Minireview

Glia–immune interactions post-ischemic stroke and potential therapies

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Impact statement

This article reviews glial cell interactions with the immune system post-ischemic stroke. Research has shown that glial cells in the brain play a role in altering phenotypes of other glial cells and have downstream immune cell targets ultimately regulating a neuroinflammatory response. These interactions may play a deleterious as well as beneficial role in stroke recovery. Furthermore, they may provide a novel way to approach potential therapies, since current stroke drug therapy is limited to only one Food and Drug Administration-approved drug complicated by a narrow therapeutic window. Until this point, most research has emphasized neuroimmune interactions, but little focus has been on bidirectional communication of glial–immune interactions in the ischemic brain. By expanding our understanding of these interactions through a compilation of glial cell effects, we may be able to pinpoint major modulating factors in brain homeostasis to maintain or discover ways to suppress irreversible ischemic damage and improve brain repair.

Abstract

Although the primary responsibility of the immune system has for over a century been perceived as the protector of the host against infection in the peripheral organs, we now know the immune system also plays a vital role in recovery pathways associated with central nervous system (CNS) injury. There is mounting evidence that the blood–brain barrier does not preclude the CNS from immune surveillance. Of particular interest for this review is how microglia and astrocytes interact with the cells of the immune system to modulate repair and recovery mechanisms in ischemic stroke. Our review argues that by deepening our understanding of neuroimmunity, specifically the bidirectional glial–immune cell communications, a plethora of new therapeutic targets and mechanisms may be revealed. Consequently, this review instigates novel experimental approaches to neuroimmunology and fosters a more rapid discovery process for the treatment of stroke.

Keywords: Ischemic stroke, neuroimmunology, astrocytes, microglia, histamine, T-cells, natural killer cells, monocytes, glutamate, interleukin-15, glial–immune interactions

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Introduction

The brain has long been considered a site of immune privilege established by the presence of the blood–brain barrier (BBB) and lack of conventional lymphatic vessels. It was thought that the BBB barricaded peripheral immune cells from extravasating into the cerebrum;[1,2] however, recent findings in animals and humans have indicated that immune cells can enter the central nervous system (CNS) and may contribute to immune surveillance in the brain.[3–9]

The interactions between glial cells and immune cells in the brain have opened the floodgates for a new area of research that is deepening our understanding of immunity in neuroscience and how the two systems play a role in regulating each other. Furthermore, this area of research is still in its infancy and has the potential to present us with novel therapies for ischemic stroke.[3,5,6,10] Post-ischemic stroke, immune cells infiltrate the brain and interact with glial cells.[5,10–12] Multiple points of contact and

communication between glial cells of the nervous system and various immune system cells or markers have been identified.[5,11–13] The role of immune cells in the brain as it relates to the progression of cerebral infarction or repair and recovery mechanisms remains unresolved. The immune cell to brain cell interaction has received notable attention for providing evidence that an innate immune response exists between peripheral immune cells such as neutrophils and brain cells.[2,14,15] On the other hand, there has been a more limited amount of research delving into the role of the adaptive immune response between immune cells and glial cells. Nevertheless, there is mounting evidence that adaptive immune cells indeed impact ischemic stroke outcome,[16] which provides the rationale to extend the focus of this research to include studying the interaction of adaptive immune cells and glial cells. Here, we review the interaction between glial cells and the immune system in brain ischemia, examine the pro- and anti-inflammatory effects, and potential therapies to reduce damage caused by cerebral infarction.

Stroke, neuroimmunity, and current therapy

Stroke is a disease associated with aging. It is the fifth leading cause of mortality and the leading cause of long-term disability in the United States.[17,18] Globally, it is the second leading cause of death.[18,19] In the United States, by 2050, the population over age 64 years will approach 84 million people.[20] This is nearly a two-fold increase for this age group, as compared to 2012.[20] Therefore, as the aged population increases, it is expected that the incidence of stroke will also rise. Of the two types of stroke, hemorrhagic and ischemic stroke, ischemic stroke predominates and consists of approximately 87% of cases; hence, this review will focus on ischemic stroke.[18]

Ischemic stroke is characterized by occlusion of an artery that restricts cerebral blood flow causing oxidative stress and glucose deprivation in the damaged brain region and triggering necrosis and apoptosis leading to functional deficits.[21] When cells die, they release damage-associated factors in prescribed molecular patterns (DAMPs).[22] DAMPs such as high-motility group box 1 (HMGB1), transcription factor A, mitochondrial (TFAM), and ATP are biomolecules that trigger activation of a non-infectious inflammatory response. HMGB1 and TFAM have been shown to play roles in upregulation of cell adhesion molecules and drive immunogenic cell death.[23] Beyond this brief introduction, DAMPs are a complex topic meriting a review of its own and is therefore beyond the scope of this mini-review. Nevertheless, these sets of events can stimulate toll-like receptors, which in turn activate a signaling cascade leading to production of pro-inflammatory mediators that recruit immune cells from the periphery to the brain.[21]

Post-ischemic stroke, macrophages, neutrophils, B-cell, and T-cell of the immune system enter the brain to engage in bidirectional communication with glial cells.[6] This glial–immune interaction was likely intended to be a protective mechanism to maintain homeostasis and prevent invaders in the CNS, although when over activated it appears to contribute to the damaging affects seen in ischemic brain injuries.[6] Hence, inflammation and immunity play an undeniable role in the pathogenesis of stroke.[24]

Recent research has rebutted the common mantra that the brain via protection of the BBB was held on a pedestal of immune privilege. The immune system has been shown to play a role in a variety of CNS diseases including multiple sclerosis, psychiatric disorders, and stroke.[13,23] In stroke, signaling generated by cerebral ischemia may activate the immune system, contribute to tissue damage and neuroinflammation, and may be responsible for changes in BBB permeability, leading to activation of macrophages and mast cells, thereby causing the release of pro-inflammatory cytokines and histamine.[13,25,26] Pro-inflammatory mediators are known to play a role in leukocyte trafficking making it possible for peripheral monocytes, neutrophils, natural killer (NK) cells, or lymphocytes to infiltrate the brain.[5,11,14,27]

A non-specific defense mechanism that is initiated immediately or within hours by the presence of an antigen is known as the innate immune response.[6] This first line of defense against pathogens involves phagocytes such as monocytes, macrophages, microglia, and neutrophils. The initial insult to the brain during ischemia involves the disruption of the BBB sending signals for upregulation of adhesion molecules on endothelial cells to promote extravasation of neutrophils and monocytes into the brain.[6] Neutrophils are considered first responders of the immune system, as they are triggered within minutes of ischemic insult.[6,14] Following the first response, blood monocytes migrate to brain tissue becoming active macrophages as second responders.[6]

The adaptive immune response is a more complex delayed antigen-specific response involving T- and B-cells, which are used to provide memory for subsequent exposure to an antigen. This response involves a specific antigen, which is recognized and processed prior to presentation on antigen-presenting cells (APCs). The APCs of the peripheral immune system present antigens on their major histocompatibility complex (MHC) to T-cells, causing them to become activated.[28,29] MHC II is found on the surface of both astrocytes and microglia.[12,30,31] Therefore, it is possible that astrocytes and microglia are the important regulators of ischemic injury, activating T-cells in a cascade that mimics adaptive immunity in the periphery.

Fostering our understanding of the intricate relationship between the immune system and glial cells post-ischemic stroke is essential to unveiling novel immunomodulating therapies that may reduce or prevent irreversible brain damage associated with ischemia or other brain injuries. Therefore, there is an urgent need to develop alternative therapies that may work alone or in conjunction with the current limited treatment, recombinant tissue plasminogen activator (rtPA). This would expand treatment modalities blocking the deleterious effects of stroke via an alternate pathway. Presently, rtPA, a thrombolytic, is the only drug approved by the Food and Drug Administration for the treatment of ischemic stroke.[30] While this drug is valuable if used within the narrow therapeutic window of 4.5 h, this time limitation allows only about 2% of stroke patients to

benefit from this infusion.[30,32,33] Beyond this short treatment window, rtPA can increase hemorrhagic risk.[34] Thus, finding alternative routes to prevent and treat ischemic stroke by using combination therapies or by lengthening the therapeutic window is crucial to prevent stroke-related disability, premature death, and enhance recovery.

In this review, two main types of glial cells will take center stage: microglia and astrocytes. The justification for this emphasis is that these cells have been identified as key CNS cells important in modulating interactions between neurons and peripheral immune cells and have characteristics themselves that mimic immune cells in the periphery. Understanding the function of each glial cell in its physiological and pathological conditions and how it interacts with immune cells during ischemic stroke may lead to effective therapies modifying the post-stroke immune response. The subsequent discussion will be outlined first by glial cell and second by innate and then adaptive immune interactions so as to follow the natural course of events.

Microglial cells

Microglia, characterized as the resident macrophages of the CNS, actually enter the CNS after birth linking the CNS to the immune system; however, the exact function of microglia during immune challenged states continues to be up for debate.[6,35,36] Much like macrophages acting as first responders in the peripheral immune system, microglia cells become activated and respond within hours following brain damage,[37,38] suggesting that this cell type is also a component of the innate immune system in the brain. Currently, two activation states have been proposed: classically activated microglia (M1) and alternatively activated microglia (M2).[39] For ease of discussion, the M1 and M2 terms will be used; however, it should be acknowledged that the M1/M2 nomenclature is under refinement, as it has been recognized that microglial phenotypic switching of M1 and M2 states exists on a continuum with more than two polarization states.[40,41]

Lipopolysaccaraides (LPS), a microbial endotoxin used to simulate neuroinflammation, has been shown in 3- to 4-month-old C57B6/J mice to exacerbate infarct volumes playing a role in stroke severity[42] and worsening neurological outcomes following 30-min transient middle cerebral artery occlusion (tMCAO). In contrast, repeated or single administration of LPS prior to stroke in rodents, termed LPS pre-conditioning, has demonstrated a shift from an early pro-inflammatory M1 microglial phenotype in association with an elevation in inducible nitric oxide synthase (iNOS) and tumor necrosis factor-alpha (TNF-α) to a later anti-inflammatory M2 phenotype associated with an increased arginase-1 and interleukin 10 (IL-10)[43,44] lending to LPS pre-conditioning as neuroprotective following stroke.[45,46]

Animal and human studies have shown that aging plays a role in microglial phenotype specificity, whereby M1 over M2 microglial phenotypes are selected in response to immune challenges.[39] Consequently, this may suggest that the aged brain is compromised by its inability to respond to stressors such as injury or ischemia due to a pro-inflammatory mechanism overriding the repair mechanism provided by M2 microglial cells.[39] Additionally, aged mice demonstrate a reduction in Iba1^{+} cortical microglia, which cluster in an uneven distribution pattern.[44] Dystrophic changes in microglia have also been reported in 68-year-old human subjects compared to 38 year olds.[47] Since ischemic stroke is a condition disproportionally affecting the aged population, it is possible that this senescent selectivity could alter signaling that impacts the glial–immune interactions during ischemia.[18,48] Microglia are known to be cells that respond to environmental stimuli; however, it was reported that the microglial response to micro-laser lesion injury has a reduced reaction speed in aged 26- to 27-month-old compared to 3 and 11- and 12-month-old Iba-1-EGFP transgenic mice.[49] Aged microglia in humans and the retina of aged mice demonstrated slower acute response time, processing motility in response to laser induced injury, and reduced dendritic arborization compromising the ability of aged microglia to survey the environment.[50,51] Furthermore, in mice, microglia express receptors which when activated induces pro-inflammatory signaling cascades, stimulating microglia and leading to upregulation and secretion of pro-inflammatory markers.[52] Additionally, microglia from animals and humans_ express inhibitory receptors to prevent overactivation of inflammation and to reduce injury-driven inflammatory response.[53–55] With age, microglia from C57/B6 mice have impaired phagocytosis, and inhibitory receptors are deficit in their ability to maintain microglial quiescence.[56] Therefore, since response to injury, and receptor function of microglia alters with age, it is likely that these cells when aged may not be able to adapt to their environment and interact in the same capacity as non-aged microglia interfering with the ability of the microglia cells to provide neuroprotection.

Microglia: The innate immune response—Interactions with monocytes

Under normal physiological conditions, monocytes are formed in the bone marrow, after which they extravasate through blood vessel walls to mature into macrophages or dendritic cells in the tissues. Upon extravasation, macrophages act as APCs in the periphery, phagocytizing debris, producing and releasing cytokines, and are first responders against foreign invaders.

Unlike microglial cells that are activated within hours post-brain ischemia, monocyte-derived macrophages are recruited to the ischemic region within three to seven days post-ischemic stroke.[57] The anti-inflammatory monocyte-derived macrophage subset, Ly6C^{low}, has not been thoroughly investigated in ischemic stroke, but depletion of this macrophage subtype did not affect brain injury nor increase infarct size in one study whereby adult mice were subjected to cerebral hypoxia-ischemia using the Levine/Vannucci model.[58] On the other hand, Ly6C^{hi} monocytes, also known as inflammatory monocytes which are recruited to sites of inflammation,[59] express CC chemokine receptor 2 (CCR2), which plays a role in the ability of monocytes to extravasate and migrate into the brain following

ischemic insult.[60] Hence, it is not surprising that Ly6C^{hi} monocytes in mice were elevated in the blood and brain post-MCAO.[11,61] Depletion of these monocytes or their inhibition by INCB3344 which binds to CCR2, leads to increased infarct volumes, and decreased M2 polarization resulting in a more damaging state and poorer functional outcomes, suggesting that monocytes play a role in reducing damage in brain ischemia.[11,61]

Manipulation of the M1 to M2 phenotype opens the possibility of targeting microglial activation states as potential therapeutic targets for neuroprotection. This is, however, a challenging endeavor as disease, medications, age, gender, or other environmental stressors may alter microglial activation states.

Microglia: The innate immune response—Interactions with histamine

Histamine is a widespread neurotransmitter and neuroimmune modulator in the brain with functions in the CNS and the periphery, playing roles in immune system regulation and brain disorders. Mast cells in the periphery and brain release a variety of mediators, including histamine, which is activated or released by neuropeptides, cytokines, serotonin, or histamine itself.[62] In the brain, approximately 50% of histamine is derived from non-neuronal cells, and changes in stress, behavior, or endocrine fluctuations can modify histamine production.[13] Furthermore, microglia have also been identified as histamine producing cells.[63]

In addition, microglia express all known histamine receptors.[64] Specifically, histamine can stimulate microglia[65] via histamine 3 receptors (H3R) to produce increased amounts of pro-inflammatory cytokines, which regulate microglial chemotaxis and phagocytosis in the brain.[13] Histamine has also been shown to trigger microglial phagocytosis via histamine 1 receptors (H1R) and reactive oxidation species production via H1R and histamine 4 receptor (H4R).[65,66] Histamine plays a role in multiple physiological functions, including interleukin 1 beta (IL-1β) and TNF-α cytokine-induced neuroinflammation as well as inhibition of LPS-induced IL-1β on microglia at H4R.[13,64] Changes in TNF-α, IL-1β, microglial activation, and apolipoprotein E-related Cluster of Differentiation 95 death receptor (CD95) expression on T-cells contribute to neuroimmune dysregulation and modify cell recruitment.[13] Therefore, this may impact CNS recovery or damage from ischemia.

Microglia: The adaptive immune response—Interactions with T-cells

The cell-mediated adaptive immune response is characterized by a delayed antigen-specific immune response. B and T lymphocytes play a pivotal role in cell-mediated immunity and thus play a secondary role in response to brain ischemia. While there has been little focus on the effects of B-cells in the ischemic brain, there has been recent work on the effects of T-cells. In the periphery, T-cells respond to specific antigens by releasing pro- or anti-inflammatory cytokines via regulatory T-cells (Treg), and CD4+ T-helper cells or by destroying virally infected cells themselves via CD8+ cytotoxic T-cells.

In one animal study, Forkhead box P3 (Foxp3)$^+$ Treg cells present in the rat cerebrum inhibited the LPS-induced M1 pro-inflammatory response of microglia, implying that certain subsets of T-cells may inhibit neuroinflammation by interacting and possibly altering microglial phenotypes which may also indirectly affect astrocyte reactivity.[8]

Crosstalk between APCs expressing programmed death receptor-1 (PD-1) and programmed death-ligand 1 (PD-L1) on CD8$^+$ T-cells, including those that infiltrated the brain during CNS injury, has been shown to cause T-cell exhaustion or suppression. One study demonstrated that activated microglia and astrocytes express PD-L1; therefore, it is not surprising that *in vitro* primary murine co-cultures of these cell types combined with blockade of PD-1 to PD-L1 communication caused increased production of T-cell interferon gamma (IFNγ) and interleukin 2 (IL-2).[12] These findings point out a potential area to target glial immune interactions in developing therapies to reduce effects of CNS insult including stroke.

Astrocytes

Astrocytes, another type of glial cell, and the most abundant cell residing in the CNS, have diverse morphology and can be classified into two main groups in the cortex: fibrous (elongated) astrocytes or protoplasmic (radial) astrocytes.[67] Fibrous astrocytes, in the white matter, tend to be in close proximity to myelinated axons and oligodendrocytes.[67] Protoplasmic astrocytes, located in the grey matter, interact directly with neurons, blood vessels,[67] and participate in the formation of the BBB, making them a prime target for immune cell exposure. Following ischemic stroke, the BBB becomes permeable, increasing the likelihood of glial–immune interactions.[68] One month post-ischemic stroke, T-cells were found in close proximity to active astrocytes in the ischemic region.[68] Astrocytes, once thought to be passive support cells for neurons,[69] are now known to respond to CNS insults, whereby they may undergo morphological and functional changes referred to as reactive gliosis.[70] Astrocyte reactivity is a way of maintaining homeostasis in the CNS and works as a defense mechanism to limit damage caused by ischemic stroke. On the other hand, it can also hinder recovery systems in the brain. Recently, reactive astrocytes have been categorized into A1 or A2 cell types. This nomenclature is a morphological distinction and may or may not reflect a functional distinction, nonetheless these terms will be used for the sake of simplicity. The A1 astrocytes upregulate complement cascade genes thought to play a role in CNS damage and the A2 neuroprotective astrocytes upregulate neurotrophic factors.[41] LPS-induced classical activation of microglia caused the release of interleukin 1 alpha (IL-1α), and TNF, which when combined with complement component 1q (C1q) to instigate astrocyte reactivity, steered astrocytes to a neurotoxic (A1) state.[41] A recent study showed that LPS directly added to astrocyte culture media was insufficient to drive astrocytes to the A1 state, and this was confirmed by measuring the upregulation of

astrocyte genes leading to the production of neurotoxins that are lethal to neurons following CNS damage.[41]

Therefore, mechanisms involved in regulation of astrocytes and astrogliosis are of particular interest, as they may provide another avenue for drug treatment to reduce post-ischemic stroke damage. Astrocytes are brain cells that bridge interactions between lymphocytes and neurons and communicate with immune system cells via cytokines.[5,23]

Astrocytes: The innate immune response—Interactions with neutrophils

Polymorphonuclear cells (PMNs) are the most abundant leukocyte and generally the first immune cell to be recruited to sites of inflammation; however, their function is at least partially determined by direct or indirect interactions with astrocytes.[14] For the purpose of this review, direct contact refers to cell-to-cell communication via touching, such as through cell receptors, while indirect contact refers cell-to-cell communication through non-touching means, such as cytokine secretion. PMNs isolated from C57BL/6 mice were placed in primary astrocyte cultures at a 1:1 ratio.[14] Direct and indirect astrocyte contact to PMN contact, resulted in attenuated PMN apoptosis, enhanced phagocytosis and decreased degranulation. However, differences between indirect and direct contact emerged demonstrating that direct astrocyte to PMN contact resulted in increased pro-inflammatory cytokine expression and decreased respiratory burst, while indirect contact encouraged PMN necrosis and increased respiratory burst.[14] The complexity of the interaction between PMNs and astrocytes warrants further investigation since it could be important in the innate immune response and be a target to reduce neuroinflammation and transmigration of other leukocytes into the brain during stroke.

Astrocytes: The innate immune response—Interactions with histamine

Astrocytes have been receiving increasing attention for their role as neuroinflammatory modulating cells, and histamine has been shown to play a part in astrocyte function including neuroprotective effects on astrocytic cell damage as a potent anti-inflammatory mediator, immune response via regulation of innate and acquired immunity, energy metabolism, homeostasis, and neurotransmitter clearance.[71–73] Current research has identified three histamine receptors (H1R, H2R, and H3R)[73] that are expressed on astrocytes indicating the importance of histamine in astrocyte function. Additionally, primary human astrocytes express organic cation transporter 3 (Oct3) and plasma membrane monoamine transporter on their cell surface.[74–76] This signifies the importance of histamine transport in astrocytes since both of these transporters are responsible for histamine reuptake. Nevertheless, little data are available describing the immunomodulatory role of histamine on astrocytes and even less is understood within the pathology of ischemic stroke.

Only one study evaluated the outcome of disruption to the histamine transporter, Oct3 in the reuptake of histamine and its effects on ischemic brain damage and Tregs.[77] In this study, an adult male 10- and 12-week-old homozygous Oct3 knockout (Oct3 KO) and wild-type (WT) mouse model was used, and mice either received a 1-h MCAO or sham.[77] Tetrazolium chloride staining showed a reduced infarction volume in Oct3 KO mice compared to controls following MCAO.[77] Histamine levels in the ischemic cortex (IC) during occlusion and 3 and 24 h after reperfusion were increased in the Oct3 KO mice compared to sham controls; however, this histamine difference was not seen between groups pre-ischemia.[77] Oct3 KO mice had higher serum histamine levels 24 h after reperfusion than WT mice.[77] Following reperfusion, all brain tissue samples taken from Oct3 KO and WT mice showed elevated levels of monocyte chemoattractant protein 1 (MCP-1) and interleukin (IL-6) cytokine production; however, the level of MCP-1 and IL-6 in the IC was remarkably higher in WT compared to Oct3 KO mice.[77]

Media from primary cell cultures of astrocytes, microglia, and bone marrow-derived macrophages had increased levels of IL-6, MCP-1, and TNF-α when cells were activated with Lipid A.[77] Proportions of type 1 (Th_1) and type 2 T-helper (Th_2), and Tregs did not vary between Oct3 KO and WT mice, before MCAO, but Treg proportions increased in Oct3 KO mice post-reperfusion. L-histidine, when injected into both groups, did not change the proportion of Treg cells in Oct3 KO mice, but it did increase the Treg cell proportion in WT.[77] In turn, Oct3 disruption may inhibit uptake and clearance of histamine in the brain.

Since Tregs are recruited by histamine,[78] elevated levels of histamine will lead to higher levels of Tregs, which play a neuroprotective immunomodulating role during cerebral ischemia. Future research should investigate the role of Tregs and their migration to and in the brain as well as targeting Oct3 as a possible therapy for cerebral ischemia. Disruption of Oct3 via increased histamine and Tregs ameliorates damage caused by ischemic brain injury.[77] Furthermore, since microglia are surveillance cells in the brain, changes in the environment such as ischemic conditions may regulate astrocyte functions which may have an impact on transporter or histamine receptor expression thereby altering interactions with histamine or recruitment of T-cells complicating repair mechanisms post-stroke.

Astrocytes: The adaptive immune response—Interactions with T-cells and NK cells

In severe oxidative stress, astrocytes, which normally play a role in removal of glutamate from extracellular fluid,[79,80] stop glutamate clearance.[81] Excessive glutamate can inhibit glutamine synthetase causing intracellular glutamate accumulation in astrocytes and inducing reactive astrogliosis.[82] *In vitro* studies demonstrated that the addition of T-cells from mouse lymph nodes and spleens to primary murine astrocyte cultures rescues astrocytes from impairment by oxidative stress endowing a neuroprotective astrocyte phenotype.[81,83] The replacement of T-cells with IL-2, or IFNγ cytokines in oxidative stress induced cultures, caused increased glutamate clearance, while cultures without cytokines, or those with interleukin 4 (IL-4) did not;[81] implying that Th1 T-cells are the primary neuroprotective T-cells[81]

and that T-cell subtypes may have different effects on neuroprotection.[84] When neurons were cultured in media derived from oxidatively stressed astrocytes, there was a significant reduction in neuron apoptosis.[81] This protective effect was further enhanced when medium from T-cells was co-cultured at a 1 to 1 ratio with astrocytes under oxidative stress.[81] By adding a glutamate uptake inhibitor, L-aspartic acid β-hydroxamate (AβH), to astrocyte T-cell co-cultures, the neuroprotective effect was blunted demonstrating that T-cell to astrocyte interactions via glutamate clearance are neuroprotective and further understanding of this interplay may help identify strategies to treat ischemic brain injury.[81]

Additionally, it has been shown that trans-presentation of interleukin 15 (IL-15), a pro-inflammatory cytokine, by astrocytes[85,86] to T-cells and NK cells exacerbates brain damage post-ischemic stroke.[5] Glutamate aspartate transporter positive astrocytes isolated from lesion areas 24 h after reperfusion from male WT C57BL/6 mice after right MCAO demonstrated an approximately 38% higher level of IL-15 in the cell lysate compared to the sham (no MCAO) mice. Also, it was determined that the largest amount of IL-15 was produced by astrocytes in the brain of both mice and humans rather than by neurons or microglia cells, revealing that IL-15 from astrocytes is a key regulator in inflammation[87] post-ischemic stroke.[5] These same researchers demonstrated that their transgenic IL-15 mouse model had increased infarct volumes and neurodeficits compared to the WT mice.[5] Furthermore, $CD8^+$ T-cells and NK cells were observed to be co-localized with astrocytes in peri-infarct regions[88] 24 h after stroke, and these leukocytes had increased expression of the T-cell and NK-cell activation markers CD69, NKG2D, and IFNγ in transgenic mouse brains but not in their spleens compared to WT.[5]

In addition, *in vitro* experiments showed that placing an insert which separated $CD8^+$ T-cells or NK cells from astrocytes in co-cultures decreased or inhibited activation of T-cells and NK cells from transgenic IL-15 mice indicating that direct cell-to-cell contact of immune cells and astrocytes was necessary for a pro-inflammatory response.[5]

Pro-inflammatory cytokine link CNS to immune system

As previously mentioned, pro-inflammatory cytokines and chemokines are released by both microglia and astrocytes. Hence, it is important to note that pro-inflammatory cytokines are an obvious link between cells in the CNS such microglia and astrocytes and immune system cells such as T-cells.

Pro-inflammatory cytokines are up-regulated in the infarcted area, urine, or serum post-ischemic stroke.[16,89–94] The acute expression of cytokines varies in mice and humans[95] (granulocyte colony stimulating factor was elevated in mice but not humans).[27] It is noteworthy that the following acute cytokines were elevated post-ischemic stroke in both mice and humans: granulocyte monocyte colony stimulating factor, IL-6, interleukin 12 active heterodimer (IL-12(p70)), IFNγ-induced protein 10, keratinocyte-derived chemokine interleukin 8 homologue, MCP-1, macrophage inflammatory protein 1 alpha, macrophage inflammatory protein 1 beta, regulated on activation, normal T-cell expressed and secreted, and TNF-α.[13,27] Several weeks post-ischemic stroke during the stage of liquefactive necrosis, the inflammatory response is dampened. Several cytokines remain chronically elevated during this period: IL-6, and MCP-1.[27] Additionally, there was a chronic T-cell response in the stroked regions of both mice and humans; however, activated T-cell subtypes varied depending on mouse strain.[27,96] The $CD4^+$ Th_1 response was higher in C57BL/6 mice, while Th_2 responses were higher in BALB/c mice.[27] Furthermore, chronic cytokine and chemokine differences were not remarkably different in 18-months-old versus 3-months-old C57BL/6 mice, while T-cell infiltration was blunted in the younger mice.[27] Other studies have shown a different profile when comparing similar age discrepancies, with 18-month-old mice versus 9- to 12-week-old mice exhibiting an increased pro-inflammatory cytokine profile in the aged mice (TNF-α, iNOS, and IL-6) and anti-inflammatory profile in the young mice (transforming growth factor beta, IL-4, and IL-10) post-MCAO.[97] Lastly, recurrent stroke mildly increased cytokine response.[27] These data call to question the use of *in vivo* models since the inflammatory response post-ischemic stroke appears to vary based on an individual's genetics and previous exposure to environmental factors.[27]

Potential therapies

When it comes to drug development for ischemic stroke, the present trend is to investigate combination therapies, which use rtPA alongside immune modulators to reduce ischemic damage. This may be a reasonable approach since ischemic stroke likely involves several mechanisms integrating cells from multiple organ systems. Potential pharmacological therapies that target glial immune interactions are summarized in Table 1.

Minocycline, a bacteriostatic antibiotic, may have an effect on microglial phenotypic changes.[98,99] Several human and animal studies have indicated that minocycline is responsible not only for aiding in a M1 to M2 shift but also selectively inhibits the M1 phenotype,[23,100] suggesting that minocycline may provide benefits to ischemic stroke patients and perhaps may be given prophylactically.[101,102] In addition, it is most likely that minocycline alone is not responsible for microglial phenotypic changes,[103] but rather a multitude of factors contribute to its potential drug efficacy such as stress, genetics, and gender.

Blocking the interaction of astrocytes trans-presenting IL-15 to T-cells and NK cells with antibodies resulted in decreased infarct volume in transgenic IL-15 mice to WT levels. This is encouraging, as it suggests that inhibition of the interaction between astrocytes and the immune system cells may reduce ischemic damage post-stroke.[5] Other ways of blocking the damage caused by IL-15 were implemented by downregulation of the IL-15R and by inhibiting direct contact between astrocytes and T-cells or NK.[5,85]

Mast cells and disruption of Oct3 during stroke increase histamine release and that may target microglia in the brain at H4R receptors as well as increase migration of Tregs to

Table 1. Potential therapies to target glial–immune interactions post-ischemic stroke.

Therapy	Known use	Target/action	Overall effect
Minocycline[82–87]	Bacteriostatic antibiotic	Microglia Selectively inhibits M1 phenotype	Anti-inflammatory Neuroprotective
PD-L1:PD-1 pathway[9]	Not currently used as a therapy	Inhibits $CD8^+$ T-cell activation and cytokine production	Neuroprotective
Anti-$CD8^+$ Anti-NK1.1 antibodies Anti-IL-15 antibodies[4,68]	Not currently used as a therapy	Blocks interaction of astrocyte transpresentation of IL-15 to T-cells and NK cells	Decrease infarct volume
L-histidine/histamine[10,45–47,54–57,59,60]	Likely has significant side effects; not in use	Microglia and astrocytes Prevent disruption of Oct3 Increase migration of regulatory T-cells to ischemic regions	Decrease infarct volume Neuroprotective Anti-inflammatory

IL-15: interleukin 15; PD-L1: programmed death-ligand 1; PD-1: programmed death-1; NK: natural killer; CD: cluster of differentiation; Oct3: organic cation transporter 3.

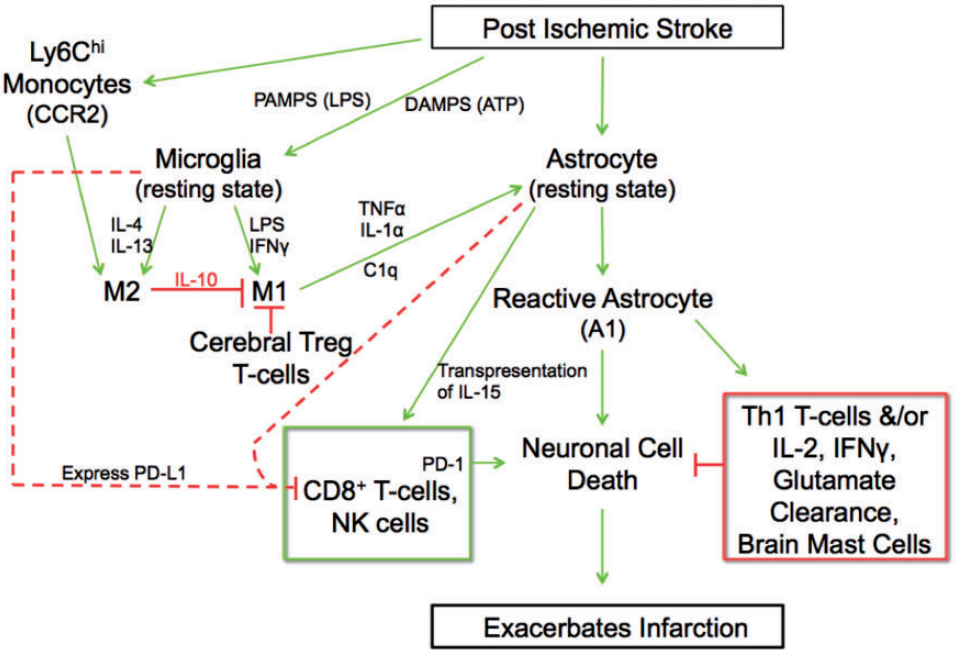

Figure 1. Glial immune interactions. Following ischemic-stroke signaling, mediators trigger glial and immune cell responses leading to inhibition or stimulation of neuronal cell death. These mediators include PAMPs, LPS, DAMPs, and cytokines: IL-4, IL-13, IL-10, IL-15, IL-1α, IFNγ, TNF-α, C1q, and IL-2. When microglia are activated by PAMPs or DAMPs, they change from a resting state to anti-inflammatory state (M2) or pro-inflammatory state (M1), respectively. Microglia in the M1 state can activate resting astrocytes causing their phenotypic shift into reactive astrocytes, resulting in neuronal cell death and exacerbating infarct damage. Microglia in the resting state which express PD-L1 can inhibit $CD8^+$ T-cells by binding to their PD-1 receptor. Activation of astrocytes post-ischemic stroke leads to trans-presentation of IL-15, NK cells, and $CD8^+$ T-cells which facilitate neuronal cell death. Monocyte activation following infarction leads to a dominant M2 microglial/monocyte phenotype resulting in the release of anti-inflammatory cytokines. Helper T-cells (Th1), IL-2, IFNγ, glutamate clearance, and mast cells in the brain have been shown to play neuroprotective roles to inhibit damaging effects of ischemic stroke. Green arrows represent stimulation. Lines with blunted ends (red) represent inhibition. The dashed red line represents inhibition of the PD-L1: PD-1 pathway only. See text for details. CCR2: CC chemokine receptor 2; PAMPs: pathogen-associated molecular patterns; LPS: lipopolysaccharides; DAMPs: damage-associated molecular patterns; IL-4: interleukin 4; IL-13: interleukin 13; IL-10: interleukin 10; IL-15: interleukin 15; IL-1α: interleukin 1 alpha; IFNγ: interferon gamma; TNF-α: tumor necrotic factor-alpha; C1q: complement component 1q; IL-2: interleukin 2; PD-L1: programmed death-ligand 1; PD-1: programmed death-1; NK: natural killer; CD: cluster of differentiation. (A color version of this figure is available in the online journal.)

regions of brain ischemia. Consequently, histamine and interactions with Oct3 on astrocytes should be investigated as a possible pharmacological therapy for cerebral ischemia since increased histamine and Treg T-cells ameliorates recovery from damage caused by ischemic brain injury.[77]

In addition, regulation of inflammatory markers and genes are important neuroinflammatory targets. Recent reports have pointed to the following potential determinants in regulating immune-related pathologies: mitochondrial factors causing systemic inflammation-related to injury, the NOTCH1 pathway associated with M1 macrophage activation and inflammation, tristetraprolin, an anti-inflammatory protein encouraging decay of pro-inflammatory mediators, release of cytokines from neutrophils, and sestrins, antioxidant proteins involved in Toll-like receptor pro-inflammatory signaling.[13] There is a network of immune signaling pathways as well as neurologic and endocrine factors that may be webbed together in stroke pathologies.[13] The complexity of these interactions suggests that neuroinflammation and neuroprotective

effects are intertwined, indicating that a delicate balance between systems is essential to avoid neurological miscommunication. Therefore, drugs targeting immune pathways may have unintended adverse effects and should be used with caution.

Conclusions

Thedialogue between glial and immune cells is complex as illustrated in Figure 1. Much of the data reported from these studies remains controversial, due to limitations in inconsistent or non-standardized *in vitro* or incompatible *in vivo* models as was demonstrated by changes in cytokine production between different strains of mice.[27] Additionally, with microglia, macrophage, and astrocyte phenotypes changing based on a multitude of environmental factors, it is difficult to confirm the role of different immune cells during cerebral ischemia or control for various environmental factors which may work together to create the final pathology. For instance, microglia can shift from neurotoxic to neuroprotective phenotypes, which can dictate alterations in cytokine levels and can also influence the function of astrocytes.

In *in vitro* studies, the ratio of glia to immune cells appears to effect ischemic damage and repair and leads to inconsistent findings between researchers. Studies have reported varied ratios of T-cells to astrocytes in co-cultures. Higher 3:1 T-cell to astrocyte ratios may be neurotoxic while a lower 1:1 ratio may be neuroprotective.[81] Furthermore, *in vitro* models do not take into consideration damaged astrocytes at the site of injury, which may be unable to respond when injured. For example, injured astrocytes may not uptake T-cell-derived glutamate, which may ultimately lead to neurotoxicity. Moreover, the method used to induce oxidative stress in many studies may not be an adequate representation of ischemic stroke in an *in vivo* model.

In addition, attention should be paid to the threshold ratio of immune cells responsible for adequate activation or phenotypic alterations in astrocytes or microglia. Still, little is known about oligodendrocytes, B-cells, and dendritic cells and their role in glial immune interactions during ischemic stroke. Current research suggests that oligodendrocytes are resistant to ischemic insult,[104] although little to no research has investigated their role in interacting with cells of the immune system. Similarly, B-cells to not exhibit a pathophysiological role in cerebral ischemia, as depletion of B-cells did not alter infarct volume or influence stroke outcomes.[105] Moreover, it is possible that only a small subset of T-cells, such as the $\gamma\delta$ T-cells, engage in stroke recovery, while another group of immune cells is part of a signaling pathway inducing a negative response. Understanding these layers of interactions will help researchers develop specific drug targets with the fewest side effects.

Despite these challenges, researchers should not be discouraged. Abundant data support cross-talk between immune signals and glia. Researchers should continue to sort out the individual components of CNS and immune cell interactions. The first step is discovering the signaling pathways that intertwine the CNS with the immune system to help regulate and reduce damage. Implications for this research extent far beyond stroke pathology to include neuroinflammatory mechanisms that influence aging, cellular metabolism, and insulin resistance.

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