REVIEW

The Local and Peripheral Immune Responses to Stroke: Implications for Therapeutic Development

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Abstract

The immune response to stroke is an exciting target for future stroke therapies. Stroke is a leading cause of morbidity and mortality worldwide, and clot removal (mechanical or pharmacological) to achieve tissue reperfusion is the only therapy currently approved for patient use. Due to a short therapeutic window and incomplete effectiveness, however, many patients are left with infarcted tissue that stimulates inflammation. Although this is critical to promote repair, it can also damage surrounding healthy brain tissue. In addition, acute immunodepression and subsequent infections are common and are associated with worse patient outcomes. Thus, the acute immune response is a major focus of researchers attempting to identify ways to amplify its benefits and suppress its negative effects to improve short-term recovery of patients. Here we review what is known about this powerful process. This includes the role of brain resident cells such as microglia, peripherally activated cells such as macrophages and neutrophils, and activated endothelium. The role of systemic immune activation and subsequent immunodepression in the days after stroke is also discussed, as is the chronic immune responses and its effects on cognitive function. The biphasic role of inflammation, as well as complex timelines of cell production, differentiation, and trafficking, suggests that the relationship between the acute and chronic phases of stroke recovery is complex. Gaining a more complete understanding of this intricate process by which inflammation is initiated, propagated, and terminated may potentially lead to therapeutics that can treat a larger population of stroke patients than what is currently available. The immune response plays a critical role in patient recovery in both the acute and chronic phases after stroke. In patients, the immune response can be beneficial by promoting repair and recovery, and also detrimental by propagating a pro-inflammatory microenvironment. Thus, it is critical to understand the mechanisms of immune activation following stroke in order to successfully design therapeutics.

Keywords Stroke · Infection · Inflammation · Immune response · Ischemia

Introduction to Stroke

Stroke is a leading cause of death and disability worldwide. Ischemic stroke is a blockage of blood vessels that supply the brain, leading to a reduction or total loss of blood flow, while hemorrhagic stroke is caused by the rupture of intracerebral vessels [1]. Regardless of subtype, stroke causes rapid necrotic death of all cells within the core, which is the region of significant loss of oxygen and nutrients [2, 3]. Ultimately, the amount of brain tissue death is affected by the absolute area at risk, whether there is any residual tissue perfusion, the length of the occlusion, and the degree of reperfusion [4]. In some ischemic stroke patients, a "no-reflow" phenomenon is observed, where even after clot removal the tissue will not fully reperfuse, causing further damage [4]. In hemorrhagic stroke, hematomas expand and surrounding edema further compresses blood vessels resulting in more tissue damage [5]. Finally, both the size and location of the ischemic lesion are responsible for patient outcomes and observed symptoms.

Risk factors for stroke include aging, hypertension, obesity, atherosclerosis, type II diabetes mellitus, and peripheral

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infection. There are also gender-specific risks within different age groups [6–8]. Each of these risk factors is independently correlated with poor short-term stroke outcome in both clinical and experimental settings [9, 10]. All of these risk factors for stroke also activate the peripheral immune system; thus, there may be cross-talk between the brain and the systemic inflammatory milieu that contributes to worse outcomes [11].

Acute treatments for arterial ischemic stroke restore blood flow to hypo-perfused tissue through chemical or mechanical thrombolysis, sometimes with dramatic success [12, 13]. Although the treatment window for thrombectomy has recently been extended through the use of modern imaging techniques, many patients are still left untreated, and once ischemia has caused irreversible damage, therapies are limited [14–16]. In the past, only a minority of patients were eligible for intervention [17], and although this is improving, there are still many patients who are not eligible, and/or who have significant infarct burden even after successful revascularization. Because of the prominent role that the immune response plays in ischemic stroke pathology in both the subacute and chronic phases, it is a promising target for any patient with a completed infarction. The immune system can further injure damaged tissue, influence clearance of dead tissue, and either hinder or facilitate neuronal rewiring, all processes that are critical to recovery during the days and weeks after stroke.

Various aspects of the immune response to stroke have been a focus for researchers, as summarized in Fig. 1. During the acute phase, the brain activates resident microglia and simultaneously recruits myeloid cells and neutrophils from the periphery. These events facilitate neuroinflammation and local repair after injury, and simultaneously activate the peripheral immune response. In the days after stroke, patients also become immunosuppressed, leading to high rates of infection. In addition, during this time, the active process of resolution is initiated to avoid excessive tissue damage. When inflammatory responses do not resolve properly, it may however result in chronic activation of the adaptive immune response, secondary neurodegeneration, and dementia in stroke survivors. Overall, understanding these different aspects of the immune response is essential for maximizing recovery of stroke patients. Finally, by modulating reperfusion-induced inflammation, these strategies have the potential to improve outcomes even after tPA therapy.

Initiating Neuroinflammation After Stroke

Damage-Associated Molecular Patterns Damage-associated molecular patterns (DAMPs), also known as alarmins, are released by necrotic cells and act as endogenous signals to stimulate the inflammatory response. Prior to secretion, these molecules also have physiological functions that are independent from their activities as rapid activators of the immune system. Immediately following arterial occlusion, intracellular ATP depletion and subsequent glutamate release to the extracellular space result in uncontrolled calcium-ion influx into cells [18]. Subsequently, dysregulated calcium signaling activates intracellular lipases and proteolytic enzymes, ultimately resulting in cell death [18]. Necrotic cells in the ischemic core
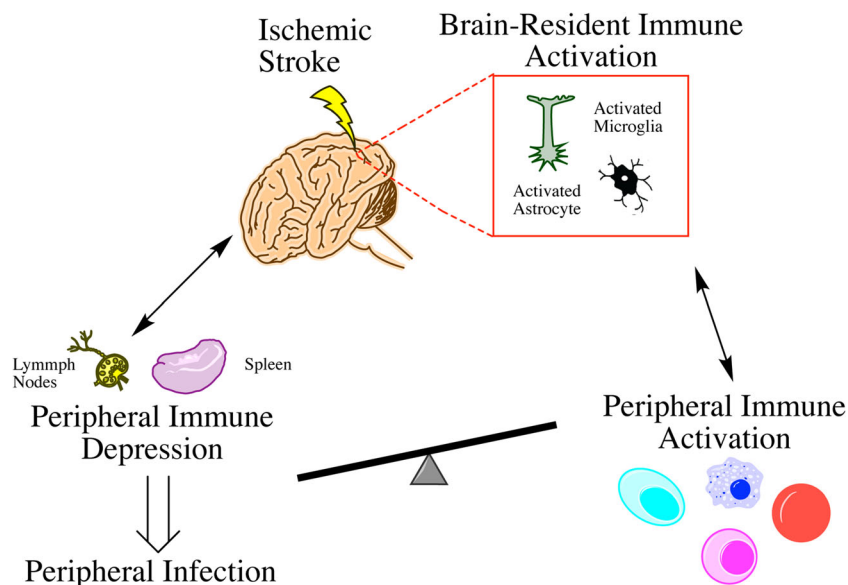
Fig. 1 Summary of local and peripheral immune responses after stroke. Immediately following stroke, brain-resident immune cells such as microglia and astrocytes are activated to respond to injury. Subsequently, peripheral immune cells are activated and recruited to the brain to assist in the immune response. In the following days, peripheral immunodepression can occur, with a subsequent increased risk for systemic infections. The extent of these local and peripheral immune responses to stroke is variable, and this plays an important role in determining patient outcomes and overall functional recovery in the acute and chronic phases after stroke

release DAMPs into the extracellular circulation to assist in the activation of brain resident immune cells (microglia) through specific receptors on the extracellular surface. In addition, DAMPs recruit peripheral immune cells to the brain once they have been released into the bloodstream. Peripheral immune cells are activated outside the brain, and then traffic to the brain to assist in repair and recovery [19].

High-mobility group box 1 (HMGB1) and heat shock proteins (Hsps) are essential DAMPs for initiating the post-stroke inflammatory response via activating receptors on peripheral immune cells [20, 21]. Specifically, HMGB1 released from activated monocytes and macrophages or necrotic cells can bind to the receptor for advanced glycation end products (RAGEs) and toll-like receptors (TLRs) 2 and 4 once secreted into the bloodstream [21, 22]. Activation of these receptors results in upregulation and subsequent secretion of multiple cytokines that promote inflammation and cellular death after ischemia, including matrix metalloproteinase 9 (MMP-9), tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β [20]. Activation of innate responses mediated by TLR-4 in monocytes, such as NF-κB activity and TNF synthesis, is associated with worse outcomes in stroke patients [23, 24]. Additionally, adequate clearance of DAMPs is required for proper resolution of inflammation following injury–deficiency in the clearance process results in severe inflammation and exacerbated neuronal injury in a murine model of ischemic stroke [25]. In contrast, astrocyte-produced HMGB1 may promote endothelial recovery [26]. HMGB1 release from activated astrocytes enhances neurite outgrowth, neuronal survival, and neurovascular recovery [26]. Taken together, these data suggest that HMGB1 likely plays a dual role in stroke pathophysiology and understanding the cell-specific role of this DAMP is critical to understanding the downstream signaling effects.

Several Hsps have also been implicated in initiating the innate immune response after ischemic stroke [20]. After secretion, Hsp70 binds TLRs on the surface of macrophages and dendritic cells in the periphery and microglia in the brain. Via TLR signaling, Hsp70 activates NF-κB in addition to other pro-inflammatory pathways [27]. It can also directly polarize CD4+ T cells to a pro-inflammatory Th1 state through interaction with extracellular receptors [28]. In contrast, overexpressing Hsp70 in mice with stroke improves long-term outcomes and knockout of Hsp70 worsens them [28, 29]. Hsp27 and Hsp32 have also been implicated in stroke. Extracellular Hsp27 binds various receptors on peripheral immune cells such as TLRs on the surface of activated macrophages, and activates the pro-inflammatory NF-κB pathway [30]. In contrast, Hsp32, or heme oxygenase-1 (HO-1), increases ischemic brain damage via formation of reactive oxygen species [31]. Interestingly, treatment of wild-type microglia with deferoxamine (a hypoxia mimetic) decreases neuronal damage, but this effect is abolished in HO-1(−/−) microglia, indicating cell-specific roles for this DAMP [32].

Due to their prominent role in the acute immune response to stroke, DAMPs have been proposed as promising therapeutic targets. However, it is often difficult to distinguish between the beneficial and injurious roles of inflammation in stroke recovery, as evidenced by the dual roles of many of these proteins, and their abilities to target multiple pathways to modulate the immune response. These studies highlight the need for further work in distinguishing cell-type specific roles and downstream signaling pathways activated by DAMPs to selectively target the harmful aspects of the immune response.

Blood–Brain Barrier Once peripheral immune cells have been activated by DAMPs, they must access the brain to assist in repair. In the healthy CNS, the blood–brain barrier (BBB) regulates the movement of nutrients and blood-borne molecules into the brain, and metabolic waste out of the brain, both maintaining a healthy environment and protecting the brain from peripheral toxins [33, 34]. Under physiological conditions, the BBB also excludes peripheral cells, but during pathological conditions such as stroke, traumatic brain injury, or multiple sclerosis, this function changes to attract immune cells [35].

Initially, circulating lymphocytes contact inflamed vessels via P-selectin glycoprotein ligand-1 (PSGL-1), and interact with endothelial cells via E-selectin and P-selectin [36]. This interaction tethers peripheral cells to the endothelial surface. Subsequently, integrins such as lymphocyte function-associated antigen 1 (LFA-1) and very late antigen-4 (VLA-4) on lymphocytes bind to ICAM-1 and VCAM-1 on endothelial cells, respectively, causing firm lymphocyte adherence to the endothelium and subsequent lymphocyte crawling [37]. Finally, PECAM-1 (CD31) and CD99 homophilic interactions facilitate structural changes to tight junctions and endothelial cell structure, and subsequently promote both paracellular and transcellular diapedesis [38]. Several animal studies indicate that trafficking of leukocytes into the brain contributes to neuroinflammation-mediated neuronal cell death [39, 40].

Independent of cellular infiltration, the structural integrity of the BBB is altered by MMP-2 activation within 6 h of reperfusion, resulting in increased permeability [41]. MMPs degrade basal lamina proteins such as fibronectin, laminin, and heparan sulfate, which contributes to the vessel damage associated with BBB breakdown and subsequent dysfunction [41–43]. Additional degradation and remodeling of tight junctions, and further increases in BBB permeability, occur within 48 h after reperfusion [41, 42, 44]. While the initial BBB opening is transient, the later increase is the result of severe damage to blood vessels by expression and activation of MMP-9 and MMP-3 [41, 45].

Innate Inflammation After Stroke

Initial Acute Brain Inflammation In the acute phase of stroke, peripheral immune cells infiltrating the brain parenchyma contribute to the inflammatory environment [33]. Signaling via the chemokine receptor CCR2 recruits both monocytes and neutrophils from the periphery to the site of injury [46]. Neutrophils are the primary cell type adhered to endothelial cells in brain vessels following the initial myeloid cell response, and they are found in significant numbers in the brain parenchyma by 3 days post-stroke in animal models [39, 47].

Accumulating evidence from both human and rodent models suggests that post-stroke accumulation of neutrophils in the brain parenchyma worsens stroke outcomes [39, 48, 49]. Neutrophils obstruct capillaries in the microvascular bed following reperfusion, suggesting that they play a causative role in the "no-reflow" phenomenon observed in humans [4]. Pharmacological ablation of neutrophils reduces infarct volumes and improves neurological outcomes, further supporting a pathogenic role of neutrophil accumulation and activation after stroke [50, 51]. Likewise, genetic deficiencies in adhesion molecules such as ICAM-1 and P-selectin reduce neutrophil trafficking into brain parenchyma, resulting in smaller infarct volumes 48 h after stroke [52, 53]. Neutrophil-mediated damage may be mediated through their high proteolytic enzyme content, their capacity to generate ROS, and ability to activate the complement system [54, 55].

Myeloid cells are also among the first immune cells to respond to injury [47, 56, 57]. Both brain resident microglia and monocyte-derived macrophages (MDMs) that are derived from peripheral monocyte ingress recognize DAMPs and other stress signals and become activated to migrate to the site of injury. Initially, microglia in the stroke penumbra are activated by cytokines and various DAMPs from the necrotic debris of local dead or dying cells. Microglia are also recruited from the surrounding brain regions by neuronal-derived chemokines such as fractalkine (CX3CL1) [58–61].

Studies investigating the beneficial or detrimental effects of microglia on the brain have been contradictory. This may be due to the different stroke models utilized and the outcomes assessed. Also, tools to distinguish MDMs from microglia in the injured brain have historically been limited [62, 63]. Newly developed chimeric models and microglia-specific markers such as Tmem119 are helping to identify the individual roles of each cellular subtype, and will hopefully lend clarity [62].

Overall, microglia likely have an early pro-inflammatory role, while MDMs primarily serve a clearance and immune resolving function [64]. However, there is more to learn about the heterogeneity within each cell population [65]. Promoting early inflammation may be critical, as when microglia are ablated before ischemic stroke it causes exaggerated neurological deficits and increased infarct volume [66], perhaps because they, via cross-talk with astrocytes, are critical for termination of inflammation by neutrophil engulfment [67].

Subacute Innate Neuroinflammation Myeloid cells can also transition from a naïve state to an anti-inflammatory phenotype in response to interleukin-4 (IL-4) release from damaged neurons via interferon regulatory factor (IRF)-4 signaling [68, 69]. IL-4 treatment of cultured microglia facilitated both trophic factor expression and peroxisome proliferator–activated receptor (PPAR)-γ-dependent phagocytosis, which are hallmark characteristics of anti-inflammatory myeloid cells [68, 70]. Anti-inflammatory microglia may also reduce activation of immune cells via release of the anti-inflammatory cytokines IL-10 or transforming growth factor (TGF)-β [71, 72]. There may also be other, as yet undiscovered, factors that induce myeloid cell polarization to an overall anti-inflammatory phenotype, and may aid in repair following injury [68, 73].

There are also factors that polarize peripheral cells to pro-inflammatory phenotypes, which are likely detrimental in the context of stroke. Monocytes are recruited from the periphery by monocyte chemoattractant protein-1 (MCP-1), also known as CCL2 [46]. Within days after the initial injury, neurons release factors including ATP and Fas ligand, which promote pro-inflammatory MDM phenotypes and activate pro-apoptotic pathways [1, 74]. These MDMs release inflammatory mediators such as TNF-α and IL-1β, various MMPs such as MMP-9 and MMP-3, and reactive oxygen species (ROS), all of which are implicated in ischemic damage [75, 76]. Clinical studies suggest that blocking the IL-1β receptor may improve long-term outcomes after stroke, suggesting these IL-1β secreting macrophages are detrimental to patient recovery [77–79]. However, the fact that clinical outcomes improved in a trial conducted prior to widespread use of thrombolysis, but were neutral in a later trial with alteplase as the standard of care with similar patient demographics, suggests a potential detrimental interaction between tPA and IL-1β pathways [78, 79]. This was suggested in the results of the SCIL-STROKE trial with IL-RA where the effect of IL1-RA on lowering IL-6 levels seemed to be beneficial but was counteracted by a potentially harmful effect. This was not proven to be due to tPA; however, 70% of the subjects were treated with alteplase [79].

There is still controversy, however, about when and which myeloid cells are pro- vs anti-inflammatory and when and why they transition from one phenotype to another. One study reported transition of both microglia and MDMs towards a pro-inflammatory state 4 days post-stroke [80]. However, using more advanced tools, Wattananit et al. suggest that this phenotypic shift may be limited to resident microglia [63]. By 2 weeks post-stroke, it appears that MDMs are primarily anti-inflammatory, and this is associated with improved long-term functional recovery. Likewise, when monocyte recruitment to the brain was inhibited, no functional recovery was observed, and there was decreased expression of anti-inflammatory

genes such as TGFβ, CD163, and Ym1 [63]. Additionally, age-related changes to IRF activity may change the polarization state of microglia, therefore altering the inflammatory milieu both prior to and post-injury [69].

In summary, therapies targeted towards innate immune cells can have a significant beneficial effect in animal models; however, accumulating evidence suggests that, in order to achieve clinical success, they must be able to promote resolution and an anti-inflammatory phenotype rather than block inflammation completely. There remain controversies and knowledge gaps as to when and how these cells change function, and also about which functions will ultimately be beneficial.

The Effects of Stroke on the Acute Peripheral Response

Immunodepression After Stroke Infection, predominantly pneumonia and UTIs, complicate stroke recovery in up to 30% of cases [81]. Pneumonia is the most common and fatal post-stroke complication, and correlates with poor stroke outcomes and possibly death (Table 1; [81, 102, 103]). It is clear that pathophysiological immunodepression, rather than pre-existing infection or aspiration of oral microbiota, is a major contributing factor [104]. For example, the severity of post-stroke infection is directly correlated with stroke size, which itself is correlated to the severity of leukocytopenia [105]. This is evident in murine stroke models, where one thousand-fold fewer bacteria are needed to cause pneumonia after stroke [106]. In addition, infection-induced inflammation exacerbates ischemic damage and acute neurological deficits in an IL-1- and neutrophil-dependent manner [9, 107].

Curiously, clinical studies with prophylactic antibiotic treatment to combat this phenomenon have yielded mixed results (Table 2). In a rodent study, treatment with ceftiofur (a β-lactam antibiotic), enrofloxacin (a fluoroquinolone antibiotic), or a vehicle control yielded no change in functional outcomes at 24 h or 1 week post-stroke [113]. Interestingly, enrofloxacin-treated rats had worse motor performance at 1 month post-stroke, suggesting this effect may be independent of the antibiotic mechanism and due to off-target effects of this antibiotic class [113]. In stroke patients, treatment with some antibiotics has reduced infarct size or infection prevalence; however, these outcomes were not associated with better overall recovery [114, 115]. In contrast, prophylactic administration of the broad spectrum mezlocillin along with the beta-lactamase inhibitor sulbactam decreased both the incidence and severity of fevers within 3–4 days of stroke, and improved long-term outcome [116]. Finally, minocycline treatment significantly improved clinical outcomes in patients, although this effect was not mediated through a decreased infection rate and actually may be due to an anti-inflammatory effect on microglia [117]. Overall, post-stroke infection is a significant risk for poor patient outcomes, and better understanding the

mechanisms of immunodepression would greatly benefit patient recovery.

Leukocytopenia, or a reduction in white blood cell numbers, has been well documented in stroke patients [118, 119]. Studies have also detected a decrease in lymphocyte proliferation in response to mitogens and decreased *ex vivo* secretion of pro-inflammatory cytokines, suggesting that lymphocyte function is also impaired by stroke [105, 120, 121]. Consistent with this idea, T cell secretion of TNF, as well as other Th1-associated cytokines such as IL-2, IL-12, and interferon (IFN)-γ may be decreased in patients in the days after stroke [122, 123]. This could be beneficial in the absence of infection as pro-inflammatory responses in peripheral organs can exacerbate brain damage and result in worse neurological outcomes in stroke patients [124]. Thus, understanding the relationship between immune activation and suppression in peripheral organs following stroke is essential to successfully treating patients.

In contrast, other studies show that despite decreased lymphocyte numbers, their function remains intact following stroke [122, 125]. Many studies show a dramatic increase in plasma and cerebrospinal fluid (CSF) levels of TNF-α and other pro-inflammatory cytokines, which correlates with stroke severity [125–128]. Thus, it is still unclear whether lymphocytes respond normally to typical infectious pathogens, such as those responsible for pneumonia or UTIs. It is clear, however, that lymphocytopenia correlates with the occurrence of post-stroke infections, suggesting a role for this phenomenon in the development of infections, and subsequent poor patient outcomes [105, 119].

Stroke-induced activation of the autonomic nervous system (ANS) may mediate immunodepression [129, 130]. The ANS is comprised of the parasympathetic nervous system (PSNS) and the sympathetic nervous system (SNS), and both affect immune responses in the periphery [102]. Ischemic injury immediately activates the SNS, leading to splenic contraction and shrinkage [131]. The PSNS antagonizes the pathways activated by the SNS, and is suppressed following stroke [129, 132, 133]. Splenectomy prior to MCAO significantly reduces infarct size, as well as the number of neutrophils and activated microglia in the brain [134, 135]. Likewise, irradiation of the spleen following stroke significantly reduces infarct size in rats [136]. Thus, in the acute phase after ischemic injury, splenic contractions contribute to immune cell efflux and mobilization in the blood stream, which contributes to inflammatory injury in the brain.

In the days after stroke, the supply of spleen-derived immune cells becomes exhausted and spleen size is dramatically reduced, contributing to immunodepression [137]. The β2-adrenergic receptor (ADRβ2), the primary adrenoreceptor on both innate and adaptive immune cells, suppresses immune function upon activation by the SNS [138]. For example, treatment with the ADRβ2 antagonist propranolol reduced

Table 1 Clinical studies evaluating incidence of infection after stroke. A summary of clinical trials, listed by year, evaluating the prevalence of infection, or specifically urinary tract infection and/or pneumonia after stroke. The outcomes assessed in each trial include the identification of risk factors associated with infection, as well as functional outcomes in patients that have acquired an infection. Studies were not included if they were not controlled. Control is either placebo or conventional therapy

Cohort Studied	Incidence of infection	Risk factors identified	Functional outcomes	Ref.
52 patients with AIS	PSP: 10% UTI: 19% Other: 2%	Elevated IL-6, CRP, abnormal leukocyte count	N/A	[82]
279 patients	PSP: 10% UTI: 11% Other: 1%	N/A	51% of deaths attributed to infectious complications; worse functional outcomes (GOS and BI) associated with infection	[83]
330 patients; 85% ischemic stroke and 15% hemorrhagic stroke	RTI: 10% UTI: 12% Other: 1%	Urinary catheterization, age, stroke severity	Infection resulted in worse functional outcomes (GOS and SSS)	[84]
119 patients	RTI: 11% UTI: 2% Other: 6%	N/A	Worse neurological deficit on admission predicts fever	[85]
3865 patients	SAI: 10%	Age, female gender, decreased SSS score	Infection does not affect SSS but does delay discharge	[86]
163 patients from SU	PSP: 12% UTI: 4%	Poor BI score, large MCA infarct, poor GCS	N/A	[87]
124 patients from NICU	PSP: 21%	PSP: mechanical ventilation, multiplelocation and vertebrobasilar stroke, dysphagia, abnormal chest x-ray findings	PSP: worse functional outcome (BI and RS), higher chance of mortality	[88]
14,293 patients with acute stroke	PSP: 7%	PSP: greater stroke severity, general frailty	PSP increasesmortality at 30 days post-stroke	[89]
1455 patients from GAIN International trial	PSP: 13.6% UTI: 17.2%	PSP: age, male gender, diabetes, stroke subtype UTI: female gender, higher NIHSS, age	PSP and UTI independently associated with worse outcome (BI, NIHSS, RS); PSP associated with increased mortality at 3 months post-stroke	[90]
52 patients in SU	SAI: 27.5% -Bronchitis/PSP: 15% -UTI: 5% -PSP+UTI: 7.5%	Age, higher NIHSS score, larger lesion volume (MRI), dysphagia, urinary catheterization	N/A	[91]
663 patients with AIS in California hospitals	PSP: 10% UTI: 13%	Age, atrial fibrillation, CHF	PSP associated with increased mortality risk and worse discharge status	[92]
229 patients with stroke admitted to NICU	SAI: 26%	Tube feeding	SAI not independently associated with worse outcome (NIHSS) at discharge	[93]
439 patients; 91% ischemic stroke and 9% TIA	PSP: 10% UTI: 7% Other: 2%	SAI: age, dependent status before admission, total anterior circulation strokes, hypoxia on arrival, urinary catheterization	SAI predicts increased mortality	[94]
412 patients, (IS: 94.9% HS: 5.1%)	PSP:18.9%	Age, aphasia, higher NIHSS score, cognitive impairment, abnormal water swallow test result	N/A	[95]

Table 1 (continued)

Cohort Studied	Incidence of infection	Risk factors identified	Functional outcomes	Ref.
236 patients with AIS	PSP:22%	PSP: dysphagia, NIHSS, non-lacunar basal-ganglia infarction, other infection present on admission	N/A	[96]
412 patients in urban teaching hospital	UTI:15.8%	Urinary catheterization, post-stroke disability, age	No relationship between infection and NIHSS or mRS scores at 3 months post-stroke	[97]
521 patients with AIS in the Netherlands	SAI: 15% PSP: 7.5% UTI: 4.4	PSP: poor functional outcome	Poor outcome (mRS) at discharge in 88% of infected patients; 47% mortality within 1 year post-stroke	[98]
94 patients with AIS	SAI: 42% - Nonserious: 51% - Serious 49% PSP: 18% Sepsis: 2% UTI: 27%	Age, higher NIHSS score	Infection is a predictor of poor functional outcome (NIHSS)	[99]
133 patients with AIS in Serbian hospitals	SAI: 47.4% UTI: 20.3%	N/A	Infection as predictor of poor functional outcome (mRS) and death 1 year after stroke	[100]
59,558 patients with AIS in Austrian SU	PSP: 5.2%	Age, stroke severity, chronic alcohol consumption, atrial fibrillation	N/A	[101]

AIS: acute ischemic stroke; *BI*: Barthel Index; *CHF*: congestive heart failure; *CRP*: C-reactive protein; *GCS*: Glasgow coma scale; *GOS*: Glasgow outcome system; *IL*: interleukin; *MCA*: middle cerebral artery; *mRS*: modified Rankin Scale score; *MICU*: medical intensive care unit; *MRI*: magnetic resonance imaging; *NICU*: neurological intensive care unit; *NIHSS*: National Institute of Health Stroke Scale; *PSP*: post-stroke pneumonia; *RS*: Rankin scale; *RTI*: respiratory tract infection; *SAI*: stroke-associated infection; *SSS*: Scandinavian stroke scale; *SU*: stroke unit; *TIA*: transient ischemic attack; *UTI*: urinary tract infection

bacterial infections and subsequent mortality, supporting a role for adrenergic signaling in post-stroke immunodepression [102, 106]. Moreover, adrenergic signaling mediates a decrease in proliferation of spleen-derived immune cells after stroke [139, 140]. Treatment of rats with carvedilol (a pan-adrenergic receptor antagonist), prazosin (an α1-receptor antagonist), or propranolol independently prevented the reduction in spleen size, but only carvedilol significantly reduced infarct volume [131]. Together, these results suggest that the splenic response to stroke is mediated through the activation of both α and β adrenergic receptors, which results in an impaired antibacterial immune response following stroke [102].

Additionally, the brain can modulate the gastrointestinal system through SNS hyperactivation following stroke [129, 141]. In various murine stroke models, this results in endothelial cell necrosis, mucosal dissociation, and inflammatory cell infiltration [129, 142]. The loss of mucosal integrity and immune cell dysfunction allows bacterial translocation and increases the risk for systemic infections [143]. SNS activation and increased risk of infection has also been observed in clinical studies [103, 144]. Another mechanism of gut-brain cross-talk is mediated via the microbiome. Microbes in the gut synthesize neurotransmitters, short-chain fatty acids, and bile acids that enter the blood stream

and signal to neurons, astrocytes, and microglia in the CNS [145]. The makeup of microbes in the gut not only influences stroke outcomes, but is also itself altered after stroke [146–148]. Many of the signaling mechanisms between the microbiome are not yet understood, and these studies are not yet translated to human populations. Both are active areas of current research.

Peripheral Immune Activation by Stroke Although post-stroke immunosuppression has been confirmed in both clinical and animal models, brain signaling to the periphery following stroke can also activate the peripheral immune system. Following CNS innate immune activation, secreted chemokines and cytokines not only assist in local immune activation but also signal back through the blood stream to recruit peripheral cells, primarily from the spleen, to the site of injury [149, 150]. This cross-talk between organ systems occurs throughout the body, and it can be detrimental, as severe neurological stress resulting from stroke, traumatic brain injury, or infection can cause multiple organ failure [124]. In humans, there is a long-lasting peripheral immune activation, and the degree of activation is associated with later cognitive decline [151].

Table 2 Clinical studies of prophylactic antibiotics after stroke. A summary of clinical trials, organized by year, that evaluated the efficacy of prophylactic antibiotic treatment in reducing infections rates in stroke patients. Studies were not included if they did not include control treatments. Control is either placebo or conventional therapy

Study name	Drug(s)	Patient cohort	Treatment regimen	Outcomes measured	Results	Ref.
Early Systemic Prophylaxis of Infection After Stroke (ESPIAS)	Levofloxacin	240 patients	IV: 500 mg/100 mL/day or placebo for 3 days	Incidence of infection 7 days post-stroke; NIHSS score and mortality 90 days post-stroke	No effect	[108]
Preventive ANtibacterial THERapy in acute Ischemic Stroke (PANTHERIS)	Moxifloxacin	80 patients	IV: 400 mg/day or placebo for 5 days within 36 h post-stroke	Infection within 11 days; neurological outcome, survival, development of stroke-induced immunodepression, and induction of bacterial resistance	Reduces infection rates	[109]
The Mannheim Infection in Stroke Study (MISS)	Mezlocillin plus sulbactam	60 patients	IV: 2 g mezlocillin and 1 g sulbactam over 20 min; every 8 h over 4 days	Incidence and height of fever; rate of infection and NIHSS over 3 days post-stroke	Decreased body temp, reduced infection rate, and better clinical outcome (NIHSS)	[110]
Prophylactic antibiotics after acute stroke for reducing pneumonia in patients with dysphagia (STROKE-INF)	Amoxicillin or co-amoxiclav with clarithromycin	1217 patients, n = 615 antibiotics, n = 602 control	7 days treatment within 48 h of stroke	PSP within 14 days	No effect	[111]
Preventive Antibiotics in Stroke Study (PASS)	Ceftriaxone	2538 patients total; n = 1268 ceftriaxone, n = 1270 control	IV: 2 g every 24 h for 4 days	mRS at 3 months post-stroke	No effect	[112]

IV: intravenous; *mRS*: modified Rankin Scale; *NIHSS*: National Institutes of Health Stroke Scale; *PSP*: post-stroke pneumonia

In addition to the SNS, the brain and spleen communicate via activation of the hypothalamic–pituitary–adrenal (HPA) axis. Both induce release of catecholamines and steroids that trigger immune cell efflux from the spleen and trafficking to the brain [152]. Cytokines such as IL-1, IL-6, IL-10, and TNF-α are released by both brain resident and peripheral immune cells following stroke, and mediate immune activation [103, 153–155]. For example, IL-1 increases after stroke, and signals to the hypothalamus to release corticotropin-releasing hormone (CRH) into the blood [156, 157]. Subsequently, CRH stimulates secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which circulates to the adrenal glands and induces secretion of glucocorticoids by the adrenal cortex [158–160]. Interestingly, the adrenal gland may also activate peripheral immune responses through humoral mechanisms or catecholamines [103].

Adaptive Immune Cell Roles in Neuroinflammation After Stroke

T Lymphocytes Adaptive immune cells such as B and T lymphocytes also traffic from the blood into the brain after stroke. T cells are heterogeneous and include subsets with various functions—regulatory (Treg), pro-inflammatory (Th1 and Th17), and anti-inflammatory (Th2). Upon entering the brain, their roles are determined by the specific populations of cells that become activated. Although it has been suggested that infiltration of T cells into the brain begins 3–4 days post-stroke, following monocyte and neutrophil infiltration [161], Tregs accumulate within 24 h in rodent stroke models [61, 162, 163].

T cells are a key mediator of acute stroke pathology, and ablating these cells after stroke may be neuroprotective [164]. In Rag1(−/−) mice, a reduction in infarct volume and an improvement in the neurological deficit of wild-type mice occurred 24 h post-injury. This protection was reversed in Rag1(−/−) mice reconstituted with wild-type splenocytes [162]. Inhibition of lymphocyte trafficking results in fewer T cells in the CNS following stroke, and reduced pro-inflammatory cytokine production [162].

T cells can be subdivided into different populations based on surface markers and cytokine expression, which determines their impact following stroke. For example, CD4+ CD25+FoxP3+ T cells proliferate and accumulate in the ischemic brain up to 30 days post injury and promote an anti-inflammatory environment [165]. Additionally, adoptive transfer of Tregs reduces inflammatory responses both intrinsic and extrinsic to the CNS [166]. Moreover, Tregs provide neurovascular protection against stroke by inhibiting peripheral neutrophil-derived MMP-9 production [166]. Thus, Tregs may be promising candidates for cell-based therapies targeting post-stroke inflammatory dysregulation and neurovascular dysfunction. In contrast, CD8+ T cells are first observed in

the brain at 3 h post-stroke and maintain their numbers in the following days. CD8+ effector T cells are likely detrimental in the acute phase of stroke due to pro-inflammatory properties [165]. Thus, a primary role of Tregs is likely to provide neuroprotection by suppressing these effector cells [165].

Interestingly, Treg-mediated neuroprotection might depend primarily on their ability to produce the anti-inflammatory cytokine IL-10. Treg transfer from IL-10-deficient mice into mice with stroke had a minimal effect on recovery, whereas IL-10-producing Tregs reduced stroke damage by antagonizing the pro-inflammatory effects of IFN-γ and TNF-α [167, 168]. Several studies in various disease states have demonstrated that antigen-specificity is necessary for Tregs to produce IL-10, which may further clarify why this cell population is only beneficial in certain microenvironments [2, 169]. In contrast, Treg depletion immediately after stroke may improve stroke outcomes up to 1 week later, as Tregs are adherent to cerebral blood vessels in the hours after stroke, and may contribute to microvascular dysfunction and the "no-reflow" phenomenon [170]. These discrepancies may be due to the different stroke models utilized resulting in different stroke sizes, and different methods of Treg depletion [167, 170]. Ultimately, it is apparent that the immune response is specific to the stroke model utilized and the extent and severity of ischemia accomplished in a given model [171].

Inhibiting specific T cell subtypes associated with detrimental cytokine signaling reduces infarct size [172–174]. Adoptive transfer of Th1- and Th17-type T cells into stroke mice increases infarct volume suggesting that both IFN-γ- and IL-17-mediated responses impact stroke damage [1, 164, 174]. Supporting this, post-mortem sections of human stroke tissue identified IL-17 localization in CD4+ T cells and astrocytes [166]. Th1 cells are major producers of IFN-γ, and potentiate damage by increasing monocyte expression of IFN-inducible protein 10 (IP-10), or CXCL10, which is itself a chemoattractant for activated T cells [162, 175]. Similarly, mice lacking CCL5, which is critical for recruiting and activating T lymphocytes, had smaller infarct volumes compared to wild-type mice, supporting a detrimental role of T cells following stroke [176]. Furthermore, CD4+ T cell–induced IL-21 has been observed in post-mortem human stroke tissue [173]. Inhibition of IL-21 before transient MCAO in mice is neuroprotective, supporting a detrimental role [173]. Overall, these findings highlight the importance of identifying and targeting specific T cell subpopulations in order to develop appropriate therapeutic targets for stroke.

B Lymphocytes After Stroke B cells also infiltrate the brain in the days after ischemic injury. B cell deficiency worsens histological damage and functional outcomes after transient cerebral ischemia [177]. B cell–deficient μMT mice had larger infarct volumes, higher mortality, more severe functional deficits, and increased numbers of activated T cells,

macrophages, microglia, and neutrophils compared to wild-type mice, and this effect was reversed following adoptive transfer of B cells from wild-type mice [177]. In contrast, other studies have not observed any differences in stroke size or severity in μMT mice [178, 179]. Interestingly, when IL-10-deficient B cells were transferred into μMT mice, this effect was not observed, supporting a role for IL-10-producing B cells in stroke recovery [177]. Although regulatory B cells represent only ~ 0.5% of CD19+ B cells, they are an important subset that secrete high levels of IL-10 [180]. There is evidence that early regulatory B cell responses to stroke are beneficial. Regulatory B cells also limit inflammation in animal models of MS via the production of IL-10 [181]. Together, these data suggest that the role of B lymphocytes in cerebral ischemia may be protective rather than pathogenic. It is notable, however, that this effect was observed only 48 h after MCAO, before B cells are typically observed in the brain. In a different study, B cell–deficient mice failed to show improvement against ischemic injury, although this may be because the analysis was performed only 24 h post-injury [162]. It may be that early effects exerted by B cells take place outside the brain, perhaps in lymphatic tissue. CNS antigens can indeed be detected in the cervical lymph nodes of stroke patients within 3 days of stroke in concentrations proportional to the extent of brain damage [182].

Overall, it is clear that all of these inflammatory components (DAMPs, myeloid cells, and lymphocytes) are inter-related, and altering the activity of one population will influence the others. While T cell responses following ischemic stroke can directly affect stroke recovery, they also influence B cell activation. For B cell activation and auto-antibody responses to occur in response to stroke, CNS antigens released from dead and damaged cells must bind the receptor of autoreactive cells that have escaped negative selection. These CNS antigens may reach secondary lymphoid tissue directly, or within antigen-presenting cells that have phagocytosed dead tissue. An impaired BBB may also facilitate direct antigen exit to circulation. Additionally, autoreactive Th cells that have also escaped negative selection must be present to provide a reciprocal secondary signal, and to fully activate the B cell [183]. Furthermore, DAMP-mediated activation of TLRs can increase the expression of co-stimulatory molecules on B cells, to further facilitate activation and autoimmunity [184].

Resolution Versus Chronic Neuroinflammation After Stroke

Resolution of Neuroinflammation Proper resolution of inflammation and subsequent repair require both debris clearance and pro-inflammatory signaling [185]. Phagocytosis of cellular debris by myeloid cells results in the production of cytokines such as TGF-β and IL-10 [186, 187]. While these

cytokines do have pro-inflammatory properties, in the context of inflammatory resolution they suppress Th1 responses and promote Treg development, thereby promoting repair and survival rather than cell death and damage [186, 187]. Similarly, the same cells (astrocytes, microglia, macrophages) that initially secrete pro-inflammatory cytokines transition phenotypically to become critical suppliers of growth factors and other molecules required for neurogenesis, angiogenesis, and neuronal sprouting, among other repair-oriented processes [188]. For example, astrocyte production of vascular endothelial growth factor (VEGF) is essential for angiogenesis, and microglial production of insulin-like growth factor-1 is required for neuronal sprouting following ischemia [188].

Resolving inflammation is an active process, rather than simply a decline in inflammatory signaling. In the brain, one cue for resolution of the inflammatory response is phagocytic removal of apoptotic neutrophils [189]. Interestingly, as in neutrophil activation, it appears macrophages require both integrin-ligand interactions and TNF-α in order to properly induce apoptosis [189]. Thus, proper inflammatory resolution relies on each of these signals, and consequent removal of cytotoxic cells. Unfortunately, however, the initiation of inflammatory resolution is otherwise not well described after stroke.

There may be similar mechanisms to those important for immune resolution in other conditions, such as wound healing. Following tissue damage, glucocorticoid production increases, which can induce macrophages to transition from a pro-inflammatory to an anti-inflammatory phenotype [190, 191]. Moreover, IL-4 secretion by Th2 cells provides an additional signal for macrophages to transition to a phenotype that suppresses inflammation and favors wound healing [192]. The role of these molecules in triggering this transition following ischemia still needs more attention.

Resolving inflammation after stroke is crucial. When resolution does not properly occur, a deleterious chronic condition develops. This happens more frequently after brain ischemia than ischemia in other organs [193], and several mechanisms may be involved. Inflammation may be exacerbated by a failure of neutrophils to undergo apoptosis, or by a failure of macrophages to phagocytose and clear the apoptotic cells or extracellular myelin and cholesterol in a timely manner [188, 193]. Additionally, premature dampening of T cell effector functions may allow inflammatory stimuli to persist, and result in non-resolving inflammation [188]. A chronic condition may then develop and result in further tissue damage.

Post-stroke Dementia and B Lymphocytes Chronic neuroinflammation is associated with cognitive decline and dementia after stroke. Dementia is a significant problem in stroke survivors [194]. Furthermore, stroke not only has the potential to immediately affect cognition due to direct tissue damage in the acute phase, it also worsens the trajectory of cognitive

decline [195]. Much work has been done to understand acute recovery from the initial infarct, but this later chronic stage is less well studied. While initial B cell activation likely occurs in the periphery, the ischemic lesion may become an additional site of B cell activation and auto-antibody production in the weeks after stroke if the immune response does not resolve properly.

In models of permanent distal MCAO, adult wild-type mice with a cortical stroke are cognitively normal at 1 week post-stroke [178, 196]. They then develop a cognitive deficit 8 weeks later [178, 196]. Importantly, the fact that mice are cognitively normal 1 week after stroke suggests that cognitive decline is not a direct effect of damage from the stroke itself. When this same stroke model is utilized in μMT mice which lack mature B cells, or in wild-type mice treated with anti-CD20 to abolish B cells, the cognitive deficit does not develop [178]. Together, these data suggest that B cells are critical to the development of a delayed cognitive deficit following stroke. Interestingly, there are increased numbers of B cells in the brain in approximately two thirds of human stroke survivors who develop dementia after stroke [178].

In both animal models and in humans, antibodies specific for CNS self-antigens can develop following stroke [197–199], although negative studies have also been recently published [200]. The specific B cell isotype is determined by cytokines produced by Th cells [201]. Auto-antibodies of the IgG, IgM, and IgA isotype have all been detected at an increased frequency in the CSF of stroke patients, suggesting an important role for T cell signaling in this pathogenesis [202, 203].

In the months and years following the initial injury, the neuroimmune response may trigger a progressive neurodegenerative process driven by dysregulated adaptive immune responses, and this may be a factor in vascular cognitive impairment. Post-stroke cognitive impairment is indeed very common, impacting 30–50% of stroke survivors [194, 204, 205], but a tight link to autoreactive antibodies against brain proteins, found in both the periphery and the CNS in patients, has not been established. Still, there are a few small studies that strongly suggest a connection.

For example, one study analyzed serum antibodies from a cohort of stroke patients who were subjected to cognitive testing at multiple time points up to 365 days after stroke [206]. It found that 17% of patients had a decline in cognitive ability in the year after stroke, while 83% were stable or improved [207]. Cognitive decline was predicted by antibody titers to anti-myelin basic protein (MBP) but not anti-proteolipid protein (PLP) [207]. Additional clinical studies have identified auto-antibodies against neurofilament, glial fibrillary acidic protein, S100B, MBP, and PLP in plasma and CSF of stroke patients [208–210]. The presence of auto-antibodies in the CNS could be harmful via

Table 3 Clinical trials of immunomodulation after stroke. A summary of clinical trials targeting various aspects of the immune system after stroke. Studies are grouped by general mechanism of action of the pharmacological agent. Studies were not included if they did not have placebo or conventional therapy control arms

Drug; target	Study cohort	Treatment regimen	Outcome evaluation	Trial results	Ref.
Enlimomab: anti-ICAM-1 monoclonal antibody	32 patients with ischemic or hemorrhagic stroke	IV loading dose within 24 h of symptom onset; followed by 4 daily doses ranging from 140 to 480 mg	Risk of adverse events 30 days post-stroke	No change in risk of adverse events with increasing dose	[211]
	625 patients with ischemic stroke enlimomab (n = 317) or placebo (n = 308)	Treatment within 6 h of stroke onset given over 5 days	mRS evaluated at baseline and on days 5 and 90 after initiation of treatment; 6 and 12 months	Worse outcomes	[212]
Fingolimod: S1P analog	23 patients with primary supratentorial ICH	0.5-mg oral dose for 3 days within 72 h of stroke	Stroke volume (MRI), GCS and NIHSS at 7 days and 3 months post-stroke	Drug was safe and well tolerated, reduced stroke volume and improved neurological outcomes at 3 months post-stroke	[213]
	22 patients with AIS; n = 11 fingolimod, n = 11 placebo	0.5-mg oral dose for 3 days over 4.5 h post-stroke	Circulating lymphocyte counts, neurological function (NIHSS) 7 days post-stroke	Safe and well-tolerated, attenuated neurological deficit and promoted recovery	[214]
	22 patients with hemispheric ischemic stroke	0.5 mg for 3 days with alteplase, within 4.5 h of stroke	Circulating lymphocytes, lesion volumes, NIHSS score at 7 days and 90 days post-stroke	Combination therapy was well-tolerated, attenuated reperfusion injury and improved NIHSS scores 90 days post-stroke	[215]
Natalizumab: anti-α4-integrin monoclonal antibody	161 patients with acute ischemic stroke natalizumab (n = 79) or placebo (n = 82)	Administered a single IV infusion up to 9 h after stroke	NIHSS, mRS, BI, SIS-16, and MoCA scores assessed at baseline (5 days post-stroke), 24 h, and days 5, 30, and 90 post-stroke	No change in infarct size; slight improvement in cognition at 90 days post-stroke	[216]
Anakinra: human recombinant IL-1 receptor antagonist	34 patients	100 mg IV loading dose over 60 s, followed by 2 mg/kg/h infusion over 72 h, within 6 h of stroke	NIHSS score; adverse events up to 90 days; 5–7 days infarct volume measured by CT	Drug is safe and well tolerated; improved outcomes at 90 days in patients with cortical stroke	[78]
	80 patients with AIS	Subcutaneous administration of 100 mg twice daily for 3 days within 5 h of stroke	Levels of inflammatory markers by 7 days post-stroke; mRS, survival, and length of stay at 90 d post-stroke	Drug is safe and well tolerated; reduces inflammatory markers in plasma by 3 days post-stroke	[79]
UK-279,276: recombinant glycoprotein with selective binding to CD11b	966 patients with stroke (887 ischemic)	Patients received 1 of 15 doses ranging from 10 to 120 mg treated within 6 h of stroke	SSS score from baseline to 30 days post-stroke	No effect; terminated early; no difference in adverse events	[217]
Fasudil: Rho kinase inhibitor	160 patients with AIS	IV: 60-mg dose over 60 min, twice daily for 14 days within 48 h of stroke	JSS score at end of treatment and mRS score 30 days post-stroke	Improved clinical outcomes at 14 days, and 30 days post-stroke	[218]
Minocycline: semi-synthetic tetracycline antibiotic	152 patients with AIS; n = 74 minocycline, n = 77 placebo	200-mg oral dose for 5 days; 6–24 h post-stroke	NIHSS, mRS, and BI score at 90 d post-stroke	Improved scores on days 7 and 30 post-stroke	[219]
	60 patients with AIS; 41 at 10 mg/kg dose	IV dose of 3, 4.5, 6, or 10 mg/kg daily over 72 h; within 6 h of stroke	Adverse events	Safe and well tolerated	[220]
	139 patients with AIS; n = 69 minocycline, n = 70 placebo	Oral dose 3–48 h post-stroke	NIHSS, mRS, BI score, and vascular outcomes at 90 days post-stroke	No change in functional outcomes	[221]

Table 3 (continued)

Drug; target	Study cohort	Treatment regimen	Outcome evaluation	Trial results	Ref.
Simvastatin: HMG-CoA reductase inhibitor	60 patients with cortical strokes; n = 30 simvastatin, n = 30 placebo	40 mg/day for 1 week; followed by 20 mg/day for 3 months; 3–12 h after stroke	Plasma markers of inflammation, neurological outcome (NIHSS and mRS)	No effect on plasma markers; improved neurological outcomes, but increased rates of mortality and infection	[222]
NXY-059: free radical trapping agent	1722 patients with AIS	72-h IV infusion; 6 h after stroke	mRS and NIHSS score 90 days post-stroke	mRS score improved at 90 days post-stroke; no effect on NIHSS score	[223]
	5028 patients with AIS	IV infusion within 6 h of stroke	mRS sore 90 days post-stroke	No effect	[224]
Tirilazad: lipid peroxidation inhibitor	1757 patients with AIS	Administered within 24 h	GOS and BI scores	Treatment increases risk of death or disability	[225]
Ebselen: antioxidant	302 patients with AIS; n = 151 ebselen, n = 149 placebo	150-mg granules for 2 weeks, within 48 of stroke	GOS, mMS, and BI scores at 1 and 3 months post-stroke	Treatment improves neurological outcomes at 1 month but no difference at 3 months	[226]

AIS: acute ischemic stroke; *BI*: Barthel Index; *CT*: computerized tomography; *GOS*: Glasgow outcome system; *HMG-CoA*: β-hydroxy β-methylglutaryl-CoA; *ICAM*: intracellular adhesion molecule; *ICH*: intracerebral hemorrhage; *IV*: intravenous; *JSS*: Motor System of Japan Stroke Scale; *mg*: milligram; *mMS*: modified Matthew Scale; *MoCA*: Montreal Cognitive Assessment; *mRS*: Modified Rankin Scale; *MS*: Matthew scale of neurological deficit; *NIHSS*: National Institute of Health Stroke Scale; *NMDA*: N-methyl-D-aspartic acid; *SIS-16*: Stroke Impact scale-16; *SSS*: Scandinavian stroke scale

multiple mechanisms, including antibody-mediated cellular toxicity, fixation of complement, inhibition of signal transduction, or direct induction of apoptosis.

Finally, it is unlikely that B lymphocytes act alone in the pathogenesis of immune mediated post-stroke cognitive decline. Innate immune cells, T cells, and even neuronal responses likely also play a role and are present late after stroke. Larger studies with more detailed cognitive testing and longer follow-up periods will be required to determine whether serum and/or CSF auto-antibodies are predictive and causative in post-stroke cognitive decline and dementia.

A working model is that a pro-inflammatory state in the periphery acts as an adjuvant to brain proteins when they are released into the bloodstream post-stroke. To test this, we used single-cell mass cytometry to characterize the peripheral immune response to stroke in the year following stroke, and subsequently correlated this response with cognitive changes [19]. Using Elastic Net regression modelling, the systemic immune response was categorized into three distinct phases: acute, intermediate, and late. The acute phase (2 days post-stroke) was characterized by increased signal transducer and activator of transcription-3 (STAT3) signaling in innate immune cells. The intermediate phase (5 days post-stroke) was characterized by increased CREB signaling in adaptive immune cells. The late phase (90 days post-stroke) was characterized by elevated neutrophil numbers, and IgM+ B cells [19]. When aligned with changes in cognitive function between days 90 and 365 post-stroke, the acute inflammatory phase correlated with post-stroke cognitive trajectories, supporting the idea that the early immune response may influence later adaptive immune response and subsequently alter post-stroke cognition [19]. Further studies involving longer term cognitive testing and immune analyses will be critical in understanding these relationships and translating them into therapeutics.

Immunomodulation as a Potential Stroke Therapy

A variety of strategies have been utilized to target the immune response to stroke, such as antioxidant therapies, statins, and inhibition of leukocyte trafficking (Table 3). These studies in murine models of stroke have yielded conflicting results, as well as limited translational success in human patients in the acute phase. The apparently contradictory results between mouse and human studies are likely due to multiple factors: the heterogeneity in stroke pathogenesis in different murine models, the different time points assessed in each both animal and human studies, the different models of stroke utilized in the laboratory, the multitude of immune cells involved, and

the variety of factors that immune cells can produce. Additionally, most trials were done before it was possible to choose patients based on the volume of stroke core (dead tissue) and tissue at risk (the brain region not functioning due to low perfusion that is potentially salvageable). With modern imaging techniques to select patients, it is possible that some previously tried therapies might be more successful in select subgroups of stroke patients.

Antioxidant treatment in stroke hinges on the idea that free radical production, which occurs during tissue re-perfusion, causes severe tissue damage and subsequent cell death [227]. In rodent models, immediate treatment with ROS-scavenging molecules reduces infarct volume and improves acute recovery within 3 days of stroke [227]. While many of these treatments seem to be safe and well-tolerated in human patients, trials demonstrated at best only minor, short-term improvement but were given later after stroke [223, 226, 228]. In contrast, studies of the lipid peroxidation inhibitor tirilazad resulted in increased mortality and worse outcomes in patients [225]. The reason for this lack of translation is perplexing. Further work is necessary to improve the similarities between animal models and human disease and understand the differences.

Additionally, inhibition of lymphocyte trafficking has received a lot of attention due to promising results in rodent stroke models. Fasudil, a Rho kinase inhibitor, prevented neutrophil and monocyte infiltration into the brain, increased regional cerebral blood flow, and upregulated eNOS activity in endothelial cells in rodent studies [218, 229]. Animal studies have demonstrated efficacy of Fasudil in reducing pulmonary arterial pressure in models of pulmonary hypertension, but effects on systemic blood pressure were not evaluated in clinical trials targeting stroke recovery [230, 231]. While a clinical trial showed minor functional improvement in patients, the short-term study (a 30-day assessment) makes it hard to assess the overall patient benefit, as it is clear that the acute immune response plays a critical role in determining long-term outcomes [218].

In a clinical trial of UK-279,276 (a recombinant glycoprotein that inhibits leukocyte infiltration), no effect on infarct size or overall recovery was observed 90 days after stroke [217]. This is in contrast to rodent studies which observed a reduction in neutrophil accumulation and infarct volume in rats 7 days after stroke [232]. However, these studies did not assess functional outcomes in rodent models. Therefore, if future murine studies are to become translatable to human trials, they should focus on longer term outcomes and functional recovery which are the main endpoints assessed in clinical trials, rather than just a reduction in infarct size.

Antibody-mediated blockade of adhesion molecules on endothelial cells has been utilized to reduce lymphocyte trafficking into rodent brains after stroke. ICAM-1-deficient mice have reduced infarct volume, increased survival, and reduced neurological deficits following transient MCAO [52]. However, a clinical trial assessing the efficacy of a murine ICAM-1 antibody, enlimomab, revealed worsened neurological performance, an increased number of stroke-related deaths, and more adverse occurrences such as infections and fever [212, 233]. These effects are thought to be mediated by an immune response to the murine antibody treatment, however, rather than an effect of blocking ICAM-1.

Likewise, studies of VCAM-1 have yielded conflicting results. Reducing VCAM-1 expression diminished T cell infiltration into the CNS and decreased infarct volume in one rodent study [168]. However, other studies using anti-VCAM-1 antibodies revealed no change in outcomes compared to IgG-treated animals [168, 234]. Unfortunately, none of these studies examine the effects of adhesion molecule inhibition on endothelial cell activation and signaling, as lack of target engagement could explain some of these disparate results. While anti-VCAM1 has not yet been tested in humans, there have been two phase 2 clinical trials of Natalizumab (anti-VLA-4, the ligand for VCAM-1 on lymphocytes). The first 2a trial, ACTION, failed to reduce stroke size but demonstrated safety and a trend towards better 30- and 90-day outcomes [216]. ACTION II was a phase 2b trial aimed at replicating these later favorable outcomes and is not yet published but was reported to be negative [235]. In this case, animal studies utilized shorter drug administration times and yielded mixed results with different animal stroke models [168, 236–239].

Another immunomodulatory agent, the sphingosine-1-phosphate inhibitor Fingolimod, inhibits leukocyte trafficking and is approved for use in multiple sclerosis patients [214]. Both rodent and human studies showed reductions in infarct volumes and neurological recovery; however, the patient trials have been small and were open label [213–215, 240].

In addition to the specific reasons above, there are systematic issues that may be critical to improve in order to develop successful immunomodulatory therapies. Preclinical stroke researchers primarily utilize younger, healthy male mice and give drugs at the time of or 3 h after ischemia, while human trials often choose later time points (Table 3). Animal studies should include both sexes, permanent and transient stroke models, and later dosing time points, and should also consider inter-species differences in immune responses and the effects of anesthetic use [241]. In addition, comorbid conditions are understudied in animals. Common comorbidities in stroke patients include age, obesity, diabetes, and hypertension, and each is independently associated with increased peripheral inflammation, which results in worse outcomes for stroke patients [242–244] and likely would also affect the patient response to immune modulating drugs. Research incorporating these different considerations will hopefully lead to improved translation between preclinical animal and clinical human studies and facilitate therapeutic development.

Future Research Priorities

The vast majority of clinical studies of immunomodulation after stroke have focused on the acute phase, assessing outcomes up to 90 days (Table 3). While this timing matches the timing of assessments in many pre-clinical stroke models, the lack of clinical translation highlights the need for longer term, chronic follow-ups in both the clinic and the laboratory so that we can better understand outcomes following stroke. Additionally, many clinical studies begin treatment within 6 h of stroke onset. While this timing may be appropriate for therapies targeting the innate immune response, it may be detrimental in its inhibition of proper inflammatory resolution. Further work is needed to understand the complexities of immune activation and how it transitions to successful inflammatory resolution after stroke, as well as targeted therapies to promote anti-inflammatory immune cell phenotypes. Additionally, for therapies targeting adaptive immune cells, later treatment in the days/weeks after stroke, when these cells begin accumulating in the brain, may be more appropriate. Finally, pre-clinical models that better mimic the human disease by incorporating both permanent and transient ischemia together, using both sexes and humanized immune responses, and incorporate pro-inflammatory comorbidities such as age, obesity, and diabetes will facilitate clinical translation. Further human studies are also necessary to optimize these changes and lead to a subsequent redesign of human trials that will be better suited to beneficially modulate inflammation after stroke.

Required Author Forms   Disclosure forms provided by the authors are available with the online version of this article.

Abbreviations   *ACTH,* adrenocorticotropic hormone; *ADRβ2,* β2-adrenergic receptor; *AIS,* acute ischemic stroke; *ANS,* autonomic nervous system; *ATP,* adenosine triphosphate; *BBB,* blood–brain barrier; *BI,* Barthel Index; *CHF,* congestive heart failure; *CNS,* central nervous system; *CRH,* corticotropin releasing hormone; *CSF,* cerebrospinal fluid; *CX3CL1,* fractalkine; *DAMP,* damage-associated molecular pattern; *GFAP,* glial fibrillary acidic protein; *GPCR,* G-protein coupled receptor; *HMGB1,* high-mobility box 1; *HO-1,* heme oxygenase-1; *HPA,* hypothalamic-pituitary-adrenal; *Hsp,* heat shock protein; *ICAM-1,* intracellular adhesion molecule-1; *IFN,* interferon; *IL,* interleukin; *IP-10,* interferon-inducible protein-10; *IV,* intravenous; *LFA-1,* lymphocyte function-associated antigen 1; *LPS,* lipopolysaccharide; *MBP,* myelin basic protein; *MCAO,* middle cerebral artery occlusion; *MCP-1,* monocyte chemoattractant protein-1; *MICU,* medical intensive care unit; *MMP,* matrix metalloproteinase; *MoCA,* Montreal Cognitive Assessment; *MRI,* magnetic resonance image; *mRS,* modified Rankin Scale; *NICU,* neurological intensive care unit; *NIHSS,* National Institute of Health Stroke Scale; *PLA,* platelet-leukocyte aggregate; *PLP,* proteolipid protein; *PPAR,* peroxisome proliferator–activated receptor; *PSGL-1,* P-selectin glycoprotein ligand-1; *PSNS,* parasympathetic nervous system; *PSP,* post-stroke pneumonia; *RAGE,* receptor for advanced glycation end products; *ROS,* reactive oxygen species; *SAI,* stroke-associated infection; *siRNA,* small interfering ribonucleic acid; *SNS,* sympathetic nervous system; *STAT3,* signal transducer and activator of transcription-3; *SU,* stroke unit; *TGF,* transforming growth factor; *Th,* T helper cell; *TIA,* transient ischemic attack; *TLR,* toll-like receptor; *TNF,* tumor necrosis factor; *tPA,* tissue plasminogen activator; *Treg,* T regulatory cell; *UTI,* urinary tract infection; *VCAM-1,* vascular cell adhesion molecule-1; *VEGF,* vascular endothelial growth factor; *VLA-4,* very late antigen-4

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