

Neuroinflammation after Intracerebral Hemorrhage and Potential Therapeutic Targets

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Spontaneous intracerebral hemorrhage (ICH) is a catastrophic illness causing significant morbidity and mortality. Despite advances in surgical technique addressing primary brain injury caused by ICH, little progress has been made treating the subsequent inflammatory cascade. Pre-clinical studies have made advancements identifying components of neuroinflammation, including microglia, astrocytes, and T lymphocytes. After cerebral insult, inflammation is initially driven by the M1 microglia, secreting cytokines (e.g., interleukin-1 β [IL-1 β] and tumor necrosis factor- α) that are involved in the breakdown of the extracellular matrix, cellular integrity, and the blood brain barrier. Additionally, inflammatory factors recruit and induce differentiation of A1 reactive astrocytes and T helper 1 (Th1) cells, which contribute to the secretion of inflammatory cytokines, augmenting M1 polarization and potentiating inflammation. Within 7 days of ICH ictus, the M1 phenotype converts to a M2 phenotype, key for hematoma removal, tissue healing, and overall resolution of inflammation. The secretion of anti-inflammatory cytokines (e.g., IL-4, IL-10) can drive Th2 cell differentiation. M2 polarization is maintained by the secretion of additional anti-inflammatory cytokines by the Th2 cells, suppressing M1 and Th1 phenotypes. Elucidating the timing and trigger of the anti-inflammatory phenotype may be integral in improving clinical outcomes. A challenge in current translational research is the absence of an equivalent disease animal model mirroring the patient population and comorbid pathophysiologic state. We review existing data and describe potential therapeutic targets around which we are creating a bench to bedside translational research model that better reflects the pathophysiology of ICH patients.

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Introduction

Spontaneous intracerebral hemorrhage (ICH) is a catastrophic illness representing a global public healthcare problem. Of the 15 million strokes reported worldwide annually, ICH accounts

for approximately 10% to 15% of all stroke cases in the United States, Europe, and Australia; and approximately 20% to 30% of strokes in Asia.¹ This is associated with a 40% 30-day mortality rate,² and those who survive have major neurologic deficits, with only around 20% of patients reaching functional

independence at 6 months.³ Although there has been a significant reduction in overall age-adjusted incidence of stroke, the incidence of ICH has remained unchanged in the past 40 years.² In the United States alone, the annual healthcare costs of ICH is estimated at \$12.7 billion.⁴

Hospitalizations for ICH have increased by 18% in the last decade,⁵ likely due to the aging population and the increasing use of anticoagulants and antiplatelet therapy. The most important risk factor is age. With each advance in decade after the age of 50, there is a 2-fold increase in ICH incidence.⁶ ICH due to hypertension (HTN) accounts for approximately 65% of all spontaneous ICH.^{7,8} ICH in the setting of HTN and associated co-morbid states including diabetes mellitus, coronary and cerebrovascular disease, and renal insufficiency, commonly occurs in the deep cerebral locations (basal ganglia, thalamus), followed by cerebral lobes, cerebellum, and brainstem (predominantly pons).⁶ ICH in the setting of cerebral amyloid angiopathy usually presents in a lobar location. Other causes of ICH include neoplasms, arteriovenous malformations and fistulae, cerebral cavernous malformations, and aneurysms.⁷

Primary brain injury from ICH typically occurs within the first few hours of ictus and is due to hematoma formation, leading to mechanical damage of the adjacent tissues from dissection and compression. Hemorrhagic volume is an important factor in determining ICH outcome, with hemorrhage volumes >100 mL associated with worse prognosis, often with high intracranial pressure, decreased cerebral blood flow and brain herniation.⁹ Location of the ICH is also important for outcome, and while guidelines support the evacuation of cerebellar hemorrhage as potentially life and function saving, thalamic hemorrhage has a notoriously poor outcome despite treatment.¹⁰ Although recent minimally invasive surgical approaches to hematoma removal while limiting surgical morbidity are underway with promising results, this does not address the secondary brain injury following ICH.¹¹⁻¹⁵

Complex immune and inflammatory cascades occur in response to acute cerebral insults, including ICH.¹⁶ Microglia, astrocytes, and T lymphocytes work in concert to be key modulators of the inflammatory response both in ischemic stroke and ICH. Based on a growing body of evidence, activated microglia appear to be the main source of cytokines, chemokines, prostaglandins, and other immunomodulatory molecules that can potentiate or attenuate secondary brain injury and are involved in subsequent brain repair mechanisms.¹⁶ In order to identify potential therapeutic targets and effectively treat the inflammatory sequelae of ICH, we need to better understand the timing of the inflammatory response and the interplay between the cellular and molecular components in post-ICH secondary

brain injury and repair.

This review will summarize the recent body of evidence describing the inflammatory cell populations and molecular signaling following ICH. As will be discussed, there are several targets that could prove efficacious in the amelioration of secondary brain injury after ICH. However, a common challenge in much of the current translational research is the absence of an equivalent disease animal model. Most of the current data is from healthy, young male animal models that do not represent the older age, sex, and comorbidities of the patient population affected by ICH. By better understanding the existing body of literature, we can create a comprehensive bench to bedside translational research model that would better reflect the pathophysiology and affected population of ICH.

Neuroinflammation

Secondary injury, such as cerebral edema, plays a significant role in the neurologic decline of ICH patients.¹⁷ This is triggered by the presence of intraparenchymal blood, which activates cytotoxic, excitotoxic, oxidative, and inflammatory pathways (Figure 1).¹⁸ There is an immediate inflammatory response after the presence of blood components are detected within the parenchyma, characterized by the mobilization and activation of inflammatory cells. It is believed that the microglia and astrocytes are the earliest of the inflammatory cells to respond to extravasated blood.¹⁹ The activation of microglia leads to infiltration of various circulating immune cells, most notably macrophages and T cells. This subsequently leads to the release of inflammatory cytokines (e.g., interleukin-1 β [IL-1 β] and tumor necrosis factor- α [TNF- α]), chemokines, free radicals, and other potentially toxic chemicals that are coordinated via the transcription factor nuclear factor- κ B (NF- κ B).¹⁶ NF- κ B targets include the genes of cytokines, chemokines, various adhesion molecules, cell surface receptors, and inflammatory enzymes (e.g., inducible nitric oxide synthase [iNOS], cyclooxygenase-2 [COX-2], phospholipase A2 [PLA2]).²⁰ These chemicals, together with cell death products, further activate resident and migrating lymphocytes, leading to increased infiltration of lymphocytes and a continued cycle of inflammatory response. There is increasing evidence that this inflammatory response contributes to the formation of edema through increased blood brain barrier (BBB) permeability around the hematoma, which exacerbates mass effect, augments the cell death process through secondary ischemia, and further produces inflammatory insults to the surrounding brain tissue.²¹ Although it would be appropriate to present the inflammatory cascade in a temporal fashion, it is presently difficult to do given the current incomplete

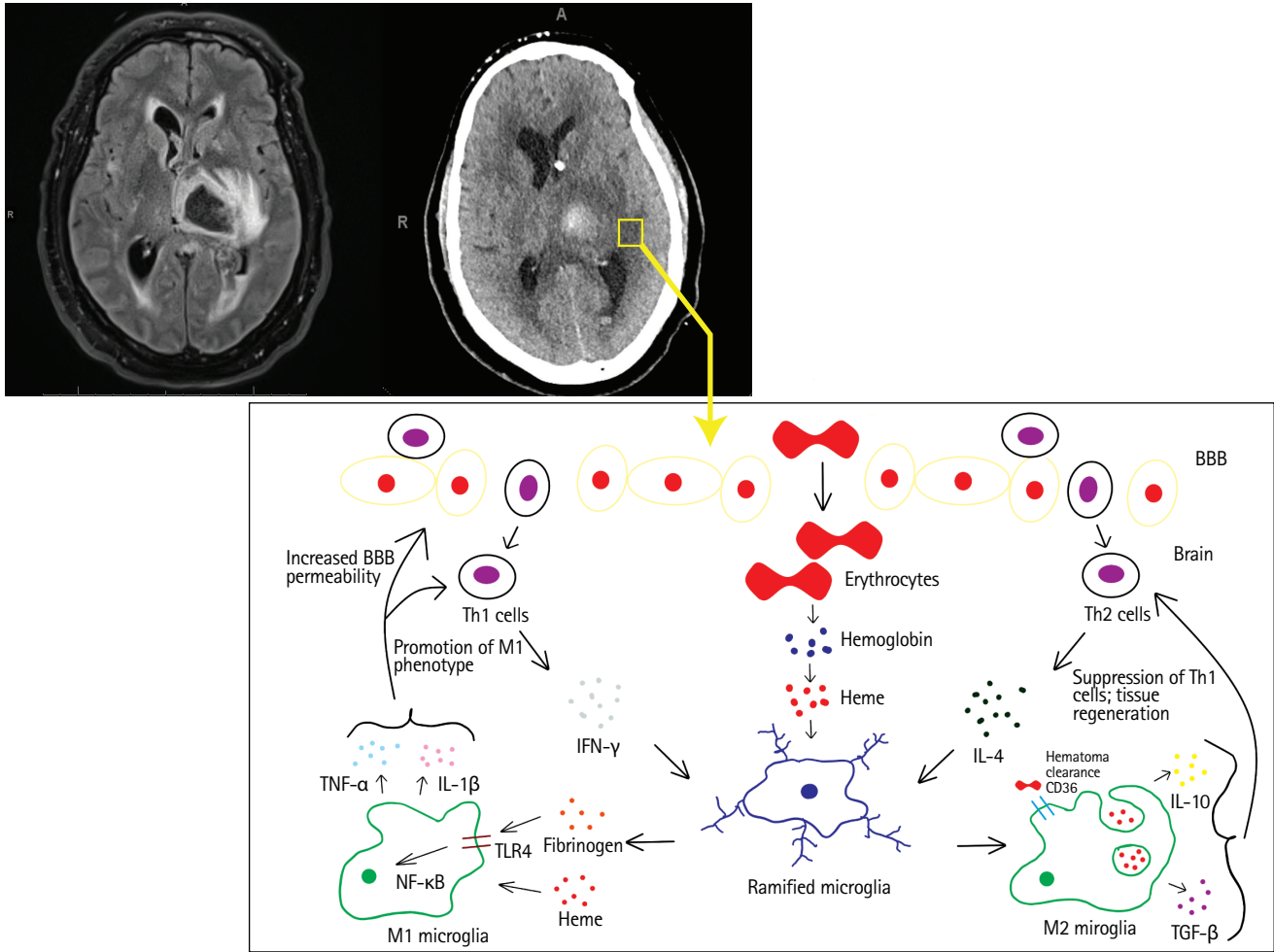


Figure 1. Magnetic resonance imaging fluid attenuated inversion recovery (left) and computed tomography (right) illustrating cerebral edema surrounding the primary intracerebral hemorrhage. Cerebral edema is the result of the ongoing inflammatory response occurring in the presence of the breakdown products of the hematoma. The initial pro-inflammatory phase involves the activation of M1 microglia, upregulating the production of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and other inflammatory products that are involved in potentiating cellular damage and the breakdown of the blood brain barrier (BBB). Once the M2 microglia are activated, there is release of anti-inflammatory cytokines IL-4, IL-10, and transforming growth factor-β (TGF-β), all involved in the regeneration of tissue and overall resolution of neuroinflammation. IFN-γ, interferon-γ; Th, T helper.

understanding of the mechanism. Instead, we will focus on three cellular components of the innate and adaptive immune systems as it relates to the two phases of inflammatory response associated with injury: the pro-inflammatory/cytotoxic phase (M1 microglia, A1 astrocytes, T helper 1 [Th1] and Th17 cells) and the anti-inflammatory/neuroprotective phase (M2 microglia, Th2 and T regulatory (Treg) cells) (Figure 1).

Cellular components of the innate immune system

Microglia

Microglia are the resident macrophages of the central nervous system (CNS) and account for approximately 5% to 20% of the total CNS glial population.²² They continually surveil the brain for infection and damage; and play an important role in the-

inflammatory and recovery phases of injury. In the non-activated state, they move frequently with intermittent extension of fine processes to assess and sample the microenvironment.²³ They quickly activate to respond to changes in their environment induced by factors secreted by neurons and astrocytes. Once activated, they gather around the site of damage or pathogen, develop stout processes, and become highly phagocytic.²¹

Microglia are vital in maintaining homeostasis of the CNS, including clearance of pathogens, damaged CNS tissue, and remodeling of the extracellular matrix and synapses.²⁴ Microglia also play a critical role in overall brain development and adult learning (activity-triggered synaptic plasticity).²⁵⁻²⁷ Following activation, microglia appear morphologically indistinguishable from infiltrating macrophages. However, recent studies revealed a receptor specific to microglia; P2Y12, an ad-

enosine diphosphate/adenosine triphosphate (ADP/ATP)-responsive G-protein coupled receptor, can be found on the resting, ramified processes of surveillant microglia.^{28,29} Extracellular ATP, when released from damaged neurons, acts as a danger signal, and upon binding with microglial P2Y12, causes migration of microglia to the site of injury.^{30,31}

Once tissue damage occurs, microglia can be activated within minutes via neurotoxins released into the extracellular space by necrotic neurons.³² By 72 hours, microglia/macrophage numbers have reached their peak.¹⁹ By day 7, the numbers begin to reduce, and by 21 days, the microglia/macrophages have returned to baseline numbers (Figure 2).³³

Based on ischemic injury and traumatic brain injury models, two microglia phenotypes have been identified. The M1 phenotype is considered pro-inflammatory/injury-inducing and is

known to produce inflammatory cytokines (IL-1 β , IL-12, IL-23, and TNF- α), chemokines, nitric oxide (NO), and reactive oxygen species which initiate breaking down the BBB.^{32,34} Their macrophage markers include CD86, CD16, major histocompatibility complex (MHC) II, and iNOS (Table 1).²⁴ The M2 phenotype is considered to be anti-inflammatory and is generally involved in phagocytosis, release of trophic factors, and the overall regeneration of tissue and resolution of inflammation.³⁵ The presence of anti-inflammatory cytokine IL-4 can prime the polarization to the M2 phenotype, leading to the production of IL-10 and upregulation of surface phenotype markers arginase-1 (Arg-1), CD206, Ym-1, and CD36 (Table 2).³⁵

In ischemic stroke murine models, it has been noted that both phenotypes are present at the site of injury, with a transient initial M2 activation giving way to a predominance of the M1 phenotype within the first week of injury.³⁶ Upregulation of M1-related genes (iNOS, CD11b, CD16, CD32, and CD86) is seen from day 3 to 14 post-stroke. Conversely, mRNA expression for M2 markers (e.g., CD206, Arg-1, Ym-1/2, IL-10, transforming growth factor- β [TGF- β]) is observed on day 1, peaks around day 3 to 5, and returns to pre-injury levels by day 14.³⁷ In contrast to ischemic stroke, ICH murine models have demonstrated a rapid increase in M1 microglia within 6 hours of ictus that slowly decreases over 14 days, whereas the M2 phenotype was found at low levels at day 1 and increased over 14 days.^{38,39} Following ICH, there is a M1 to M2 phenotype switch occurring within the first 7 days, but the exact timing and driving force of the phenotype change remains unclear.³⁵ The microglia have a great deal of plasticity, allowing variation between phenotypes based on cues from the microenvironment.^{36,40}

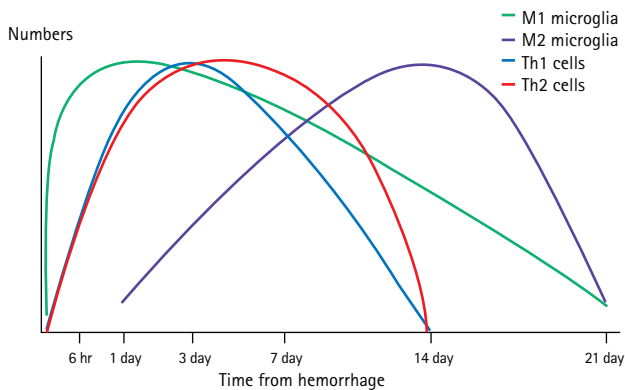


Figure 2. Timeline of inflammatory cells after intracerebral hemorrhage. These timelines are based on young, healthy male animal models with data based on normal immune responses. When accounting for age and comorbidities, the timing and activation of different immune cells may differ in the target population of patients with intracerebral hemorrhage. Th, T helper.

Table 1. M1 microglia functions

Phenotype	Molecule	Type	Role
M1	IFN- γ	Cytokine	Pro-inflammatory, induces M1 phenotype
	IL-1 β , IL-6, IL-23, TNF- α	Cytokine	Pro-inflammatory
	IL-12	Cytokine	Implicated in the differentiation to Th1 cells
	IL-17	Cytokine	Pro-inflammatory, modulating factor of cytokine production
	ROS, iNOS	Metabolic enzyme	Oxidative damage
	CD11b, CD16, CD32	Surface receptors	Phagocytosis, chemotaxis
	CD68	Surface receptor	Scavenger receptor
	CD86	Surface receptor	Surface receptor interacting with CD28
	MHCII	Surface receptor	Presentation of extracellular proteins
	NF- κ B	Transcription factor	Involved in the modification of the M1 phenotype
	STAT1, STAT3	Transcription factor	Promote the expression of NF- κ B/p65

IFN- γ , interferon- γ ; IL, interleukin; TNF- α , tumor necrosis factor- α ; Th1, T helper 1; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase; MHCII, major histocompatibility complex II; NF- κ B, nuclear factor- κ B; STAT, signal transducer and activator of transcription.

(1) Classically activated microglia (M1 phenotype)

Classically activated microglia/M1 cells have been strongly associated with worsening cerebral edema surrounding ICH seen on radiography.⁴¹ Upon activation, M1 cells transiently develop a primarily phagocytic function to clear necrotic neurons and cellular debris to diminish the deleterious release of inflammatory cytokines and chemoattractants.⁴² However, as the number of M1 cells increases, the phagocytic ability appears to significantly decrease, and there is increased secretion of inflammatory cytokines, chemokines, and other neurotoxic mediators (e.g., NO), leading to widespread cellular damage.³⁵ Additionally, proteolytic enzymes (e.g., metalloproteinases and collagenases) lead to the breakdown of the extracellular matrix and cellular integrity.⁴³ In the ramified resting state, microglia play a role in angiogenesis and development of the BBB.⁴⁴ Following insult, oxidative stress from activated microglia likely plays an important role in the impairment of the BBB.⁴⁴ Other suggested mechanisms of BBB hyperpermeability in the setting of ICH include the excessive production of NO induced by the presence of hemoglobin⁴⁵ and thrombin-induced injury via the activation of proteinase activated receptor-1 (PAR-1) mechanisms.⁴⁶ In the setting of increased BBB permeability, the secretion of M1-specific chemokines allow infiltration and recruitment of hematogenous leukocytes that perpetuate the inflammatory response, aggravating neuronal death induced by excitotoxicity or oxygen-glucose deprivation.⁴⁷ Additionally, M1 microglia can also create microglia-T cell crosstalk via antigen presentation through the M1 surface MHCII expression.⁴⁸

M1 microglia are activated following hemorrhage via dam-

age-associated molecular pattern molecules (DAMPs), including the high mobility group box 1 (HMGB1), family of extracellular peroxidoreductin proteins, galactin-3, other danger signals such as hemoglobin, heme, and methemoglobin.^{23,49} These are sensed by the family of Toll-like receptors (TLR), present at low levels on ramified microglia and vigorously upregulated during activation.⁵⁰ Upregulation of TLR4 and subsequent activation of the TLR4-mediated NF- κ B pathway have been seen in ICH animal models^{51,52} and are associated with poor outcomes.⁵³ Once TLR4 binds to its ligands, it interacts with the adaptor proteins myeloid differentiation primary response protein (MyD88) and TIR-domain-containing adapter-inducing interferon- β (TRIF) in order to activate two parallel signaling pathways that initiate the activation of transcription factors to regulation pro-inflammatory cytokine genes. The MyD88 pathway is crucial for the activation of the NF- κ B pathway,⁵⁴ which occurs within 15 minutes of ICH ictus⁵⁵ with subsequent upregulation of downstream target genes of IL-1 β , TNF- α , and heme oxygenase-1.²⁰

(2) Alternatively activated microglia (M2 phenotype)

In the face of anti-inflammatory cytokines, specifically IL-4, IL-10, and IL-13, microglia can undergo alternative activation to the M2 phenotype, resulting in hematoma/debris removal, healing (extracellular matrix deposition), neurogenesis/angiogenesis, and neuroprotection, which correlates with resolution of cerebral edema and neurologic improvement. The M2 phenotype can be broken down into subtypes M2a, M2b, and M2c, each with their unique biologic functions and cell surface markers.⁵⁶ IL-4 and IL-13 can induce downstream processes

Table 2. M2 microglia functions

Phenotype	Molecule	Type	Role
M2	IL-4	Cytokine	Anti-inflammatory, induces M2 phenotype
	IL-10	Cytokine	Anti-inflammatory, human cytokine synthesis inhibitory factor
	TGF- β	Cytokine	Regulation of lymphocyte proliferation, differentiation, and survival; regeneration; upregulate Bcl-2 and Bcl-x1
	Ym-1	Protein	Binds heparin/heparin sulfate, possibly prevents the degradation of extracellular components
	Arg1	Protein	Downregulation of nitric oxide synthesis. Induces fibrosis and tissue regeneration
	FIZZ1	Protein	Associated with reconstruction of extracellular matrix
	CD36	Surface receptor	Scavenger receptor involved in hematoma resorption in setting of upregulation of PPAR- γ
	CD163	Surface receptor	Scavenger receptor for haptoglobin-hemoglobin complex
	CD206	Surface receptor	Antigen internalization and processing/mannose receptor type 1
	PPAR- γ	Transcription factor	Induce M2 phenotype
	STAT6	Transcription factor	Inhibit the activation of NF- κ B
	SOCS3	Signaling modulator	Cytokine-inducible negative regulators of cytokine signaling

IL, interleukin; TGF- β , transforming growth factor- β ; Bcl-2, B-cell lymphoma 2; Bcl-x1, B-cell lymphoma-extra large; Arg1, arginase 1; FIZZ1, found in inflammatory zone 1; PPAR- γ , peroxisome proliferator-activated receptor- γ ; STAT6, signal transducer and activator of transcription 6; NF- κ B, nuclear factor- κ B; SOCS3, suppressor of cytokine signaling 3.

with anti-inflammatory properties, such as Arg-1 upregulation (important part of wound healing and matrix deposition), NF- κ B isoform inhibition (leading to inhibition of the M1 phenotype),⁵⁷ and increased production of scavenger receptors, such as CD36 via upregulation of the transcription factor peroxisome proliferator-activated receptor γ (PPAR- γ), a key component of phagocytosis of extravasated erythrocytes and overall hematoma resorption.⁵⁸⁻⁶⁰ These functions have been described as the M2a activation, the opposite of the M1 phenotype with described roles in suppression of inflammation⁶¹ and contribution to cellular regeneration.⁶² A second phenotype, M2c, becomes active when exposed to IL-10, TGF- β , and glucocorticoids, which is involved in remodeling (via removal of dysfunctional synapses to allow formation of new functional synapses⁶³) and matrix deposition once inflammation is downregulated.³⁵ A third M2 sub-phenotype, M2b, has been described in the last stage of injury and is the least understood. It appears to have both pro-inflammatory and anti-inflammatory properties, but most closely resembling the M1 phenotype. They lack the typical M2 markers such as Arg-1, Ym-1, or found in inflammatory zone 1 (FIZZ1), but express the usual anti-inflammatory cytokine IL-10.^{35,56,62} They also appear to have higher levels of MHCII and CD86, suggesting they have retained the ability to stimulate T cells and initiate the M2 response.⁶⁴ M2b activation also leads to the recruitment of regulatory T cells.⁶⁵

Recent studies have described a family of transcription factors known as signal transducer and activator of transcription (STAT) that serve as crucial signals to achieve M1 or M2 polarization.⁶⁶ Generally speaking, the STATs are activated by Janus kinase (JAK) and inhibited by feedback inhibitors called suppressor of cytokine signaling (SOCS). STAT1 increases M1 polarization in the presence of interferon- γ (IFN- γ).⁶⁷⁻⁶⁹ Conversely, STAT6 helps establish the M2 phenotype once activated by IL-13 and IL-4.^{70,71} The functions of STAT3 are more diverse and appear to have both pro- and anti-inflammatory roles^{66,72} through M1 polarization in the setting of IL-6⁷³ and M2 polarization in the setting of IL-10.⁷⁴

Because of the anti-inflammatory function of M2 microglia, there have been efforts to find pharmacologic agents that could inhibit M1 activation, driving microglia towards the M2 phenotype. Current agents under investigation include the hypoglycemic drug class thiazolidinediones (rosiglitazone and pioglitazone), which act as PPAR- γ receptor agonists. In support of this approach, preclinical autologous ICH models have demonstrated thiazolidinedione-mediated decreases in the production of pro-inflammatory factors, driving M2 activation.⁷⁵ Experimental treatment in these models has shown upregulation of CD36 expression with improved hematoma resolution, de-

creased pro-inflammatory gene expression, and reduction in subsequent neuronal damage.⁶⁰ Another agent under investigation is the tetracycline antibiotic minocycline. In *in vitro* models, minocycline was found to inhibit the upregulation of NF- κ B, indicating selective inhibition of M1 activation without affecting the expression of M2 markers.⁷⁶ Minocycline also reduces the early upregulation of pro-inflammatory factors such as TNF- α and matrix metalloproteinases (MMPs).⁷⁷

Astrocytes

There is emerging evidence that there is another key cellular component of neuroinflammation. Astrocytes are abundant within the CNS and perform the following key functions: (1) neurotrophic support; (2) synaptogenesis and maintenance of these synapses; (3) synaptic pruning; and (4) control of BBB functions, including bidirectional control of blood flow and neuronal function and restricting the entry of immune cells.⁷⁸ Activated microglia induce the activation of astrocytes and will determine their functional fate, either neuroprotective or detrimental for neuronal function. Conversely, astrocytes also secrete pro-inflammatory (e.g., IL-1 β , IL-6) and anti-inflammatory cytokines that can control the activation and functions of microglia.⁷⁹ The pro-inflammatory state induced by M1 microglia, the A1 astrocytes, can lead to the upregulation and expression of pro-inflammatory cytokines, chemokines, and growth factors.⁸⁰ This results in abnormal neurotransmitter synthesis and release, synaptic destruction, and loss of other homeostatic functions, specifically, the failure to promote neuronal growth and survival and the release of neurotoxins, leading to apoptosis of neurons and surrounding oligodendrocytes.⁸¹ It has been hypothesized that there is an A2 astrocyte state that is induced under ischemic conditions, encouraging upregulation of neurotrophic factors and the secretion of proteins promoting synaptogenesis.⁸²

Astrocytes have been observed to accumulate in the perihematomal region within 1 to 3 days after ICH ictus.⁸³ There are several mechanisms by which reactive A1 astrocytes may be detrimental in the setting of ICH. The presence of hemoglobin in the brain parenchyma is a powerful trigger of oxidative stress, which then induces MMP-9 on astrocytes.⁸⁴ MMPs have been implicated in blood barrier injury and subsequent cerebral edema in ICH.⁸⁵ Another consequence of blood extravasation on astrocytes is the presence of thrombin in the extracellular matrix, which also induces cerebral edema after ICH⁷ and leads to the activation of PAR-1, which can be primarily found at perisynaptic endfeet of the astrocyte.⁸⁶ In addition to causing further cerebral injury, PAR-1 on astrocytes causes rapid remodeling of synapses, resulting in migration of astrocytic pro-

cesses away from excitatory glutamatergic synapses.⁸⁷ This reduction of glutamatergic receptors in the setting of astrocytic PAR-1 impairs long-term plasticity.⁸⁷ This particular detrimental mechanism may be involved in the cognitive impairment that is seen after ICH. Although we have evidence that suppression of astrocytic activity decreases hematoma volume, BBB destruction, and improves neurologic outcomes,⁸⁸ therapeutic targets as it relates to astrocytes have not been explored and warrant further investigation.

Cellular components of the adaptive immune system

T lymphocytes

T lymphocyte functions have been well-characterized in ischemic stroke models,⁸⁹ but their role in ICH is less defined. As such, much of our understanding of T cell function in ICH is extrapolated from pre-clinical ischemic models. In the healthy brain, there are very low numbers of T cells functioning in CNS surveillance.⁹⁰ Those present appear to enter the cerebrospinal fluid through choroid plexus veins or meningeal blood vessels to monitor the subarachnoid space, allowing for initiation of a local immune response or return to secondary lymphoid organs.⁹¹ Following stroke, immunohistochemistry has identified different T cell subtypes invading the infarcted region. It is theorized that activated T cells can cross the BBB once a T cell response is activated in the presence of a CNS autoantigen in the lymphoid organs.⁹² In the ischemic stroke model, the influx of CD4⁺ and CD8⁺ T cells are thought to play a detrimental role in perpetuating ischemic tissue injury by contributing to the microvascular dysfunction caused by cerebral ischemia/reperfusion injury.⁹³ Models of either CD4⁺ or CD8⁺ deficiency resulted in decreased stroke volume and improved neurologic performance.^{18,94} Alternatively, some types of T cells can play a neuroprotective role, such as regulatory T cells following ischemia, which promote recovery via their immunomodulating properties.^{95,96}

Temporally, there have been reports of an increase in CD8⁺ cytotoxic T cells as early as 24 hours after ICH, peaking after 2 to 7 days.⁹⁷ There is an increase in CD4⁺ Th cells approximately 3 to 4 days after a hemorrhagic event.⁹⁸ Guo et al.⁹⁹ confirm the presence of Th cells in the perihematoma region in surgical specimens of patients with ICH. These data support the radiographic findings of cerebral edema, with peak swelling days 3 to 7 after ICH.

Th cell differentiation in CNS injury and neuroregeneration has been an area of extensive research. In the 1980's, two subtypes of CD4⁺ T cells were described based on their cytokine secretion patterns: Th1 and Th2 cells (Table 3).⁹⁰ Th1 cells are

programmed to fight intracellular pathogens by supporting cell-mediated responses, including cytotoxic T cells and natural killer (NK) cells.¹⁰⁰ Th1 cell differentiation is initiated by IL-12 and IFN- γ , known to perpetuate pro-inflammatory reactions. These cells are typically characterized by C-C chemokine receptor type 5 (CCR5), C-X-C motif chemokine receptor 3 (CXCR3), and T-bet markers.¹⁰¹ T-bet is the primary transcription factor to determine the Th1 phenotype by upregulating the production of IFN- γ and plays an important role in suppressing differentiation to Th2 and Th17 cell types.¹⁰²⁻¹⁰⁴ Additionally, there is an IL-12 induced STAT4 pathway that promotes Th1 differentiation. These 2 pathways work in tandem rather than in a sequential fashion.¹⁰² Although the Th1 cells are known to be present within the perihematoma region, their role in the pathogenesis of ICH-induced secondary injury remains to be defined. It is theorized that these cells promote vascular permeability and may induce apoptosis of neurons via production of inflammatory cytokines.⁹² Th2 cells function to fight extracellular pathogens via the humoral response and are critical for wound healing and repair¹⁰⁵ following the time course of radiographic evolution of edema from post-bleed day 7 onward. Their differentiation is typically initiated by IL-4 and IL-2, and they secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, which are involved in various anti-inflammatory functions. Th2 cells can be identified by CCR3, CCR4, chemoattractant receptor-homologous molecule (CRTH2), and transcription factor GATA binding protein 3 (GATA3) expression.¹⁰¹ STAT6 is the primary transcription factor involved in the Th2 cell differentiation through upregulation of GATA3.¹⁰⁶ It is presumed that Th2 cells act by producing the above cytokines to down-regulate, and in some cases, directly suppress Th1 cell activation.⁹² Given the delayed infiltration of Th cells,⁹⁸ it is postulated that they directed the later stages of the immune response after ICH, fostering either a highly inflammatory microenvironment or a regenerative microenvironment. However, we currently lack the preclinical evidence for the exact role of these T-helper subtypes.

Another set of functionally opposing lymphocytes involved in the inflammatory response in ICH is Th17 and Treg cells. This set of cells has a reciprocal relationship in the promotion and suppression of inflammation. Th17 cells differentiate in the presence of IL-6, IL-21, IL-23, and TGF- β . These cells express CCR6 and ROR γ t markers and secrete IL-17, IL-21, and IL-22. Th17 cells have been strongly linked to various autoimmune diseases (e.g., multiple sclerosis [MS]) via production of IL-17 and IL-22. It is theorized that a key pathophysiologic mechanism is the disruption of the BBB via binding of cytokines secreted by Th17 cells to IL-17 and IL-22 receptors found on endothelial cells, allowing for the further recruitment and migration of Th 17 cells

Table 3. T lymphocyte populations and functions

Phenotype	Molecule	Type	Role
Th1	IL-12, TNF- α	Cytokine	Activates macrophages, responsible for cell-mediated immunity and phagocyte-dependent protective responses
	T-bet	Transcription factor	Induces Th1 phenotype, enhances production of IFN- γ and mediates repression of IL-4 expression
	STAT1	Transcription factor	Primarily regulates T-bet and mediates IL-4 repression to mediate Th1 cell commitment
Th2	IL-4, IL-5, IL-10, IL-13	Cytokines	Responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, providing phagocyte-independent protective responses
	GATA3	Transcription factor	Key for Th2 cytokine production; inhibition of Th1 responses
	STAT3	Transcription factor	Required by STAT6 for interaction with appropriate gene loci
	STAT5	Transcription factor	Independent of IL-4 signaling; coordinates with GATA3 to induce Th2 phenotype
	STAT6	Transcription factor	Upregulates expression of GATA3 and mediates Th2 cell phenotype
Th17	IL-17A/F, IL-21, IL22, IL-23, TGF- β	Cytokines	Creates inflammation and tissue injury in autoimmune diseases; unclear of function in ICH
	ROR γ t	Transcription factor	Master regulator to induce Th17 phenotype via STAT3 activation
	STAT3	Transcription factor	Activated by IL-6 and IL-23 to induce expression of ROR γ t
Treg	IL-2, IL-10, TGF- β	Cytokines	Essential for the maintenance of peripheral tolerance, prevention of autoimmune disease, and limiting chronic inflammatory disease
	Foxp3	Transcription factor	Major lineage-specific transcription factor involved in Treg differentiation
	STAT5	Transcription factor	Enhances Foxp3 expression to promote Treg development

Th, T helper; IL, interleukin; TNF- α , tumor necrosis factor- α ; IFN- γ , interferon- γ ; STAT, signal transducer and activator of transcription; GATA3, GATA binding protein 3; TGF- β , transforming growth factor- β ; ICH, intracerebral hemorrhage; ROR γ t, retinoic acid receptor-related orphan receptor γ t; Foxp3, forkhead box P3; Treg, T regulatory.

and other pathogenic cell types, including Th1 cells.¹⁰⁷ However, the actual role of Th17 cells in the pathophysiology of post-ICH neuroinflammation remains unknown. Th17 cells express a high degree of plasticity, allowing for functional adaptation and phenotypic switching to Treg cells depending on the inflammatory milieu. TGF- β alone can induce the differentiation of Treg cells, or conversely, in combination with IL-6 or IL-21, can lead to the differentiation of Th17 cells.¹⁰⁸

Treg cells are known for their anti-inflammatory function by mediating immune tolerance to self-antigens. Existing data suggest that Treg cells are beneficial in the context of stroke, both ischemic and hemorrhagic. Depletion of Treg cells led to increased brain injury and neurologic deterioration⁹⁵ and boosting the presence of Treg cells decreased ICH-induced inflammatory injury.⁹⁶ In a rat ICH model, Treg transfusion resulted in the inhibition of IL-1 β , TNF- α , and MMP-2 in the perihematomal tissue and increased the presence of the anti-inflammatory cytokines IL-4, IL-10, and TGF- β .¹⁰⁹ Transfused Treg cells decreased perihematomal edema and BBB permeability, leading to improved short- and long-term neurologic function.¹¹⁰

It is clear that the function of microglia and T cells are intertwined, i.e., they have the potential to cross-regulate the differentiation and phenotype of the other population.^{49,102} This

occurs largely through the production of cytokines. M1 cells produce TNF- α , IL-6, IL-12, and IL-23,¹¹¹ which can recruit and induce the differentiation of Th1 and Th17 cells. In a positive feedback loop, Th1 cells secrete IFN- γ and Th17 cells secrete IL-17, continuing the cycle of augmenting M1 polarization and potentiating the inflammatory response.¹¹² Similarly, M2 cells secrete IL-4, IL-10, and TGF- β , which drives differentiation into Th2 and Treg cells. As a result, M2 polarization is maintained by the production of IL-4 and IL-10 by Th2 cells and IL-10 and TGF- β by Treg cells. This results in downregulation of the inflammatory response and promotes tissue repair.¹⁰¹ In a murine autologous injection ICH model, Treg cells have been seen to modulate microglia activation and polarization with decreased expression of TNF- α , IL-1 β , MMP-2, pro-inflammatory cytokines and chemokines necessary for the activation of microglia, particularly the M1 phenotype.¹¹³ Similarly, boosting the Treg population increased the presence of M2 associated markers (TGF- β and IL-10) with a resultant increase in CD206 expression, indicating increased conversion to M2 phenotype of microglia, therefore ameliorating ICH-induced inflammatory injury.¹¹⁴

Other cells of the adaptive immune system

To date, there is very little literature regarding the pathophysiology

ologic role of B cells and NK cells after ICH. It has been observed there is minimal CNS infiltration of these 2 cell types after ICH,¹¹⁵ indicating a minor role in the neuroinflammatory process after ICH.

Programmed death-1 pathway

Programmed death-1 (PD-1) is a member of the CD28 family of T cell regulators and functions as a negative regulatory system to fine-tune T and B cell activity and plays a critical role in the maintenance of central and peripheral immune tolerance. The PD-1 pathway is essential for the maintenance of peripheral T cell tolerance and is crucial in the attenuation of autoimmunity and preservation of T cell homeostasis. It is expressed at low levels on naïve T cells, and can be induced in most immune cells upon activation.¹⁸ PD-1 binds 2 ligands, programmed death-ligand 1 (PD-L1-CD274) (found on T cells, B cells, dendritic cells, NK cells, macrophages, monocytes, and non-hematopoietic cells) and PD-L2 (CD273). PD-L1 expression is upregulated in the presence of several pro-inflammatory factors, including TNF- α , types I and II IFNs, granulocyte-macrophage colony-stimulating factor, vascular endothelial growth factor, IL-4, and IL-10, of which IFN- γ is the most potent.¹¹⁶

The PD-1/PD-L1 ligation complex mediates immune suppression via several mechanisms. First, the PD-1 complex can lead to the inactivation of T cells. The presence of PD-L1 on antigen presenting cells (APCs) is key in the induction and maintenance of anergy of autoreactive T cells.¹¹⁷ The binding of PD-L1 to PD-1 blocks the downstream signaling events that occur after T cell receptor (TCR)-mediated cell activation, impairing T cell activation (block of T cell proliferation) and IL-2 production.¹¹⁸ The PD-1/PD-L1 complex also decreases T cell survival by impacting genes involved in apoptosis. This pathway prevents the expression of the anti-apoptotic gene B-cell lymphoma X_L (Bcl-X_L) and upregulates the pro-apoptotic factor Bim.¹¹⁹ Finally, the PD-1 pathway is key in the generation of anti-inflammatory Treg cells. PD-L1+ APCs, in the presence of TGF- β , can convert naïve peripheral CD4+ T cells to inducible Treg cells.¹¹⁹ PD-L1 maintains the survival and anti-inflammatory activity of Treg cells by upregulating the expression of the Treg transcription factor forkhead box P3 (Foxp3). Additionally, mature Th1 cells can be converted to Foxp3+ Tregs cells by engaging the PD-1 pathway. PD-1 deficiency or PD-1/PD-L1 blockade can lead to the development or exacerbation of autoimmune diseases.¹²⁰ In addition to its regulatory activity on lymphocytes, the PD-1/PD-L1 interaction may also regulate the function of macrophages, leading to increased IL-10 (anti-inflammatory) and decreased IL-6 (pro-inflammatory) production.^{121,122}

Again, much of the work highlighting the importance of PD-1 and PD-L1 signaling in post-stroke inflammation first comes from studies in the pre-clinical ischemic stroke animal model. In a murine experimental stroke model (transient middle cerebral artery occlusion [MCAO] in 8 to 12 week old otherwise healthy male animals) after a 96-hour reperfusion period, Upregulation of PD-1 expression was observed in resident microglia and macrophages within the ischemic CNS lesion on wild-type (WT) mice, and was not seen in PD-1 knockout (KO) mice.¹¹⁴ The loss of PD-1 led to significantly increased numbers of MHC class II+ and TNF- α secreting microglia and macrophages after activation, resulting in larger infarct volumes and worsened neurologic deficit, compared to WT animals with an intact PD-1 pathway.¹¹⁴ Alternatively, the same research group performed another study with PD-L1 and PD-L2 KO mice and noted that the presence of PD-Ls exacerbated post-stroke inflammation, increased infarct size, and worsened neurologic outcomes.¹²³ Mechanistically, the protective effect of PD-1 may be due to its expression on B cells and resulting inhibition of other immune effectors cells, and the detrimental effect of PD-L1 may be due to its ability to inhibit recruitment of immunoregulatory CD8+CD122+ suppressor T cells from the spleen into the brain.¹²⁴

In contrast, a mouse collagenase injection ICH model has shown upregulation of PD-1 expression in the perihematomal tissues, expressed mainly on macrophages, and in PD-1 KO mice, there was significant increase in mRNA and protein expression of pro-inflammatory cytokines compared to WT ICH groups, manifesting as increased brain water content and worsened neurologic impairment in the PD-1 KO group.¹²⁵ PD-L1 administration significantly attenuated the severity of neurologic deficits, decreased cerebral edema by decreasing brain water content, and decreased hemorrhage volume.¹²⁶ PD-L1 also downsized the number of CD4+ T cells infiltrating the brain and overall percentages of Th1 and Th17 cells while increasing the overall percentages of the Th2 and regulatory T cells. The overall pro-inflammatory cytokine production was decreased with down-regulation of IL-1 β , IL-6, and TNF- α , and upregulation of IL-10.¹²⁶

As mentioned earlier, microglia polarization plays a very important role in ICH-induced secondary brain injury. In response to ICH, selective polarization to the pro-inflammatory type occurs.¹²⁷ Overexpression of PD-1/PD-L1 was able to decrease this pro-inflammatory phenotype, with evidence of inhibited STAT1 phosphorylation, suggesting a possible method of modulation to the anti-inflammatory phenotype via inhibition of STAT1 (Figure 3).¹²⁷

The PD-1 pathway, as it relates to stroke, warrants further investigation as a therapeutic target to drive the phenotypic

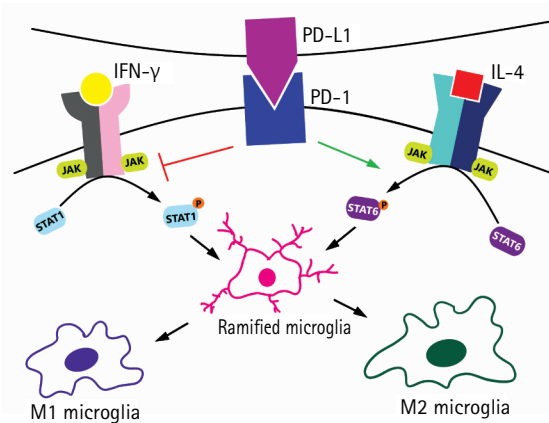


Figure 3. Programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) driven polarization of microglia. In the setting of intracerebral hemorrhage, upregulation of PD-1 has been noted in the perihematoma tissue. After the administration of PD-L1, the overall number of T cells infiltrating the central nervous system was decreased, with decreased total T helper 1 (Th1) and Th17 cells and increased Th2 and T regulatory cells. There is also increased polarization to the M2 phenotype in the setting of signal transducer and activator of transcription 1 (STAT1) inhibition. IFN- γ , interferon- γ ; JAK, Janus kinase; IL-4, interleukin-4.

change from a pro- to anti-inflammatory immune profile in order to mediate perihematoma edema and improve overall outcomes. As noted, there is discrepancy in the function of the PD-1 pathway in the neuroinflammatory process between acute ischemic stroke (AIS) and spontaneous ICH, as well as discrepancy within the AIS literature itself. As a result, there is a need for a model of stroke, both ischemic and hemorrhagic, that can be used to better understand this pathway in the context of the actual disease state, which includes associated risk factors and co-morbidities that are representative of a low-level chronic pro-inflammatory state.

Sphingosine 1-phosphate receptor modulation

Sphingosine 1-phosphate (S1P) engagement of sphingosine 1-phosphate receptors (S1PRs) plays a key role in the regulation of immune responses. *In vivo*, sphingosine is phosphorylated by sphingosine kinase 1/2 (Sphk1/2) to S1P, which then binds to G-protein coupled receptors S1PRs. There are five known S1PR subtypes, S1PR₁₋₅. S1PR₁₋₃ are universally expressed in the immune, cardiovascular, and CNSs. S1PR₁, and to a lesser extent S1PR₃ and S1PR₄, is highly expressed on T and B lymphocytes. S1PR₄ is generally found in the hematopoietic and lymphoid tissues. S1PR₅ is predominantly found within the CNS white matter.¹²⁸ This family of receptors participate in a wide variety of biologic processes, including leukocyte recirculation, cellular pro-

liferation and differentiation, morphologic changes, cellular motility, endothelial cell function, vascular regulation, and cardiovascular and nervous system development.¹²⁹

The role of the S1P/S1PR axis in the control of T cell retention in lymph nodes has been extensively studied.¹³⁰ In naïve T cells, S1PR₁ expression directs lymphocyte egress from lymph nodes in responses to the high concentration of S1P ligand present at exit sites.¹³¹ Following T cell activation, S1PR₁ expression is transiently decreased, inhibiting egress during the period of proliferation and differentiation. Upon re-expression of S1PR₁, activated T cells leave to traffic to sites of infection/inflammation.¹³¹ S1P also plays an important role in modulating Treg differentiation and function. S1P binding has been reported to inhibit differentiation into Treg cells as well as decrease suppressive activity,¹³² making this pathway an important potential contributor to the immune response following CNS damage.

Fingolimod (FTY720) is a structural analogue of phosphorylated sphingosine. It acts as a S1PR modulator and functions to regulate various cellular responses, including proliferation, apoptosis, and inflammation.¹²⁸ It binds with a high affinity to the following S1PR subtypes: S1PR₁, S1PR₃, S1PR₄, and S1PR₅.¹²⁸ Fingolimod is an oral immunomodulatory agent approved by the U.S. Food and Drug Administration for the treatment of relapsing-remitting MS, in which it has been shown to reduce relapses and limit loss of brain volume.¹³³⁻¹³⁵ The classic mechanism of fingolimod is based on the control mechanism of T cell retention and egress. It acts as a functional antagonist at S1PR₁, causing internalization and degradation of this receptor and removing the lymphatic S1P gradient necessary for lymphocyte egress, thereby reducing the overall number of inflammatory cells migrating to the CNS.¹³⁶ Based on the high expression of S1PRs in the CNS, a new aspect of fingolimod function is emerging, focusing on direct neurologic cellular modulation. It has been found to easily cross the blood-brain barrier in its unphosphorylated form, where it is then phosphorylated by endogenous CNS Sphk2.¹²⁸ Once phosphorylated, it is postulated that fingolimod has the capacity to influence the function of CNS biology by reducing astrogliosis and demyelination.¹³⁷

S1PRs are widely expressed on all cell lines in the CNS, although the level of receptor subtype expression varies based on cell type, and in the case of microglia, their state of activation.¹³⁸ In the ramified state, microglia express S1P receptors 1, 2, 3, and 5, with S1PR₁ expression being the dominant receptor type.¹²⁸ S1P signaling is essential in key neuron specific functions such as the regulation of transmitter release and the proliferation and survival of neurons and glia.¹³⁹ It has been re-

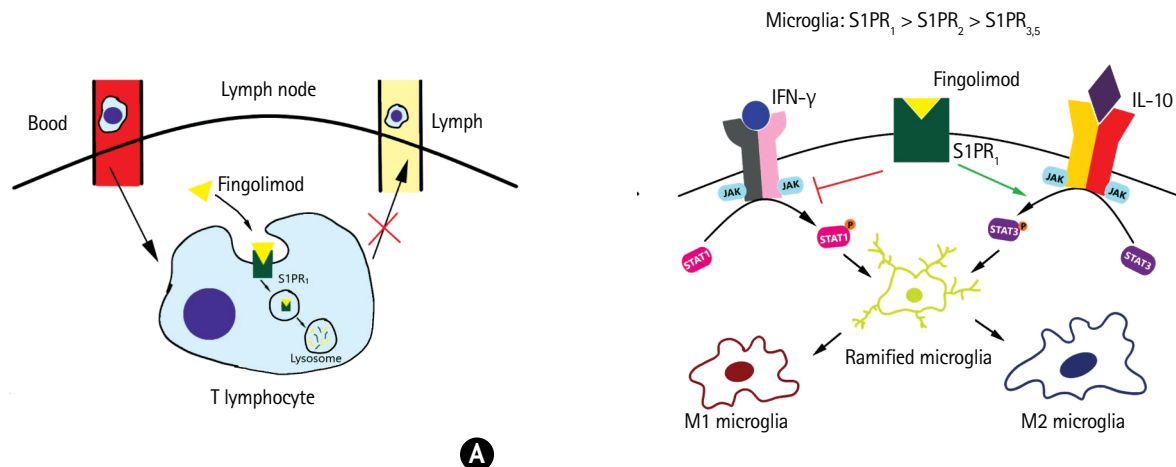


Figure 4. Fingolimod's proposed anti-inflammatory functions. (A) The sphingosine 1-phosphate (S1P) and sphingosine 1-phosphate receptor 1 (S1PR₁) interactions on lymphocytes are key for exiting the lymph nodes. In the inactivated state, S1PR₁ undergoes cyclical expression on circulating T cells. In the blood and lymph, S1PRs are typically downregulated in the presence of a high concentration of S1P. When the T cells circulate within the lymph nodes, S1PR expression is upregulated in the setting of low S1P concentrations. If an appropriate antigen is not encountered, T cells will exit, following the gradient of S1P. In the setting of fingolimod, the receptors internalize and are degraded. This prevents the sensing of the S1P gradient, thereby preventing the egress of the T cells. (B) In the central nervous system, S1PR₁ is noted to be expressed in high concentrations on microglia. It is proposed that fingolimod increases the expression and phosphorylation of signal transducer and activator of transcription 3 (STAT3) and inhibiting STAT1, potentiating the polarization the M2 phenotype. IFN- γ , interferon- γ ; JAK, Janus kinase; IL-10, interleukin 10.

ported that Sphk1 plays a critical part in the inflammatory processes mediated by TNF- α in immune cells.¹⁴⁰ *In vitro*, once microglia have been activated, S1P has been noted to amplify the release of pro-inflammatory cytokines, and with the suppression of Sphk1, there was suppression of TNF- α , IL-1 β , and iNOS gene expression.¹⁴¹ This was confirmed *in vitro* with the binding of S1PRs with fingolimod, which downregulated the production of pro-inflammatory cytokines and promoted the increased expression and release of neurotrophic factors. The effect on gene expression was also shown to be dose-dependent.^{142,143}

In addition to regulation of inflammatory and chemoattractant molecule gene expression, functional antagonism of the S1PRs with fingolimod may also limit the inflammatory response of activated microglia by polarizing microglia to the M2 phenotype, which suggests that S1P could be involved in the activation of the JAK/STAT3 cascade that is typically associated with M2 polarization (Figure 4).¹⁴⁴ Additionally, gene expression profiling has been performed to better understand the molecular effects of fingolimod. It has been found that fingolimod inhibited the expression of critical transcription factors, most importantly STAT1, thereby suppressing the activation of microglia (M1 phenotype).¹⁴⁵ The effect of fingolimod on microglial activation was confirmed *in vivo* in MS patients, where serial positron emission tomography imaging was performed, showing decreased microglial activation at the sites of focal inflammatory lesions.¹⁴⁶

Based on literature showing fingolimod and its protective effects on ischemia-reperfusion injury in heart, liver, and kidney,^{147,148} several groups hypothesized a potential neuroprotective effect of fingolimod in the setting of cerebral ischemia. It showed a neuroprotective effect after MCAO in the rat model with significantly diminished infarct volume, improved overall neurologic scores at 24 and 72 hours¹⁴⁹ and fewer activated neutrophils and microglia/macrophages.¹⁵⁰ Based on this pre-clinical evidence, two small human pilot studies were recently performed to evaluate the safety and efficacy of fingolimod in the treatment of acute ischemic strokes. Their clinical results mirrored those seen in animal models, with reduced infarct size and improved clinical outcomes.¹⁵¹

Beyond ischemic insults, fingolimod has also been shown to potentially reduce the incidence of reperfusion hemorrhage in the setting of delayed tissue plasminogen activator administration via enhancement of the BBB integrity.¹⁵² In light of this evidence, Lu et al.¹⁵³ studied the effects of fingolimod in the setting of ICH in the mouse model. It was found that fingolimod significantly reduced perihematomal edema, apoptosis, and associated brain atrophy, but they did not find a difference in the number of inflammatory cells between the treated and control groups.¹⁵³ However, Rolland et al.¹⁵⁴ found significant reduction in hematogenous and intracerebral lymphocytes compared to controls. They also found decreased expression of intercellular adhesion molecule-1 (ICAM-1), IFN- γ , and IL-17

at 72 hours. At 8 and 10 weeks post-ICH, treated rats were noted to have significantly reduced spatial and motor learning deficits, brain atrophy, and neuronal cell loss.¹⁵⁴ In experimental ICH models, selective S1PR₁ immunomodulation led to significant attenuation of neurologic deficits and improved post-ICH survival. Overall lesion volume was reduced. Decreased edema and midline shift were noted on magnetic resonance imaging, most likely due to the resultant reduced counts of microglia and T cells with associated decreased production of pro-inflammatory cytokines IL-1 β and TNF- α .¹⁵⁵

A small clinical pilot study of fingolimod in ICH patients also exhibited radiographic evidence of smaller perihematomal edema, significant reduction in neurologic impairment and improvement in Glasgow Coma Scale by day 7; and at 3 months, a greater proportion of fingolimod-treated patients achieved full neurologic recovery. There were no differences in adverse event rates between the treated and control groups.¹⁵⁶

Summary and future directions

Given the devastating nature of ICH, there has been increased interest in treating both the primary ICH and resultant cerebral edema. It has become increasingly clear that the cellular and molecular components of inflammation are involved in post-hemorrhagic secondary brain injury and are key components to the overall outcome of ICH. However, as mentioned previously, much of the current evidence regarding the inflammatory response surrounding ICH has been gleaned from healthy, young male murine models with normal immune responses. Given that age and specific medical co-morbidities are associated with the development of spontaneous ICH, we must develop ICH animal models that better mimic the clinical disease, as these factors may affect the inflammatory pathways and the switch from the pro-inflammatory to anti-inflammatory state. Additionally, it is important to note that current animal models do not account for differences in sex and the likely hormonal influences on the inflammatory pathways, warranting future investigations in both male and female animal models. In order to improve on the translation of preclinical findings into effective therapeutic targets, we are currently investigating the utility of the hypertensive transgenic rat line TGR (mREN2)27 in conjunction with autologous blood injection to simulate ICH. These animals overexpress renin and become chronically severely hypertensive around week 12. As they age, they also develop the other hallmarks of metabolic syndrome (obesity, insulin resistance, and dyslipidemia) and end organ changes (heart failure, chronic renal failure).¹⁵⁷ As we are discovering the importance of inflammation in these chronic conditions,

we are now discovering that the different factors in metabolic syndrome most likely represent a chronic low-level inflammatory state.¹⁵⁸ As such, we believe that the presence of these chronic conditions in an animal model may yield a better understanding of neuroinflammation after ICH.

Translation of preclinical findings to the clinical setting remains challenging, in part due to current ICH animal models' limitations and incomplete understanding of the pathophysiology of this complex disease. It is clear that there are pro- and anti-inflammatory phenotypes that may be leveraged in the treatment of ICH. With the advancement in surgical technology and techniques allowing for safe evacuation of the hematoma, it is possible to define the immune cell populations and phenotypes in ICH patients and follow their inflammatory profiles over time, ultimately correlating the change from pro- to anti-inflammatory phenotypes with long-term functional status to better understand the relationship of inflammation to clinical outcomes and utilize therapeutic targets to drive this change. Modulation of described and new pathways as promising therapeutic targets, such as S1PR and PD-1/PD-L1, and the optimal timing for intervention to mitigate the inflammatory cascade surrounding ICH prove hopeful to improve patients' functional outcomes.

Disclosure

The authors have no financial conflicts of interest.

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