cell population in the adult murine brain (16.6% in humans) and are primarily found in brain gray matter [3]. Once activated by extracellular signals, they function to sweep debris and toxic substances by phagocytosis, thereby helping maintain normal cellular homeostasis in the brain [4, 5]. Activated microglia also increase secretion of cytokines and leukocyte adhesion molecules within cerebral vasculature, all within 24 hours of the ischemic insult [6, 7]. Astrocytes are the most abundant cells in the brain. In uninjured brain tissues, astrocytes provide structural and nutritive support for neurons. After ischemic stroke, astrocytes play an important role in wound healing and repair by mediating

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reactive gliosis and glial scar formation [8]. Both astrocytes and microglia may also produce inflammatory cytokines and toxic mediators such as excitotoxic glutamate.

Within a couple days to a week after ischemic stroke, peripheral circulating leukocytes are also recruited to the injury response. Neutrophils, monocytes, and lymphocytes infiltrate the CNS by binding to adhesion molecules on activated endothelial cells. Activated endothelia further enhance adhesion binding of circulating leukocytes by causing microvascular occlusion and infiltration of immune cells into the brain parenchyma [9, 10]. Once in the CNS, leukocytes release inflammatory cytokines at the site of injury such as tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) [11]. Several studies have shown that mechanism of leukocyte infiltration could be a possible way to widen the therapeutic window after ischemic stroke [12-14]. Given that the immune system consists of a variety of different cell populations with a range of different functions at different time points following tissue damage, detailed analyses of specific immune cell infiltration is certainly needed.

This review examines the role of glia and leukocytes in ischemic stroke, exploring how these two sets of immune cells work in tandem to both mediate repair and augment injury. Better understanding these mechanisms may help expand stroke's narrow therapeutic window and lead to the discovery of novel pharmacologic interventions involving one or more immune cell population(s).

DYNAMIC MICROGLIA AND ASTROCYTE IN ISCHEMIC STROKE

Microglia

Microglia are key modulators of the immune response in the brain and are considered the resident immune cell of the central nervous system. Under normal conditions, microglia are primarily involved in activity-dependent synaptic pruning and repair [15]. In the event of acute brain injury, microglia can quickly undergo morphologic transformation from a ramified resting state, characterized by many branching processes, to an active, motile ameboid state, where they become virtually indistinguishable from circulating macrophages [16, 17]. Therefore, activated microglia are often called brain macrophages. Active microglia can then phagocytose foreign organisms as well as injured brain cells [18-20]. In ischemic stroke, microglial activation occurs in the early stages of neuroinflammation; activated microglia can be detected in lesions as early as 2 hours post-ischemia and can be detected up to 1 week after brain injury [21]. Several reports have demonstrated that the direct application of activated microglia has been shown to effect cell death in neurons [22, 23]. In *in vitro* and *in vivo* models, these activated microglia release cytotoxic factors such as superoxide, nitric oxide, and TNF-α [24-26]. The cytotoxic effects begin shortly after insult and can continue to exacerbate injury for a few days afterward. It is thought that the later effects of activated microglia may be important for tissue repair and wound healing [27, 28].

In experimental stroke models, activated microglia have been shown to migrate toward the ischemic hemisphere of the cerebral cortex [29]. The precise mechanisms of microglial activation following ischemia are unclear. However, the literature to date strongly indicates that activate microglia have predominantly harmful effects in the acute stages of ischemic stroke and that most beneficial effects appear in delayed stages. Numerous studies indicate that different signals lead to two primary activation phenotypes: classically activated (M1) and alternatively activated (M2) [30-32]. The M1 phenotype microglia, activated by lipopolysaccharide (LPS) and the proinflammatory cytokine interferonc (IFNc), induces transcriptional activation of nuclear factor-kB and makes high levels of proinflammatory cytokines and oxidative metabolites such as TNF-α, interleukin (IL)-12, IL-6, IL-1β, and nitric oxide (NO), formerly indicated to cause additional damage (Fig. 1) [33]. In contrast, the M2 phenotype microglia is promoted by anti-inflammatory cytokines such as IL-4 or IL-13 [34], which are considered to prevent inflammation and improve tissue repair and wound healing (Fig. 1) [35]. Microglial activation may begin with an M1 phenotype which mediates an innate or an adaptive immune response and ultimately exacerbates neuronal damage [36, 37]. At later timepoints after injury, microglia may also transform the M2 phenotype microglia which facilitate repair-oriented functions by secreting growth factors such as vascular endothelial growth factor or brain-derived neurotrophic factor, and by clearing cellular debris via phagocytosis. M2 phenotype microglia tend to limit proinflammatory signal production. The timing and mechanism of M1 vs M2 phenotype activation is an important element to consider when manipulating microglia in stroke, and it is important to identify and understand the different microglial phenotypes and their unique functions at different time points [38, 39].

Recent research has also demonstrated that microglia can switch from the M1 phenotype to the M2 phenotype [40-42]. One such study showed that HIV-associated dementia initiates and maintains M1 phenotype microglia in the event of CD40 ligation by CD40L and TNF. These glia may later switch glia to the M2 phenotype via upregulation of CD45 [43]. In another study of aged mice subjected to brain injury, histological results indicated that aged brains of injured mice had not only larger lesions and worsened outcome, but also showed microglial polarization

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toward M1 phenotype compared to younger mice [44].

The dual phenotype functions make microglia a promising target for treating ischemic stroke. Future research for stroke therapies can build on studies characterizing different modes of microglia activation by, for example, dampening injury-exacerbating M1 phenotype functions or by inducing or amplifying native M2 phenotype repair functions at the appropriate time intervals following injury. For example, one recent study showed that activating microglia membrane protein triggering receptor expressed on myeloid cells 2 (TREM2) may also lead to phagocytic activity [45] and drive other anti-inflammatory functions [46]. Transgenic TREM2-deficient mice showed poorer recovery following ischemic stroke [47]. The challenge will be to discover methods for selectively suppressing the detrimental effects of microglial activation without compromising the restorative properties such as repair and remodeling. Studies in therapeutic microglial targets will also need to find ways to suppress cytotoxic mechanisms without disrupting beneficial effects. In neurodegenerative diseases, down-regulating CD40 or turning on CD45 has been shown to induce M2 phenotype and improve neurological outcome [43]. Similar strategies may also prove to protect against brain ischemia.

Astrocyte

Astrocytes are important mediators of homeostasis in the brain, including the regulation of immune reactions. In addition to their immunological functions, astrocytes have been reported to release various pro-inflammatory factors after ischemic injury, such as glial fibriliary acidid protein (GFAP) [48]. These cells also play an central role in enhancing reactive gliosis and glial scar formation (Fig. 1) [8]. Although an important part of the long-term healing, astrocytic gliosis may also be destructive after brain injury [18]. A massive astroglial response appears in the core of the lesion from hour 4 to day 1 following ischemic stroke, and reaches a peak around day 4 [49]. This glial scar has both neurotoxic and neurotrophic properties. The scar acts as a barrier which prevents axonal ingrowth and reinnervation, thus interrupting recovery. However, this scar also isolates damaged tissue from viable tissue and prevents additional damage to the surrounding brain [49]. Recent study showed beneficial effect of astrocytic gliosis which aids rather than prevents CNS axon regeneration [50]

In addition to modulating scar formation, astrocytes have also been observed releasing various immune molecules such as cytokines, chemokines and inducible nitric oxide synthase (iNOS), and inducing a Th2 (anti-inflammatory) immune response [51]. In ischemic stroke, iNOS was observed in reactive

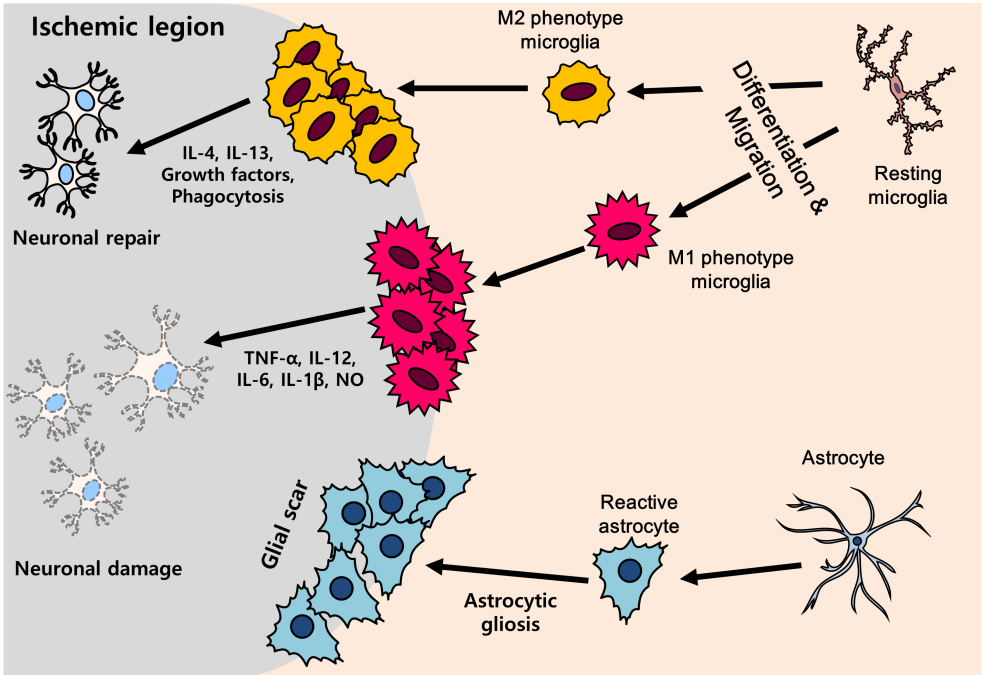

Fig. 1. Immune signaling of microglia and astrocyte after ischemic stroke. Resting microglia can be polarized to either the M1 or M2 phenotype. M1 microglia contribute to neuronal damage by pro-inflammatory mediators, whereas M2 microglia improve neuronal protection through anti-inflammatory mediators and phagocytic functions. Astrocytes accumulate at the borders of the lesion, become reactive, and start the formation of a glial scar.

hippocampal astrocytes [52]. The inflammatory role of astrocytes has also been demonstrated in a study of TNF-like weak inducer of apoptosis (TWEAK), a member of the TNF superfamily. TWEAK was detected on neurons, astrocytes and endothelial cells, where it was shown to increase pro-inflammatory molecule production through interaction with the astrocytic Fn14 receptor [53, 54]. TWEAK and Fn14 expression have been documented in experimental stroke models, where Fn14 inhibition led to decreased ischemic injury [54]. These results show that while astrocytes have long been viewed to play scaffolding and supportive roles for neurons, activated astrocytes may also be detrimental in the ischemic brain, not unlike microglia and other immune cells.

INFILTRATED LEUKOCYTES EXACERBATE ISCHEMIC STROKE

Infiltrated leukocytes promote cerebral ischemic injury in a number of different ways. First, adhesion of leukocytes to the endothelium can reduce the flow of erythrocytes through the microvasculature causing the cerebral no-reflow phenomenon and additional brain injury. Activated leukocytes at the surface of the endothelium also release proteases, ROS, gelatinases, and collagenases, and impair potentially salvageable blood vessels and brain tissues. Phospholipase activation in leukocytes leads to a production of biologically active substances, such as leukotrienes, eicosanoids, prostaglandins, and platelet-activating factor, which result in vasoconstriction and extend platelet aggregation. Lastly, infiltrated leukocytes release pro-inflammatory cytokines and other immune modulators in the penumbra surrounding the infarct core causing further neuronal injury [55-58].

Neutrophils

Neutrophils are the first blood-borne immune cells to arrive at ischemic brain tissues. These innate immune cells have important roles in acute ischemic brain injury and in the events leading up to infarction such as atherosclerosis and thrombus formation. Following ischemic stroke, neutrophils may cause sterile inflammation by interacting with endothelial adhesion molecules to slow their intravascular movement and induce polarization, which causes adhesion to the pro-inflammatory endothelium [59]. Neutrophils attach to the endothelium by binding various adhesion molecules including the selectins (P-, E-, and L-selectin), intracellular cell adhesion molecule-1 (ICAM-1) and integrins (CD11a, b and c) within 15 minutes of ischemic stroke [60, 61]. By 2 hours, neutrophils rolling and adhesion appear in the pial vessels of brain [62]. After initial adherence, neutrophils will follow a chemokine and activator gradient produced by the injured tissue. Neutrophils reach peak numbers at 2~4 days after ischemic stroke and then decrease thereafter [63, 64]. During this period, pro-inflammatory neutrophil activation contributes to disruption of blood brain barrier, increased infarct size, hemorrhagic transformation, and worse neurologic outcomes.

Neutrophil adhesion is an important step in the immune response to ischemic brain injury [65]. Adhesion molecules attach immune cells tightly to the endothelial wall, thereby stimulating and facilitating diapedesis through the vessel wall to the site of ischemic brain injury [65]. These neutrophil adhesion factors include ICAM-1, MAC-1 (CD11b/CD18), and selectins. Expression of ICAM-1 is increased in endothelia proximal to the injured brain within hours after stroke onset, and peaks at about 12-48 hours [66]. Mice deficient in adhesion molecules either by transgenic manipulation or pharmacologic interruption of ICAM-1 activity have been shown with decreased infarct areas and reduced brain leukocyte infiltration in experimental stroke [67, 68]. Neutrophils express CD11b/CD18, also known as MAC-1, which are integrins that contain a common β2 chain (CD18) and are thus classified as β2 integrins. Integrins are located in neutrophil plasma membranes, where they bind to endothelial ICAM-1 and enable cell migration through the vessel wall. In MAC-1 deficient transgenic mice, infarct size, mortality, and neutrophil infiltration into ischemic brain are reduced after ischemic stroke [69]. Another study inhibited MAC-1 via monoclonal antibodies in experimental stroke models and found decreased infarct sizes and improved functional outcomes in rabbits [70]. Similarly, blocking MAC-1 with recombinant neutrophil inhibitory factor (rNIF) is associated with reduced infarct size, and improved neurological outcomes [71]. However, this benefit only occurs when rNIF is administered early after cerebral reperfusion (2 to 6 hours) and is not effective in models where no reperfusion occurs. Lastly, selectins are calcium-dependent, transmembrane glycoproteins that bind to carbohydrate residues (sialyl-Lewis X), and mediate rolling and adhesion to vascular endothelium. E-, P-, and L-selectin work together to coordinate neutrophil trafficking after brain ischemia [72]: E-selectin and P-selectin participate in initial neutrophils rolling and recruitment [73], whereas L-selectin guides unstimulated neutrophils to areas of activated endothelium [74]. Various experimental stroke models have found positive correlations between P- and E-selectin upregulation and the promotion of post-ischemic inflammatory responses as well as injury severity [75, 76].

Inflammatory neutrophil activity after ischemic stroke is further modulated by interactions with chemokines and other cytokines, both of which are released in brain tissues after injury. These

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signaling ligands can increase the neutrophil recruitment and migration mechanisms mediated by adhesion factors by binding and activating neutrophils. Chemokine receptors are expressed on neutrophil plasma membranes, and their activation drives neutrophils to sustain or even amplify inflammatory pathways at brain lesion. Different classes of chemokines are identified by their structures, the main classes being CXC, CC, XC and CX3C. The "C"s refer to N-terminal cysteine residues, and the classes are divided depending on whether these residues flank an amino acid between them (CXC) or whether they are adjacent (CC). The different classes of chemokines act through unique and overlapping receptors which are a part of a superfamily of G-protein-coupled receptors [77]. The CXC class can be further split into ELR+ or ELR- groups based on whether the glutamate-leucine-arginine motif is present between the N-terminus and the first cysteine [78]. Chemokine receptors can also be flexible, binding multiple classes or subtypes of ligands, and many different chemokines are capable of activating neutrophils [79]. However, the ELR+ CXC chemokine subfamily are thought to be mainly neutrophil chemoattractants, whereas the CC chemokines more typically attract monocytes and T lymphocytes [80]. Though as a whole, their signaling is associated with more general pro-inflammatory mechanisms and pathways, several chemokines in CXC group have also been directly implicated in mediating neutrophil infiltration [81]. Because of this, chemokine signaling is closely associated with worse stroke outcomes [60], and chemokine ligands and receptors have become a hot topic of research investigating potential therapeutic targets. One recent study showed large increases in expression of key members of the ELR+ CXC chemokine subfamily, the neutrophil receptor CXCR2, and its ligands CXCL1 and CXCL2, which reached maximum levels at 1 to 3 days after injury [81]. In the rodent stroke model, inhibition of CXCR1 and CXCR2 with Reparixin decreased infarct size, improved motor function, and reduced brain levels of MPO and IL-1β [82].

Monocytes

Monocytes are derived from hematopoietic stem cells (HSC) in the liver and spleen during embryonic development and primarily in the bone marrow after birth [83, 84]. Like neutrophils, monocytes are incompletely differentiated cells that have a highly phagocytic capacity and react depending on the nature of stimuli within their microenvironment [84, 85]. By expression of specific surface markers, this cell type can be divided into pro-inflammatory (classically activated) or anti-inflammatory (alternatively activated) subsets. Recruitment of circulating monocytes to the ischemic brain is similar to that of neutrophils, orchestrated by inflammatory cytokines, such as adhesion molecules and chemokines. In stroke models, the monocyte chemoattractant protein-1 (MCP-1, CCL2) and its receptor CCR2 are known to be involved in the inflammatory response of the injured brain [86]. At baseline, CCL2 mRNA expression is almost absent, but ischemia leads to a significant increase in MCP-1 mRNA expression in injured portions of the cortex after either permanent or temporary MCA occlusion around 12 h to 2 days and remained elevated up to 5 days [87, 88]. Pro-inflammatory monocytes express CCR2 with low or no expression of CX3CR1 across different species. CCR2 expression is critical for the trafficking of circulating monocytes into injured brain where they can transform into macrophages. Anti-inflammatory monocytes, which do not express CCR2 but do express higher levels of CX3CR1, patrol blood vessels in a steady state and perform in situ phagocytosis [85, 89]. Several other studies showed that CCL2 or CCR2 deficient mice reduce phagocytic macrophage accumulation with smaller infarcts in experimental stroke models, suggesting CCR2 monocytes may have a deleterious effect [90, 91]. In rodents, monocytes fall into two main subsets based on chemokine receptor and Ly-6C (Gr-1) expression levels. $Ly6C^{high}$ pro-inflammation has a short half-life and is actively recruited to inflamed tissues, contributing to the inflammatory response. $Ly6C^{low}$ anti-inflammatory has a longer half-life and is the subtype responsible for patrolling the lumen of blood vessels, contributing to the maintenance of vascular homeostasis [92]. One recent paper demonstrated an increase in $Ly6C^{high}$ monocytes at day 3 after stroke, whereas the number of $Ly6C^{low}$ monocytes was greatest at day 6, paralleled by sequential peaks of CCR2 and CX3CR1 mRNA as well as gene expression of the pro- and anti-inflammatory cytokines IL-1β and TGF-β, respectively [93]. These data may indicate the presence of a dynamic shift in the recruitment and infiltration of monocyte subsets into injured brain after ischemic stroke, or probably the differentiation of pro-inflammatory monocytes ($Ly6C^{high}$) into anti-inflammatory monocytes ($Ly6C^{low}$). Better understanding the mechanisms mediating differentiation into these two monocyte subsets may reveal additional therapeutic strategies for controlling inflammation after ischemic injury.

Lymphocyte

The patterns and consequences of lymphocyte activity after stroke are not as well characterized as that of neutrophils and monocytes. A number of studies have observed that lymphocytes may negatively contribute to brain injury pathogenesis. Similar to neutrophils and monocytes, these cells also release pro-inflammatory cytokines and cytotoxic substances, such as ROS.

Several stroke studies have shown that the number of lymphocytes spikes in the ischemic brain at later time-points than neutrophils [94, 95]. Interrupting lymphocyte entry into infarcted brain tissues decreases the severity of the injury, and suggests that, like neutrophils, lymphocytes perform an overall harmful role [96]. T lymphocytes are a key player in amplifying inflammation after ischemic stroke, whereas B lymphocytes have been shown to play a lesser role [81]. One study showed that T lymphocyte-deficient mice had smaller infarct sizes and improved neurological outcomes relative to control groups in a model of transient focal ischemia [13, 97]. This and other studies of lymphocyte-deficient mice together suggest that the neuroprotective effects produced by lymphocyte suppression in stroke appear dependent on the absence of T lymphocytes and not B lymphocytes because the reconstitution of B lymphocytes does not affect the protection observed. By contrast, when T lymphocytes are transplanted back in to Rag1-deficient mice, this protection disappeared [13, 97, 98]. This distinction between T and B lymphocytes remains somewhat controversial since another recent study failed to see noteworthy differences in infarct size between immune-deficient mice (deficient in both T and B lymphocytes) and wildtype after stroke [99]. The cause for these conflicting observations is unclear, although they may be related to the type of stroke; the latter study adopted a permanent model of focal cerebral ischemia instead of the temporary focal ischemia model used in the first study.

Another faction of the current literature suggests that not all T lymphocytes subtypes are detrimental to acute brain injured outcome. In one study that recreated ischemia-like injury in cultured primary neurons, isolated neutrophils released excitotoxins that induced neuronal death, whereas lymphocytes were not shown to be neurotoxic and actually increased astrocyte proliferation [100]. Another group showed that natural killer (NK) cells and T lymphocyte functions that rely on T cell receptor co-activation may not influence ischemic injury at all [98]. Furthermore, the impact of regulatory T (Treg) lymphocytes is still in question. Liesz et al. reported that infarct volume and neurological deficit were significantly increased in mice given an antibody to neutralize Treg lymphocytes compared to controls Beneficial effects of Treg in brain ischemia were mediated by IL-10 [101]. These data also demonstrated that IL-10 may be important for this immunomodulatory event. However, other study could not show any modulatory role of Treg cells [102]. While there is little evidence of their impact at the site of ischemic injury, NK cells may nevertheless influence stroke patient outcomes. In the liver, resident NK cell function is reduced by augmented sympathetic neurotransmission, which leads to the immunosuppression and susceptibility to infections that occur following stroke [103].

Whether harmful or protective, the mechanisms of lymphocyte activity after stroke are currently unclear. However, among the various theorized roles of lymphocytes in stroke, injury-exacerbating mechanisms of T lymphocytes have been best characterized to date. Generally, T lymphocytes attack bacteria- and virus-infected cells by releasing cytokines or cytotoxins [104], and they may respond similarly at sterile ischemic brain lesions. These T lymphocytes secretions cause cell death via interaction with the Fas receptor [105], and a few studies reported that neutralization of T lymphocyte-derived cytokines (IL-17, IL-12, IL-23, interferon gamma) reduced brain injury and improved neurological outcomes in experimental stroke models [13, 101]. Another paper showed that mice lacking T lymphocyte-secreted perforin demonstrated significant neuroprotection, suggesting another pathway by which lymphocytes may contribute to ischemic damage [48]. In addition to these findings, yet another recent study observed circulating T cells producing 7 to 15 fold greater amounts of NADPH oxidase type 2 (Nox2)-derived superoxide after ischemic stroke, suggesting that peripheral T lymphocytes may exacerbate oxidative tissue injury at the brain lesion remotely, without having to migrate to the brain lesion, by releasing Nox-2-derived superoxide into the blood [106].

CONCLUSIONS

The role of inflammation following ischemic stroke has become an increasingly popular area for understanding interactions between the peripheral immune system and brain injury. Glia and leukocytes are considered the two major classes of immunocompetent cells involved in ischemic brain injury, and their activation and recruitment represent key stages in in initiating and sustaining neuroinflammation. However, our understanding of the mechanisms governing their activation and function after ischemia is still limited. For this reason, much effort has been directed toward an understanding of where each cell migrates and localizes in the ischemic brain, when they accumulate and infiltrate in brain injury, and how they become activated and mediate neuroinflammation (Table 1). Future studies investigating the spatial, temporal, and functional attributes of glia and leukocytes are necessary for greater insight into how these cells may exacerbate or protect against ischemic stroke. The mechanisms underlying these functions are critical to understand for developing new therapeutic strategies to treat acute ischemic brain.

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Table 1. The role of glial cells and leukocytes after ischemic stroke

Cell type	Major function(s)	Reference(s)
Microgliae	- activity-dependent synaptic pruing and repair	[15]
	- morphologic transformation in acute brain injury	[16,17]
	- sweep foregin organism in brain injury	[18-20], [21]
	- role of M1 phenotype microglia in the brain	[25], [28,29]
	- role of M2 phenotype microglia in the brain	[26], [27], [30,31]
	- swith of M1 to M2 microglia	[32-34], [35], [36]
	- microglia functionin ischemic stroke	[37], [38,39], [40-42], [45], [46], [47]
Astrocyte	- regulation of immune reactions	[48]
	- reactive gliosis and glial scar formation	[8], [18], [49]
	- releasing various immune molecules	[50], [51], [52,53]
Neutrophil	- roles in acute ischemic brain injury	[58]
	- interaction with adhesive molecules	[59,60]
	- rolling and adhesion the pial vessels of brain	[61]
	- infiltration and immune response to ischemic brain	[70], [71], [72,73]
Monocyte	- phagocytic capacity	[83,84]
	- expression of chemokines	[85], [86,87]
	- expression of the pro- and anti-inflammatory cytokines	[92]
T lymphocyte	- improvement of inflammation in ischemic brain	[68], [93,94], [95]
	- increased astrocyte proliferation	[102]
	- oxidative tissue injury	[105]

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