doi:10.1093/brain/awz393 BRAIN 2020: 143; 1297–1314 | 1297

BRAIN
A JOURNAL OF NEUROLOGY

REVIEW ARTICLE

Microglia and macrophage phenotypes in intracerebral haemorrhage injury: therapeutic opportunities

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The prognosis of intracerebral haemorrhage continues to be devastating despite much research into this condition. A prominent feature of intracerebral haemorrhage is neuroinflammation, particularly the excessive representation of pro-inflammatory CNS-intrinsic microglia and monocyte-derived macrophages that infiltrate from the circulation. The pro-inflammatory microglia/macrophages produce injury-enhancing factors, including inflammatory cytokines, matrix metalloproteinases and reactive oxygen species. Conversely, the regulatory microglia/macrophages with potential reparative and anti-inflammatory roles are outcompeted in the early stages after intracerebral haemorrhage, and their beneficial roles appear to be overwhelmed by pro-inflammatory microglia/macrophages. In this review, we describe the activation of microglia/macrophages following intracerebral haemorrhage in animal models and clinical subjects, and consider their multiple mechanisms of cellular injury after haemorrhage. We review strategies and medications aimed at suppressing the pro-inflammatory activities of microglia/macrophages, and those directed at elevating the regulatory properties of these myeloid cells after intracerebral haemorrhage. We consider the translational potential of these medications from preclinical models to clinical use after intracerebral haemorrhage injury, and suggest that several approaches still lack the experimental support necessary for use in humans. Nonetheless, the preclinical data support the use of deactivator or inhibitor of pro-inflammatory microglia/macrophages, whilst enhancing the regulatory phenotype, as part of the therapeutic approach to improve the prognosis of intracerebral haemorrhage.

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Keywords: intracerebral haemorrhage; microglia; neurotoxicity; neuroinflammation; stroke

Abbreviations: ICH = intracerebral haemorrhage; M/M = microglia/macrophages; MMP = matrix metalloproteinase

Received July 24, 2019. Revised September 19, 2019. Accepted October 20, 2019. Advance Access publication January 9, 2020
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Introduction

Intracerebral haemorrhage (ICH) accounts for ~12–20% of all strokes. It affects over 2 million individuals annually and is associated with a high mortality rate (Cordonnier *et al.*, 2018). Moreover, ICH carries a worse prognosis than the predominant type of stroke caused by ischaemia. ICH associated with hypertension remains the most common form of haemorrhagic stroke. ICH may also be caused by coagulopathy, cerebral amyloid angiopathy, brain tumours, vascular anomalies, brain trauma, or premature birth. The mortality rate at 30 days for ICH is 43–51% (An *et al.*, 2017). Recovery following ICH is poor and most survivors retain a considerable functional handicap related to the specific site of haemorrhage. The most common sites of hypertensive ICH are the caudate/putamen (basal nuclei), thalamus, cerebellum and pons. When ICH occurs, blood collects in the brain and causes brain damage, neuronal death and varying degrees of functional impairments, associated with neuroinflammation.

Microglia constitute 5–10% of the total cellular population within the normal brain; they act as the first and main form of active immune defence intrinsic to the CNS (Poon *et al.*, 2017). In response to pathology signals, they can change morphologically and functionally, and can migrate towards these signals. Unless specifically differentiated by lineage markers, microglia and monocyte-derived macrophages in the injured CNS parenchyma are not readily distinguished from one another so we refer to them here as microglia/macrophages (M/M). Microglia primarily use phagocytic and cytotoxic mechanisms to destroy foreign materials, similar to macrophages. Both microglia and monocyte-derived macrophages contribute to pro-inflammatory and homeostatic mechanisms within the brain through the secretion of cytokines and other signalling molecules. Recently, border-associated macrophages in the meninges, choroid plexus and perivascular cuffs have been described, and they are distinct from parenchymal microglia or monocyte-derived macrophages (Prinz *et al.*, 2017). As these have not been specifically addressed in their potential roles in ICH, they have not been included in this review.

In health, the ramified microglial cell is commonly found throughout the entire CNS with a small soma that remains fairly motionless; nonetheless, the long-branched processes of microglia are in constant motion to survey the microenvironment (Nimmerjahn *et al.*, 2005). Herein, we describe the activation of M/M following ICH in animal models and clinical subjects, consider their multiple roles after haemorrhage including pro-inflammatory (also referred to in the literature as inflammatory) and regulatory (also cited as homeostatic, non-inflammatory, anti-inflammatory or reparative) phenotypes, and review therapeutic strategies to improve the prognosis of ICH based on modulation of these M/M roles. Where possible, we evaluate the strength of evidence and translational potential of proposed medications from animal studies to human subjects to treat ICH.

Pathophysiology of brain damage after intracerebral haemorrhage

Brain injury immediately following ICH is generally described as primary injury, which includes direct mechanical disruption due to the enlarged haematoma and physical stretch, compromise of local cerebral blood flow through mechanical and chemical factors, and raised intracranial pressure and reduced cerebral perfusion pressure resulting from cerebral oedema (Kingman *et al.*, 1987). Secondary injury occurs in a temporal dynamic progression of intertwined degenerative, inflammatory and biochemical cascades culminating in tissue damage and cell death (Shao *et al.*, 2019). The most widely appreciated component of the intracerebral inflammatory response is the influx of blood-derived monocytes that become macrophages in the brain; concordantly, CNS-intrinsic microglia are activated (Lan *et al.*, 2017*a*). The activated M/M phagocytose haematoma breakdown products. Subsequently, the haematoma becomes a cavity that contains proteinaceous fluid, scar components such as extracellular matrix deposition, and M/M, surrounded by reactive astrocytes (Altumbabic *et al.*, 1998).

Figure 1 encapsulates the inflammatory cascades of neuroinflammation in ICH with the focus on M/M (summarized from Mracko and Veltkamp, 2018; Shao *et al.*, 2019; Shi *et al.*, 2019), providing the background for the interventions to be described in this review. Immediately following the primary injury with pronounced death of neurons, there is release from degenerating neurons and the neuropil of damage-associated molecular patterns (DAMPs) including heat shock proteins, high-mobility group box 1 (HMGB1) and extracellular matrix fragments, many of which act on toll-like receptors (TLRs) on microglia. In response, the activated microglia release factors such as reactive oxygen species (ROS, e.g. superoxide) and reactive nitrogen species (RNS, e.g. nitric oxide), as well as matrix metalloproteinase (MMPs), chemokines and cytokines. The latter include interleukin 1β (IL-1β) and -6 (IL-6), and tumour necrosis factor alpha (TNF-α), which activate local endothelial cells as in other tissues to upregulate levels of the adhesion molecules selectins, and integrin-binding intercellular cell adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1). Together with a chemokine gradient, the elevated vascular adhesive proteins trap leucocytes, particularly neutrophils that then transmigrate into the CNS parenchyma within the early minutes to hours of injury; lymphocytes enter hours to days later. Monocytes in the circulation are recruited in the early hours of ICH, and they mature into macrophages in the CNS parenchyma.

Besides the immune cell recruitment, blood and blood components enter into the brain parenchyma immediately after ICH. When erythrocytes begin to disintegrate within

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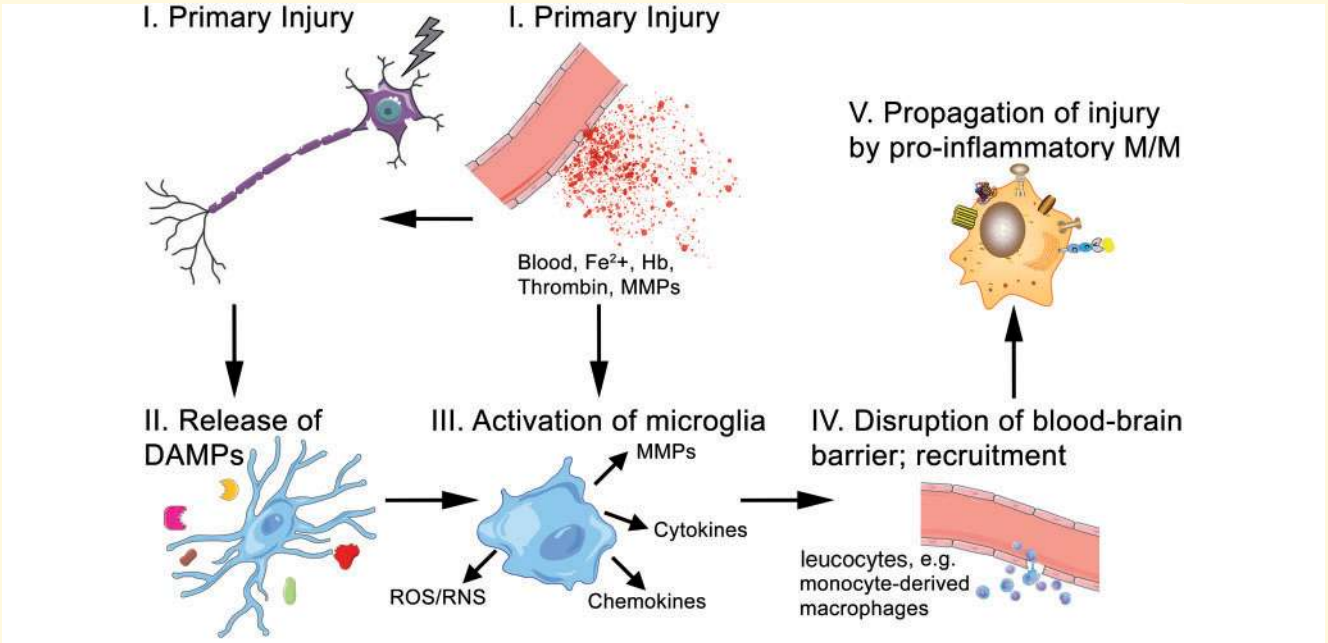

Figure 1 The early cascades of ICH with focus on M/M. Following the primary injury (I), damage-associated molecular patterns (DAMPs) released from compromised neural cells and the extracellular matrix engage pattern recognition receptors on microglia (II), resulting in the latter's activation. Blood products leaked into the circulation during primary injury (I) also activate microglia in addition to causing further damage. The activated microglia release a variety of products that exacerbate degeneration (III) and these disrupt the blood–brain barrier and help attract leucocytes including monocyte-derived macrophages (IV) into the CNS parenchyma. The result is the generation of pro-inflammatory microglia/macrophages (M/M).

the haematoma, haemoglobin (Hb) is released and it can cause brain damage through iron-dependent formation of oxidative species and heme (Wang and Dore, 2007). Other components of blood that are toxic or cause brain inflammation include thrombin, MMPs, plasmin, and complement proteins. Agonists of TLRs from plasma that enter the brain parenchyma and/or produced by brain cells also contribute to neuronal injury (Nishino *et al.*, 1993).

Overall, an inflammatory response that includes microglia activation and leucocyte infiltration, enzyme activation, and release of many mediators of injury such as Hb, iron and reactive oxygen and nitrogen species (Garcia *et al.*, 1994) propagates the cell death resulting from primary injury within the CNS. Together, these primary and secondary events culminate in extensive damage to neural cells.

Animal models of intracerebral haemorrhage

Animal models of ICH have been developed and used to explore the pathogenesis of ICH. They are also used to evaluate preventive or therapeutic strategies in ICH in several species including mouse, rat (Xue and Del Bigio, 2000), pig (Wagner *et al.*, 1999) and primate (Bullock *et al.*, 1988). However, rodent models have been used predominantly in studies of M/M in ICH. Three main models are used to mimic spontaneous ICH (Table 1).

Intracerebral haemorrhage animal model induced by collagenase

A collagenase proteolytic enzyme is injected into the cerebrum to induce ICH by destruction of the extracellular matrix-rich basement membrane around capillaries in the brain (Rosenberg *et al.*, 1990); this leads to opening of the blood–brain barrier causing intraparenchymal bleeding (Rosenberg *et al.*, 1990; Wang and Tsirka, 2005). The advantage of this model is that it produces highly reproducible haemorrhage and mimics spontaneous ICH without significant blood leakage along the needle track. A disadvantage of this model is that the collagenase seems to cause greater inflammatory reactions than autologous blood injection, another model of ICH to be discussed later. This collagenase model has been used to test treatment and to study the activation of M/M following ICH in rodents (Rosenberg *et al.*, 1990; Rosenberg and Navratil, 1997; Ohnishi *et al.*, 2011; Lively and Schlichter, 2012; Wang *et al.*, 2017*a*; Yang *et al.*, 2019).

Intracerebral haemorrhage animal model induced by thrombin

Thrombin toxicity activates microglia and promotes cytokine production that causes neuroinflammation and cell death. Thrombin released from haematoma is a major contributor to secondary brain damage in acute ICH (Xue and

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Table 1 Animal models of ICH

Mediators of injury	Results	Reference
Collagenase	Opens the blood–brain barrier and causes intraparenchymal bleeding; activates microglia and promotes cytokine production	Rosenberg *et al.*, 1990; Rosenberg and Navratil, 1997; Wang and Tsirka, 2005
Thrombin	Activates microglia and promotes cytokine production that causes neuroinflammation and cell death	Xue and Del Bigio, 2001; Yang *et al.*, 2015*b*
Autologous whole blood	Blood toxicity and the resulting inflammation activates microglia and promotes cytokine production	Bullock *et al.*, 1984; Nath *et al.*, 1986; Xue and Del Bigio, 2000; Xue *et al.*, 2003*a*, 2006

Measurements commonly used in all three models include: haematoma size, blood–brain barrier disruption, neurological deficit scale, wire-hanging test, inflammatory reactions and cell death.

Del Bigio, 2001). Intrastriatal thrombin injection that impairs neurogenesis and spatial memory function is partly mediated by inflammation, characterized by the activation of CD68 positive M/M (Yang *et al.*, 2015*b*). This model has been used to study the mechanisms of thrombin toxicity that cause neuroinflammation and cell death (Yang *et al.*, 2015*b*). A disadvantage of this model is that it provides minimal utility beyond thrombin toxicity research.

Intracerebral haemorrhage animal model induced by autologous whole blood

The autologous whole blood-induced ICH model has been used extensively in rats (Bullock *et al.*, 1984; Nath *et al.*, 1986) and mice (Xue *et al.*, 2003*a*, 2006). Autologous whole blood may be obtained from the animal's tail or femoral artery, then directly injected into selected brain areas. Alternately, the femoral artery may be attached directly to a cannula to simulate pressure/pulsation. The intracranial-deposited blood can cause brain oedema, cell death, leucocyte infiltration, M/M activation, and behavioural impairment (Xue and Del Bigio, 2000; Xue *et al.*, 2006). A disadvantage of this model is that autologous blood deposition does not reproduce the rupturing of a blood vessel in spontaneous ICH. An advantage is that this method allows anatomically localized haematomas to be created. This model has been used to study the brain injury mechanisms following ICH including activation of M/M in rodents.

Phenotypes of microglia/macrophages

Microglia typically respond to acute brain injury by developing classic pro-inflammatory signatures. However, while potentially neurotoxic, there are benefits of neuroinflammation in fostering CNS recovery after neural injury, as highlighted by examples from multiple sclerosis, traumatic spinal cord injury, stroke, and Alzheimer's disease (Yong *et al.*, 2019). Indeed, more and more studies demonstrate that microglia can play protective roles after stroke, in both the subacute and chronic phases (Yang *et al.*, 2016; Li *et al.*, 2017; Zhou *et al.*, 2017), through a regulatory/homeostatic phenotype.

Animals treated with IL-4, a cytokine that generates regulatory/homeostatic cells, exhibit elevated numbers of non-inflammatory and regulatory M/M phagocytes that remove cellular debris (Lively *et al.*, 2016). Neuroprotective strategies aimed to convert pro-inflammatory to regulatory phenotypes reduce inflammatory injurious processes and improve phagocytosis of debris and functional recovery, as discussed below.

Figure 2 depicts soluble mediators released by pro-inflammatory or regulatory M/M (Mishra and Yong, 2016). In general, molecules released by pro-inflammatory M/M tend to propagate an inflammatory response. These molecules include IL-1β, IL-6 and TNF-α, which activate endothelium and promote leucocyte recruitment as aforementioned, and chemokines including CXCL8, CCL2 and CCL5, which serve to attract neutrophils, monocytes and lymphocytes, respectively. The regulatory M/M favour the release of anti-inflammatory cytokines (Fig. 2), including IL-4, IL-10 and IL-13; and other mediators such as IL-1 receptor antagonist (IL-1Ra) and transforming growth factor-β1 (TGFβ1) that counter the activity of pro-inflammatory molecules.

Pro-inflammatory and regulatory M/M also have cell-associated molecules and enzymes that differ from one another (Fig. 3), and that help account for their differential properties (Mosser and Edwards, 2008; Mishra and Yong, 2016; Wynn and Vannella, 2016; Beaino *et al.*, 2017). These include, for pro-inflammatory versus regulatory cells, the enzymes inducible nitric oxide (iNOS) and arginase-1 (Arg1); receptors CD16 (FcγRIIIa) and CD32 (FcγRII) on the former and scavenger receptors CD163 (for haemoglobin-haptoglobin complex), CD204 (macrophage scavenger receptor 1) and CD206 (mannose receptor) on the latter; purinergic receptors P2X7 versus P2RY12; adenosine receptor (R) A2R, IL-1R1 and CSF-1R versus IL-4R/IL-10R; CCR2/CCR7 versus CX3CR1; and the CD80/CD86 co-stimulatory ligands on pro-inflammatory M/M in contrast to the checkpoint ligand PD-L1 on regulatory cells. The transcription factor STAT1, and to a lesser extent STAT3, tend to polarize M/M to the pro-inflammatory type, while STAT6, and to a smaller extent STAT4, are associated with the regulatory phenotype. It should be noted that the markers described herein may change in relative abundance dependent on the disease condition or tissue microenvironment, that they can also be

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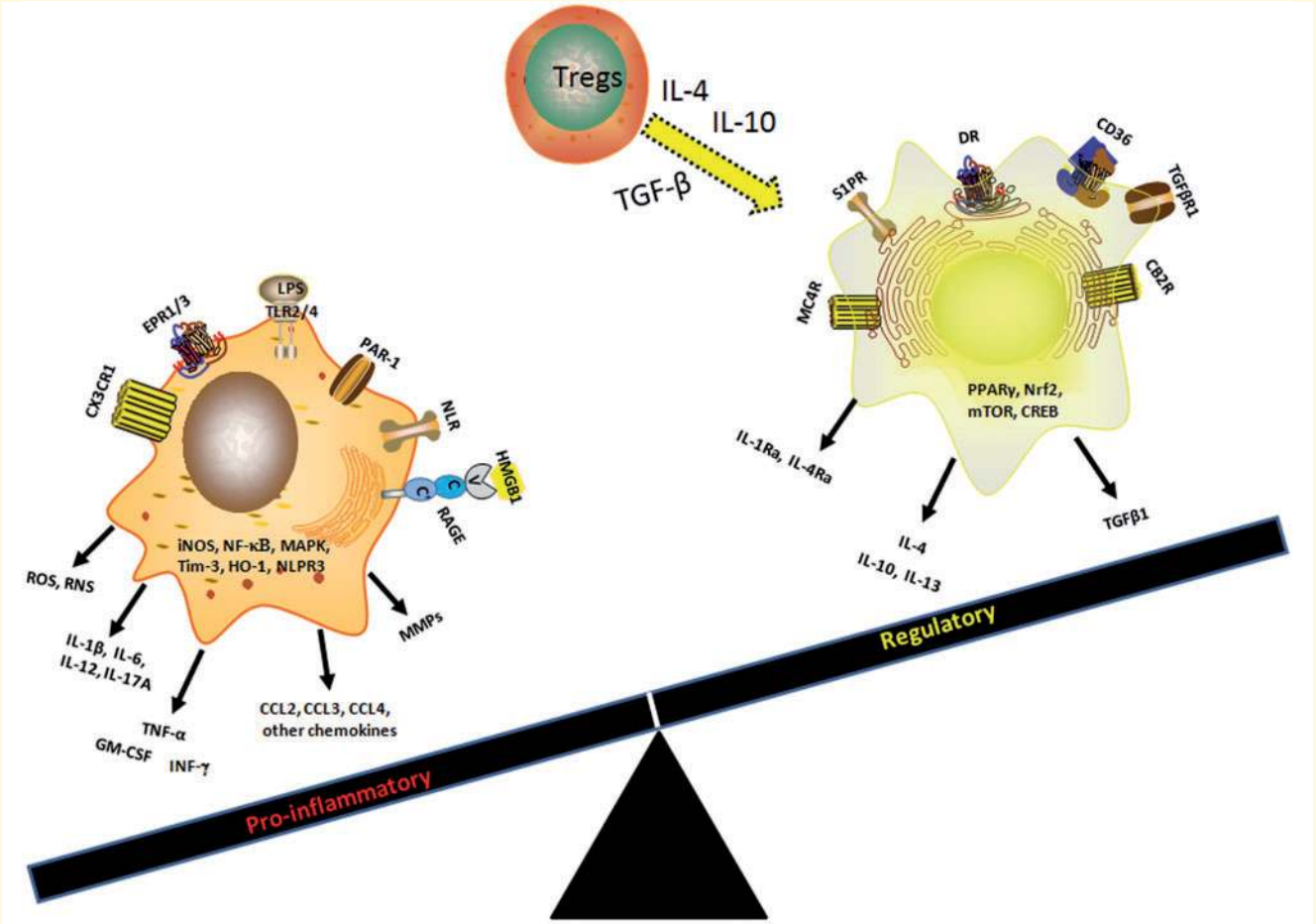

Figure 2 Pro-inflammatory M/M predominate over their regulatory counterparts in ICH. Early after ICH, the representation of pro-inflammatory M/M, generated by ligands acting on receptors including CX3CR1, EPR1/3, TLR2/4, PAR-1 NLR and RAGE, is elevated and in excess of regulatory M/M. The pro-inflammatory M/M secrete inflammatory cytokines, chemokines, MMPs and other molecules capable of inflicting injury and exacerbating neuroinflammation. A goal in ICH is to shift the M/M towards a regulatory phenotype that produces anti-inflammatory cytokines; regulatory M/M may be generated through regulatory T cell activity or by stimulating receptors such as MCAR, S1PR, CD36, TGFβR1 and CB2R. Intracellular mediators of both phenotypes are indicated within the respective cells.

expressed in the other subtype, and that multiple markers should be used simultaneously to more accurately delineate their polarized function. Moreover, the majority of studies to subclassify M/M subtypes have used macrophages rather than microglia, although the principles do appear to hold for both cell types (Michell-Robinson *et al.*, 2015).

Activation of pro-inflammatory microglia/macrophages in intracerebral haemorrhage

Pro-inflammatory cytokines

Activated M/M increase their production of numerous pro-inflammatory and potentially neurotoxic mediators which may contribute to ICH neuronal injury. The production of cytokine and chemokine mRNA coincident with or preceding the infiltration of neutrophils and monocytes has suggested a role for these molecules in the pathology of brain injury (Arvin *et al.*, 1996).

The role of individual cytokines in brain disease has been studied by injecting the purified protein into brain or by studying mutant mice. Direct injection of the pro-inflammatory cytokines IL-1α, IL-1β and TNF-α into the CNS causes local inflammatory responses and neuronal degradation. TNF-α is released by activated M/M and other cell types in the brain such as neurons and astrocytes. The elevated expression of TNF-α in M/M has been shown in ICH in rats (Mayne *et al.*, 2001). In one study, there was no significant elevation of cytokines IL-6, IL-1β and TNF-α 1 h after autologous blood ICH in the brain, CSF or serum of dogs (Qureshi *et al.*, 2001); however, others found profoundly increased levels of these cytokines 24 h after ICH in animals (Chen *et al.*, 2018) and in a clinical study of

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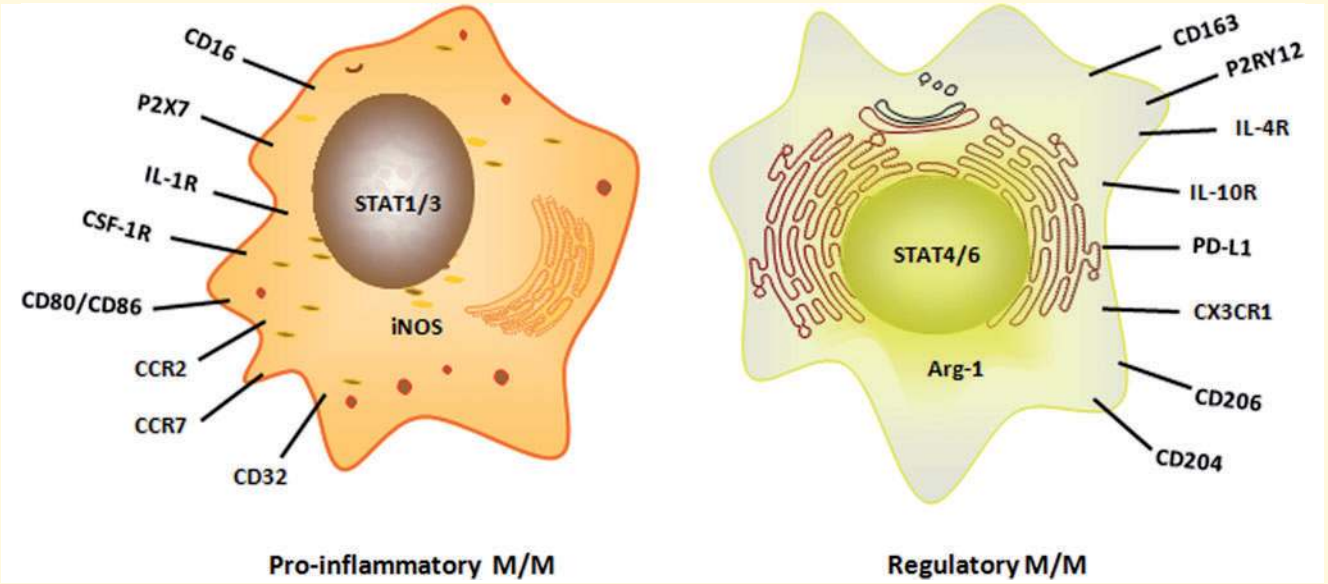

Figure 3 Cell surface markers and transcription factors of pro-inflammatory and regulatory M/M.

peri-haematomal brain tissue (Zhang *et al.*, 2014). A TNF-α-specific antisense oligodeoxynucleotide reduces cell death and improves neurobehavioural deficits after ICH in rodents (Mayne *et al.*, 2001), linking expression of cytokine to pathophysiology of ICH.

The pro-inflammatory IL-17A has also been reported to be involved in ICH-induced microglial activation and neuroinflammation, and an IL-17A antibody has been suggested to be a promising therapeutic strategy in ICH (Yu *et al.*, 2016). The transcription factor NF-κB is closely related with secondary brain damage after ICH in patients (Zhang *et al.*, 2014). NF-κB is involved in the transcription of many pro-inflammatory cytokines, including TNF-α and IL-1β, after ICH (Wagner, 2007).

Interferon (INF)-γ is mainly produced by T lymphocytes but also by activated M/M. The immune effects of INF-γ include the enhancement of the activation of M/M, cytotoxic T lymphocytes and NK cells, and the upregulation of class I and II MHC antigen expression on a variety of immune cells (Benveniste, 1998). INF-γ also enhances apoptosis through perforin/granzyme or Fas ligand mechanism, and INF-γ knockout mice have significantly reduced neuroinflammation and neuronal cell death compared to wild-type mice following ICH (Xue and Del Bigio, 2005).

Proinflammatory chemokines

Chemokines are a family of regulatory polypeptides with roles in cellular communication and inflammatory cell recruitment in host defence. Chemokines are divided into four main subfamilies: C, CC, CXC, and CX3C. Activated M/M are sources of some chemokines and express the monocyte chemoattractant protein-1 (MCP-1, CCL2) chemokines in particular. Inflammatory cytokines including IL-1β and TNF-α, and lipopolysaccharide (LPS), may stimulate microglia to produce CCL2, macrophage inflammatory protein-1α (MIP-1α, CCL3), and MIP-1β (CCL4). Inhibition or deficiency of CCL2 and CCL3 is associated with reduced brain ischaemic injury (Garau *et al.*, 2005). In humans after ICH, high level of serum CCL2 at 24 h is correlated with poor functional outcomes at 7 days (Hammond *et al.*, 2014).

The CX3CR1 chemokine receptor is expressed on microglia and modulates microglial activation (Wolf *et al.*, 2013). Mice with myeloid-specific CX3CR1 deficiency show no defect in functional recovery compared to wild-type after blood-induced ICH (Taylor *et al.*, 2014).

In addition to chemotactic properties, chemokines are found to directly affect blood–brain barrier permeability. CCL2 may play a role in 'opening' the blood–brain barrier by altering tight junction proteins (Stamatovic *et al.*, 2005). Overall, manipulating chemokine expression by M/M and chemotactic signals may constitute an approach to the treatment of ICH injury.

Thrombin

Thrombin is a serine protease that is generated by the cleavage of prothrombin. Thrombin converts fibrinogen into fibrin, which is involved in formation of a blood clot. Thrombin is produced immediately in the brain after ICH (Gingrich and Traynelis, 2000). Some of the detrimental effects of blood following ICH have been attributed to activity of thrombin (Xue and Del Bigio, 2001). In this regard, thrombin induces local M/M activation and in nearby brain tissues (Yang *et al.*, 2015*b*), and it promotes

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neuronal death (Xue and Del Bigio, 2001; Xue *et al.*, 2003*b*, 2006). Microglia proliferate in response to thrombin acting through protease-activated receptor-1 (PAR-1) (Noorbakhsh *et al.*, 2003) or PAR-4 (Suo *et al.*, 2003). PAR-1 is upregulated after ICH in neurons and microglia. PAR-1 knockout mice have less pro-inflammatory polarization and reduced proinflammatory cytokine levels in the brain compared with wild-type mice (Wan *et al.*, 2016). Thrombin potentiates NMDA receptor function (Gingrich *et al.*, 2000) and activates rodent microglia *in vitro* (Moller *et al.*, 2000) through MAPK signalling (Ohnishi *et al.*, 2013). Thrombin inhibitors reduce thrombin-induced M/M activation in ICH, culminating in less brain damage and neuronal death. However, thrombin is essential for the formation of a blood clot and thus its activity is vital to prevent the further enlargement of a haematoma (Yang *et al.*, 2015*b*). By reducing haematoma growth, thrombin would inhibit oedema formation, inflammation and brain cell death. Thrombin at very low concentration is neuroprotective and it enhances the survival of hippocampal neurons and astrocytes after exposure to cellular insults (Striggow *et al.*, 2000). Overall, the role of thrombin in ICH is complex, thereby making it a difficult therapeutic target in ICH.

Matrix metalloproteinases

MMPs are a family of zinc-dependent endopeptidase enzymes that can degrade all components of the extracellular matrix (Yong *et al.*, 2001). MMPs have many substrates in addition to extracellular matrix molecules (McCawley and Matrisian, 2001) and they are implicated in diverse functions including the regulation of survival, signalling, angiogenesis, inflammation, and cell motility (Egeblad and Werb, 2002). MMPs are produced by activated microglia, infiltrating inflammatory cells, neurons and astroglia following brain injury. This widespread distribution suggests that MMPs have diverse functions in ICH. In this regard, MMPs can directly damage neural cells, cause cell injury by processing death molecules (e.g. FasL), promote demyelination, perpetuate inflammation, and produce an increase in capillary permeability and brain oedema that is secondary to ICH (Yong *et al.*, 2001; Rosenberg, 2002; Xue and Yong, 2008). Intracerebral injection of collagenase (a subgroup of MMPs) leads to ICH with blood–brain barrier disruption, oedema and tissue necrosis (Rosenberg *et al.*, 1998), and it increases the levels of multiple MMPs (Power *et al.*, 2003). As noted above, the intracerebral injection of collagenase constitutes a model of ICH (Table 1).

Bleeding increases *Mmp2*, *-3*, *-7*, *-9* and *-12* mRNA levels in a rat model of collagenase-induced ICH (Power *et al.*, 2003). MMP3, -9 and -12 are induced during the inflammatory response secondary to the immediate early genes c-fos and c-jun, and the cytokines TNF-α and IL-1β (Rosenberg, 2002). Normally, MMP9 protein is not easily detectable in the brain; however, following brain injury, MMP9 is prominently elevated and produced by activated M/M, infiltrating inflammatory cells, and astrocytes (Romanic *et al.*, 1998). MMP3 has been detected in microglia and in neurons during ischaemia (Rosenberg *et al.*, 2001), and it can activate microglia and cause neuronal apoptosis (Kim *et al.*, 2005). MMP12 is expressed by activated M/M in ICH (Power *et al.*, 2003). That these proteases are, on balance, detrimental is demonstrated in the autologous blood-induced model of ICH where the extent of brain damage is reduced in MMP3, -9 or -12 single null mice compared to wild-type controls (Wells *et al.*, 2005; Xue *et al.*, 2009).

MMPs are usually present in pro-forms and are activated by autocatalysis or cleavage by membrane-type MMPs, thrombin or plasmin (Yong *et al.*, 2001; Rosenberg, 2002; Xue *et al.*, 2006). ROS also activate MMPs (Rajagopalan *et al.*, 1996) through acting on the pro-forms, or by inducing the elevation of their mRNA through signalling via NF-κB (Yong *et al.*, 2001). In the rat model of collagenase-induced ICH, there is increased activation of MMP2 and MMP9 at 16–24 h after the onset of haemorrhage (Rosenberg and Navratil, 1997). The activated MMP9 has been shown to induce neuronal cell death via apoptosis (Gu *et al.*, 2002).

While MMPs appear neurotoxic in the initial periods of ICH and inhibition of MMPs early after stroke reduces brain damage, there are concerns that prolonged inhibition of MMPs during recovery from stroke could do more harm than good, as MMPs are involved in some of the remodelling and reparative processes after injury (Zhao *et al.*, 2006). Overall, the biology of MMPs is complex and the long-term inhibition of MMPs may be inappropriate because of the potential to inhibit the beneficial roles of MMPs in regulating neurogenesis, myelin reformation, and axonal regrowth (Yong, 2005; Zhao *et al.*, 2006).

Collaboration of proteases

Our group studied the role and collaboration of proteases in mediating neuronal death in culture and in ICH injury. We found that human neurons die when exposed to thrombin or MMP9 in isolation but that their combination increases neurotoxicity further in cell culture, in part by thrombin converting proMMP9 to active MMP9 (Xue *et al.*, 2006). Moreover, the concordant antagonism of thrombin using hirudin alleviates further the reduced injury found in MMP9 null mice, emphasizing the collaborative role of MMP9 and thrombin in promoting ICH injury (Xue *et al.*, 2006). Furthermore, we noted that brain damage and neuronal death induced by blood is reduced further in MMP3/9 double null mice treated with hirudin (Xue *et al.*, 2009). Treatments targeting proteases in ICH will require activity on multiple enzymes that collaborate to mediate injury.

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Other pro-inflammatory mechanisms in intracerebral haemorrhage

Inducible nitric oxide synthase

Inducible nitric oxide synthase (iNOS) is upregulated as early as 3 h after ICH (Yang *et al.*, 2013), leading to increased levels of its product, nitric oxide, that can cause free radical-mediated injury to neurons. Lan *et al.* (2017*a*) found that iNOS and other pro-inflammatory markers such as CD16 and CD32 are highly expressed on microglia at Days 1 and 3 after ICH.

High mobility group box protein 1

HMGB1 is normally a cytosolic protein that translocates into the nucleus during cellular activation to affect gene transcription. Upon injury, it can be released by cells where it acts as a DAMP that binds TLRs and the receptor for advanced glycation endproducts (RAGE) on M/M. RAGE is an early pro-inflammatory signal within the neurovascular unit during the acute phase of collagenase-induced ICH in rats (Ohnishi *et al.*, 2011; Wang *et al.*, 2017*a*). One study shows that HMGB1 is increased in thrombin-induced injury in rat cortico-striatal slice cultures and *in vivo* rat ICH model (Ohnishi *et al.*, 2011). A HMGB1 inhibitor reduces the number of activated M/M and an anti-HMGB1 monoclonal antibody ameliorates brain injury in collagenase-induced ICH in rats (Wang *et al.*, 2017*a*).

Complements

Much evidence demonstrate that the complement cascade is activated after experimental ICH (Yang *et al.*, 2006; Garrett *et al.*, 2009; Yuan *et al.*, 2017). Compared to complement C3-sufficient mice after ICH, C3-deficient mice have less brain oedema, and reduced microglia activation and neutrophil infiltration around the clot (Yang *et al.*, 2006). Administration of C5a receptor antagonist (C5aRA) alone, or the combination of C3aRA/C5aRA, provides neuroprotection and leads to synergistic improvements in neurofunctional outcome in haemorrhagic stroke (Garrett *et al.*, 2009). $C5aR^{-/-}$ mice exhibit attenuated inflammatory reactions after ICH via fibrinogen-like protein 2, and ERK1/2 and p38 pathways (Yuan *et al.*, 2017). Furthermore, the C5a receptor antagonist PMX53 provides neuroprotection in ICH associated with reduction of pro-inflammatory factors TNF, IL-6 and iNOS, leading to improvement in functional outcomes (Li *et al.*, 2014).

Prostanoids

Prostaglandin E2 (PGE2) acts via the G-protein-coupled E prostanoid (EP) receptors EP1–EP4 to activate its downstream signalling pathways. EP1 receptor is expressed on microglia, and EP1 deletion dampens microglial activity and phagocytosis (Singh *et al.*, 2013). Furthermore, treatment with the EP1 receptor antagonist SC51089 decreases microglial activation and attenuates grey and white matter injury in mouse models of ICH (Zhao *et al.*, 2015*c*). Similarly, EP3 receptor is expressed on microglia (Han *et al.*, 2016), and treatment with an EP3 antagonist reduces lesion volume, neurological deficit, cell death, MMP9 activity, neutrophil infiltration and $CD68^+$ M/M, while increasing the $Ym1^+$ regulatory M/M in a thrombin-induced ICH model (Han *et al.*, 2016).

Heme oxygenase

Heme oxygenase (HO-1, *HMOX1*) is expressed by M/M and is thought to exacerbate early brain injury after ICH. Lesion volume is significantly smaller in $Hmox1^{-/-}$ mice than in wild-type controls 24 and 72 h after ICH (Wang and Dore, 2007). Recently, several studies showed that long-term induction of HO-1 increases haematoma absorption, angiogenesis, and recovery of neurological function in the later stages of ICH (Zhang *et al.*, 2017; Li *et al.*, 2018).

Inflammasome activation

ICH activates the Nod-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome and inflammation, and NLRP3 RNAi treatment attenuates inflammation and brain injury after ICH (Yuan *et al.*, 2015; Yao *et al.*, 2017). Thrombin activates ROS/TXNIP/NLRP3 signalling in BV2 microglia-like cells, and the NLRP3 antagonist MCC950 significantly attenuates cellular apoptosis and expression of apoptotic proteins (Ye *et al.*, 2017). Increased expression of NLRP6 inflammasome is found in peri-haematomal brain tissues ranging from 6 h to 3 days, with a peak level at 1 day after ICH. However, $Nlrp6^{-/-}$ ICH mice exhibit significantly higher brain damage than wild-type mice, suggesting that the upregulated NLRP6 inflammasome in peri-haematomal brain tissues attenuates ICH-induced brain injury (Wang *et al.*, 2017*b*).

T cell immunoglobulin and mucin domain-3

T cell immunoglobulin and mucin domain-3 (Tim-3, encoded by *Havcr2*) was originally described as a T lymphocyte (T helper-1) membrane protein but it is expressed by M/M where it regulates their innate immune functions. Expression of Tim-3 increases early and peaks at Day 1 in mouse peri-haematomal brain tissue after autologous blood injection, predominantly on M/M, and is positively correlated with the elevated concentrations of TNF-α, IL-1β and brain water content (Xu *et al.*, 2013). $Havcr2^{-/-}$ mice had reduced ICH-induced brain inflammation with decreased TNF-α and IL-1β, cerebral oedema and

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neurological deficit scores (Xu *et al.*, 2013). The ligand for Tim-3 on M/M following ICH is unknown.

Inhibition of soluble epoxide hydrolase (sEH) reduces thrombin-and hemin-induced microglial activation as well as p38 MAPK and NF-κB activation in primary microglial cultures (Wu *et al.*, 2017). C1q/TNF-related protein 9 (CTRP9) is an agonist of adiponectin receptor 1 (AdipoR1); recombinant CTRP9 (rCTRP9) administered intranasally at 1 h after ICH attenuates neuroinflammation through interfering with the AdipoR1/AMPK/NF-κB pathway, thereby reducing brain oedema and improving neurological function in bacterial collagenase-induced ICH in mice (Zhao *et al.*, 2018). It is likely that other as yet unreported pro-inflammatory mechanisms occur after ICH to affect its outcome.

Activation of regulatory microglia/macrophages after intracerebral haemorrhage

Anti-inflammatory cytokines

Markers of regulatory M/M in ICH have received less attention than pro-inflammatory factors. Nevertheless, regulatory M/M have important functions in phagocytosis and clearance of toxic materials in ischaemic stroke (Lively *et al.*, 2016). Increasing evidence indicates that activated M/M in the later phase of ICH phagocytose/resorb haematoma and resolve oedema, contributing to improved white matter integrity, repair and functional recovery (Wan *et al.*, 2016; Li *et al.*, 2017). The intracerebral injection of IL-4 to enhance regulatory M/M leads to improved behavioural recovery from deficits after ICH (Yang *et al.*, 2016). Furthermore, IL-4 treatment increases striatal expression of several anti-inflammatory markers (Arg1, CCL22, CD163) and phagocytic M/M, and elevates VEGF-A-positive infiltrating neutrophils in the infarcts at Day 1 after ICH; the expression level of receptors or pathways associated with regulatory M/M such as CD206, IL-4Rα, STAT6, and peroxisome proliferator-activated receptor γ (PPARγ) remains high at Day 7 (Lively *et al.*, 2016).

IL-10 is secreted by macrophages and microglia as a regulatory cytokine, similar to IL-4 (Ouyang *et al.*, 2011). Treatment with IL-10 promotes M/M polarization to a regulatory phenotype *in vitro* (Avdic *et al.*, 2013). Interestingly, IL-10 levels in brain tissue and peripheral blood are increased in patients during the early acute phase of ICH (Shi *et al.*, 2015), as are 'M2' monocyte anti-inflammatory microparticles (Walsh *et al.*, 2017) and this may be related to the functions of TGFβ and regulatory T cells (Tregs). The peripheral frequency of Tregs in ICH patients is significantly increased, accompanied by boosted TGFβ and IL-10 levels (Shi *et al.*, 2015). Tregs ameliorate ICH-induced inflammatory injury by modulating M/M polarization to the regulatory phenotype through the IL-10/GSK3 beta/PTEN axis (Zhou *et al.*, 2017). Another study suggests that Tregs alleviate bleeding from thrombolytic therapy in stroke patients (Mao *et al.*, 2017). Microglia can produce and respond to TGFβ1, as TGFβR1 receptor is expressed on them. TGFβ1 is upregulated in the rat striatum after collagenase-induced ICH (Lively and Schlichter, 2012). Another study shows that 7 days after ICH, there is substantial TGFβR1 staining in M/M in the haematoma; as well, TGFβ1 treatment reduces pro-inflammatory mediators (e.g. *Casp1*, *Ccl3*, *Ccr5*, *Il1b*, *Ifngr1*, *Tnfa*, *Tnfrsf1b*) to a greater extent than IL-10 (Lively *et al.*, 2018). TGFβ1 treatment following ICH decreases microglial IL-6 gene expression *in vivo* and improves functional outcomes in the murine model (Taylor *et al.*, 2017). Taken together, the data show that TGFβ1 modulates microglia-mediated neuroinflammation after ICH and promotes functional recovery, suggesting that elevation of TGFβ1 may be a therapeutic target for ICH.

Sphingosine-1-phosphate receptors

Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator acting via S1P receptors (SIPR) 1–S1PR5. Microglia express all five S1PRs *in vitro* (Noda *et al.*, 2013). In tissue culture, the production of pro-inflammatory cytokines by activated microglia is reduced by the S1PR agonist fingolimod, while the neurotrophic factors usually associated with regulatory cells, brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), are elevated (Noda *et al.*, 2013). Another study using the BV-2 microglia cell line found that fingolimod suppresses the production by activated BV-2 cells of IL-1β, IL-6 and TNF-α, and also inhibits NLRP3 inflammasome (Yao *et al.*, 2019). In ischaemic white matter injury, fingolimod treatment engages STAT3 signalling and results in microglia assuming a regulatory phenotype (Qin *et al.*, 2017). Thus, S1PR engagement in microglia appears to reduce pro-inflammatory properties while elevating regulatory features. Targeting S1PR in microglia would appear to be a useful therapeutic strategy in ICH, as is discussed further below.

Peroxisome proliferator-activated receptor γ

It has been demonstrated that PPARγ agonist-induced upregulation of CD36 in macrophages enhances the ability of microglia to phagocytose red blood cells in an *in vitro* assay, helps to improve haematoma resolution *in vivo*, and reduces injury-induced deficit in a mouse model of ICH (Zhao *et al.*, 2009). Another study shows that PPARγ activators reduce proinflammatory gene (IL-1β, TNF, MMP9, and iNOS) expression, extracellular H_2O_2 level, and neuronal damage. *Pparg* (PPARγ) gene knockdown significantly inhibits microglia phagocytosis (Zhao *et al.*, 2007).

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Other regulatory mechanisms

Daily intraperitoneal injection of dopamine D2 receptor (DRD2) agonists 1 h after ICH ameliorates neurological outcome, reduces brain oedema, and lowers levels of IL-1β, CCL2 and M/M activity around the peri-haematomal region; this therapeutic benefit appears to be mediated by alpha B-crystallin (CRYAB) and enhanced cytoplasmic binding activity to NF-κB (Zhang *et al.*, 2015). DRD1 activation improves neurological outcome in part through inhibition of NLRP3-mediated inflammation in ICH mice (Wang *et al.*, 2018*a*).

The transcription factor, nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), a master-regulator of anti-oxidative defence in microglia plays an important role in augmenting their antioxidative capacity, phagocytosis, and haematoma clearance after ICH (Zhao *et al.*, 2015*a*). Moreover, the scavenger receptor CD36 associated with regulatory outcomes is upregulated in microglia treated with an Nrf2 activator (Zhao *et al.*, 2015*b*).

The cannabinoid receptor-2 (CB2R) is expressed in the brain in both glial and neuronal cells. A CB2R agonist elevates the expression of microglial regulatory markers through pPKA/cAMP-response element binding protein (CREB) signalling pathway, ameliorates brain oedema, brain damage, and neuron death and improves neurobehavioural outcomes in the early periods after ICH (Li *et al.*, 2017).

Melanocortin receptor 4 (MC4R) is expressed by microglia, neurons and astrocytes. Activation of MC4R suppresses M/M activation through AMPK/JNK/p38MAPK pathway, and diminishes neutrophil infiltration after ICH (Chen *et al.*, 2018). In general, as with pro-inflammatory mechanisms noted earlier, it is likely that more regulatory mechanisms of M/M after ICH remain to be discovered.

Treatments that target pro-inflammatory microglia/macrophages

Minocycline

Minocycline is a second generation CNS-penetrant tetracycline with several non-antimicrobial properties that could be useful to combat ICH pathophysiology, including inhibitory activity on pro-inflammatory M/M and MMPs, antioxidant capacity, and reduction of apoptotic signalling in neurons (Sanchez Mejia *et al.*, 2001; Wells *et al.*, 2003; Yong *et al.*, 2004). In culture and in mice, minocycline is observed to inhibit pro-inflammatory but not regulatory features of microglia (Kobayashi *et al.*, 2013); furthermore, it suppresses NLRP3 inflammasome activation in microglia (Lu *et al.*, 2016). In rats with ICH, minocycline administered intraperitoneally from 1 h after the injury lowers lesional MMP12 levels, microglial activation and cellular apoptosis, and this is accompanied by improvement in behavioural recovery compared to vehicle controls 7–28 days after the insult (Power *et al.*, 2003). However, when the initiation of minocycline treatment is delayed to 3 h after ICH, another group found that minocycline fails to reduce neuron loss or confers functional recovery assessed with a battery of sensory motor tests (Szymanska *et al.*, 2006). Nonetheless, in a study where minocycline is initiated 6 h after ICH, several benefits including reduction of brain damage and amelioration of neutrophil infiltration are observed (Wasserman and Schlichter, 2007). A study of ICH in mice where several treatment paradigms are varied found that early versus delayed administration, local intracerebral versus intraperitoneal delivery, and high versus low concentrations of minocycline favour recovery from ICH (Xue *et al.*, 2010). Recently, a systematic review and meta-analysis of rodent and human studies concluded that minocycline appears to be an effective therapy for stroke (Sheng *et al.*, 2018).

There is progress in translating minocycline into clinical trials in ICH. A single centre trial of 16 subjects randomized eight patients to receive 400 mg of intravenous minocycline within 24 h of injury, followed by 400 mg of oral minocycline for 4 days (Fouda *et al.*, 2017). The authors found no differences in inflammatory indices, haematoma volume or peri-haematomal oedema in minocycline subjects compared to the control arm. They concluded that intravenous minocycline was safe, that the oral doses led to delayed absorption when high concentrations of drug was desired, and that intravenous minocycline was an excellent candidate for a pre-hospital treatment trial in ICH. An earlier study of 47 ischaemic or haemorrhagic patients administered 100 mg minocycline intravenously within 24 h of stroke, and continued every 12 h for a total of five doses, did not find an efficacy signal (Kohler *et al.*, 2013); however, the dose may be too low to overcome ICH. In our own experience, in patients with traumatic spinal cord injury, intravenous minocycline initiated as an 800 mg loading dose within 12 h of trauma, and tapering down by 100 mg every 12 h until 400 mg was reached (by Day 3 of treatment), and then continued every 12 h for the next 4 days, was well tolerated (Casha *et al.*, 2012).

Overall, minocycline is a promising candidate in ICH. Besides inhibiting the pro-inflammatory features of activated microglia, its broad spectrum features including MMP inhibition, antioxidant activity, reduction of leucocyte migration and alleviation of cell death mechanisms (Sanchez Mejia *et al.*, 2001; Wells *et al.*, 2003; Yong *et al.*, 2004) counters several pathophysiological features of ICH (Fig. 1). High concentrations administered intravenously and rapidly, including in ambulance *en route* to hospitals, appear necessary for reducing the long-term sequalae of ICH.

Miscellaneous therapies

Several candidates to reduce the pro-inflammatory activity of M/M have already been introduced above, including

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complement 5a receptor antagonist (C5aRA), recombinant C1q/TNF-related protein 9 (rCTRP9), and the leukotriene B4 (LTB4) receptor antagonist. Other pro-inflammatory pathways of M/M noted earlier have been focused by potential therapeutics but as the literature has not been substantial, they are grouped herein. The NLRP3 inflammasome has been targeted by fimasartan in rats with collagenase-induced ICH, with resultant reduced oedema and improved neurological functions after the injury; activity on microglia is supported by decreased expression of NLRP3 within microglia in tissue sections of treated ICH animals, and from tissue culture studies where haemolysate-activation of microglia is mitigated by fimasartan (Yang *et al.*, 2018). A proteasome inhibitor, bortezomib, induces significant reduction of ICH-induced mRNA expression of TNF-α, IL-6, iNOS and COX2, presumably from microglia (Sinn *et al.*, 2007).

Finally, interference with TLRs impacts microglia activity and ICH outcomes but the results are varied. Resatorvid, a TLR4 antagonist, reduced ICH-induced neurogenesis and angiogenesis in rats (Lei *et al.*, 2016). Conversely, pinocembrin, an antioxidant flavonoid, inhibits the TLR4 signalling pathway, reduces pro-inflammatory microglial polarization, and protects the haemorrhagic brain (Lan *et al.*, 2017*b*).

In summary, while several medications have been proposed to inhibit the pro-inflammatory features of M/M (Fig. 4), there is scant literature on the majority of these drugs in ICH. The exception is minocycline, which counters several pathophysiological features of ICH, and where preclinical and early clinical trial results would suggest that there is room to improve the efficacy of this intervention in ICH, particularly when administered in high amounts through the intravenous route, and as early as possible after the haemorrhagic event.

Treatments that enhance regulatory features while reducing pro-inflammatory properties of microglia/macrophages

S1PR agonists

We discussed earlier that S1PR engagement in M/M reduces their pro-inflammatory properties while elevating regulatory features. The S1P agonist fingolimod thus appears to have promise in ICH, given its known safety profile as it has been used chronically in multiple sclerosis. Preclinical studies support fingolimod's activity. In mice with collagenase-induced ICH, fingolimod reduces levels of ICAM1, IL-17 and INF-γ, and improves functional outcomes (Rolland *et al.*, 2013). Another study shows that fingolimod lowers cognitive deficits in mice at 28 days of ICH (Yang *et al.*, 2019).

Given that fingolimod affects all five SIPRs, efforts have been made to use more selective agents. RP101075, a selective SIPR1 agonist unlike fingolimod that affects several S1PRs, alleviates neuronal death, oedema and neurological deficits after autologous blood-induced ICH in mice (Sun *et al.*, 2016).

Modulation of SIPR in ICH has been translated into clinical trials. Fingolimod was administered orally within 72 h of injury for three consecutive days in patients where it was found to be safe and to lower MRI-detected haematomal and peri-haematomal oedema volumes by Day 7 of ICH compared to standard management; the medication also resulted in more patients achieving full recovery of neurological functions at 3 months (Fu *et al.*, 2014).

Statins

The statins have a substantial literature as immune modulating agents, including in ICH. In rats with collagenase-induced ICH, atorvastatin inhibits the expression of pro-inflammatory markers (iNOS, TNF-α) while enhancing IL-10, TGFβ1 and sensorimotor recovery (Jung *et al.*, 2004; Ewen *et al.*, 2013; Wu *et al.*, 2017). Simvastatin increases PPARγ activation, promotes haematoma absorption and improves neurological outcomes after rat ICH; this is accompanied by the upregulation of expression of the phagocytic receptor CD36 and polarization of peri-haematomal M/M into the regulatory subtype (Wang *et al.*, 2018*b*).

A pilot clinical trial with rosuvastatin in ICH was undertaken by a Mexican group where 18 patients were drug-treated and the results compared to 57 non-treated controls in their clinical records (Tapia-Perez *et al.*, 2009). The authors reported a lower mortality rate during hospitalization in the statin versus non-treated group. A follow-up trial documented in clinicaltrials.gov has not reported results. Of interest, prior statin use may reduce the risk of developing ICH as detected in a population-based Danish registry study (Ribe *et al.*, 2019).

Miscellaneous therapies

Agonists at PPARγ receptors have been preclinically tested in ICH. Rosiglitazone, an-anti-diabetic medication, elevates CD206 expression on M/M, increases IL-10 levels in serum and peri-haematomal tissue, reduces haematoma volume and enhances neurological recovery after ICH in mice (Chang *et al.*, 2017). In rabbits, the elevation of MMP9 and disruption of blood–brain barrier integrity is decreased by rosiglitazone after ICH (Wu *et al.*, 2015).

Inhibition of the mTOR pathway represents another approach to affect M/M in ICH. Rapamycin attenuates the

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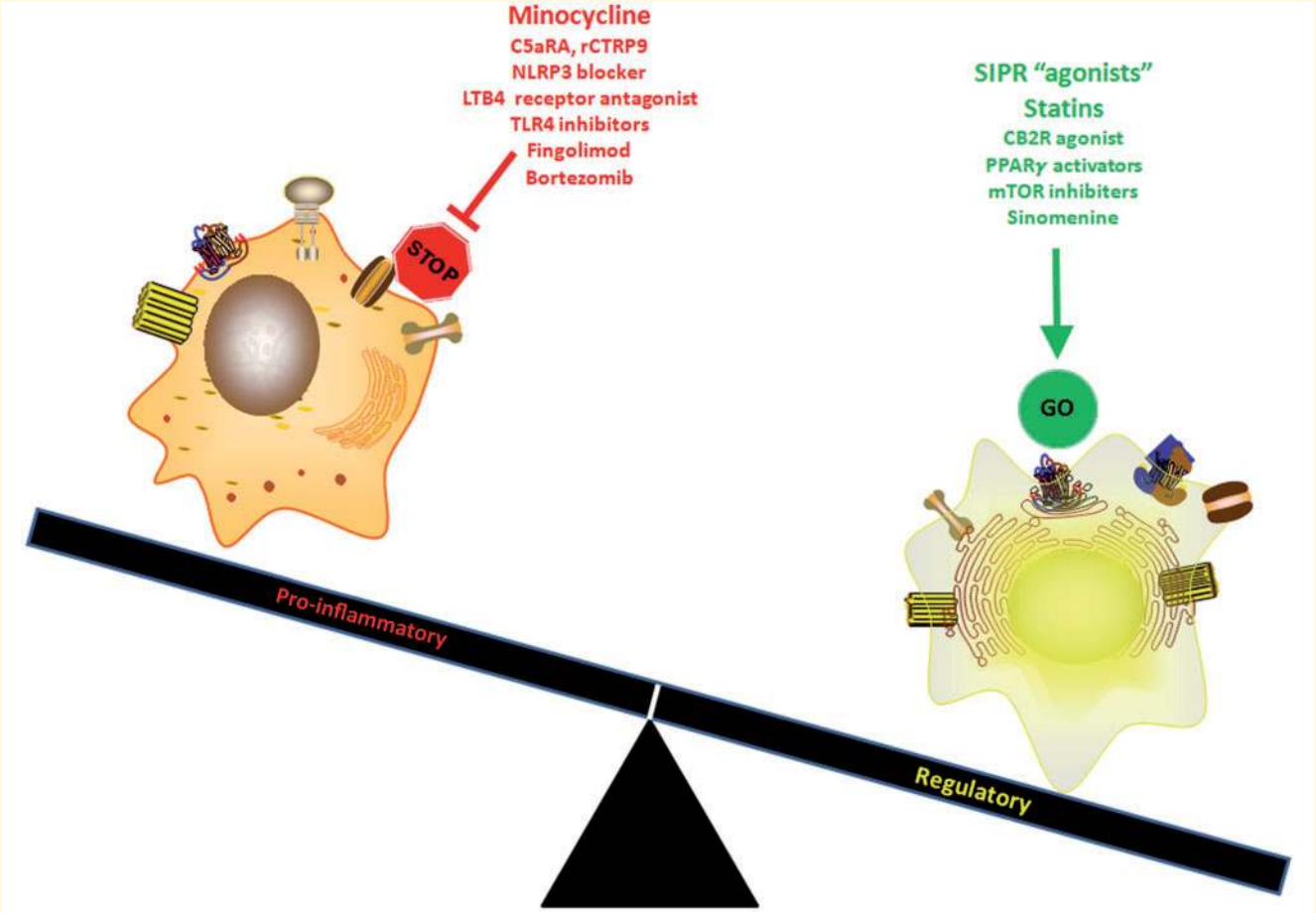

Figure 4 Medications to shift the balance of M/M to a regulatory phenotype. Illustrated are medications that either inhibit the generation or properties of pro-inflammatory cells, or promote the evolution of regulatory M/M. Medications with strong evidence and have been used in clinical trials of ICH are in bold and displayed in a larger font size.

upregulation of IL-1β, IL-6, TNF-α, Caspase-3 and INF-γ, while increasing regulatory markers such as Tregs, IL-10 and TGFβ in both peripheral blood and brain in ICH; these changes are correlated with improved recovery of neurobehavioural deficits after ICH (Lu *et al.*, 2014; Wang and Zhang, 2017).

Finally, an alkaloid from the medicinal herb *Sinomeniumacutum*, sinomenine, inhibits microglial release of TNF-α, IL-1β, IL-6 and ROS, and this is associated with reduction of NF-κB activation and attenuation of ICH-induced injury (Yang *et al.*, 2014). More recently, it was noted that sinomenine enhances the polarization of microglia into a regulatory phenotype, correspondent with protection of brain following an ICH insult; the authors propose sinomenine as a novel therapeutic for brain haemorrhage (Shi *et al.*, 2016).

In summary, while several approaches still lack the experimental support necessary for translation into humans, the statins and S1PR agonists show promise to treat ICH in patients, in part through modulating the activity of M/M by elevating their regulatory properties and reducing the pro-inflammatory functions.

Conclusion and future directions

ICH has a very poor prognosis with mortality rate of almost 50%, of which 75% of survivors are incapable of living independently after 1 year (Klebe *et al.*, 2015). This has become a great burden for society. When ICH occurs, an early inflammatory cell that responds to the injury is the microglia. The subsequent infiltration of blood-borne cells, including of monocytes that become macrophages in lesions, further propagates the neuroinflammatory cascades. Previous studies have shown that brain damage and neuronal death in ICH are always accompanied by the activation of M/M and these have multiple roles. For the early intervals after ICH, the over-represented M/M with excessive pro-inflammatory cytokines and neurotoxins appear to be on balance, detrimental. These are opposed by regulatory M/M that progressively become more numerous, and a treatment opportunity is to steer the lesion microenvironment to one that has greater representation of regulatory versus pro-inflammatory M/M (Fig. 4). These treatments

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Table 2 Pro-inflammatory and regulatory microglia/macrophage phenotypes observed in intracerebral haemorrhage

	Reference
Pro-inflammatory phenotypes	
Proinflammatory cytokines and chemokines	
IL-1β, IL-6, IL-12, IL-17A, TNF-α	Mayne *et al.*, 2001; Qureshi *et al.*, 2001; Zhang *et al.*, 2014; Yu *et al.*, 2016; Chen *et al.*, 2018
CCL2, CCL5, CCL20	Garau *et al.*, 2005; Stamatovic *et al.*, 2005; Hammond *et al.*, 2014
CXCL1, CXCL10, CX3CR1	Wolf *et al.*, 2013; Taylor *et al.*, 2014
Thrombin	
	Gingrich *et al.*, 2000; Gingrich and Traynelis, 2000; Moller *et al.*, 2000; Striggow *et al.*, 2000; Xue and Del Bigio, 2001; Noorbakhsh *et al.*, 2003; Suo *et al.*, 2003; Xue *et al.*, 2003*b*, 2006; Ohnishi *et al.*, 2013; Yang *et al.*, 2015*b*; Wan *et al.*, 2016
Matrix metalloproteinases	
MMP-3, MMP-9, MMP-12	Rajagopalan *et al.*, 1996; Rosenberg and Navratil, 1997; Romanic *et al.*, 1998; Rosenberg *et al.*, 2001; Yong *et al.*, 2001; McCawley and Matrisian, 2001; Egeblad and Werb, 2002; Gu *et al.*, 2002; Rosenberg, 2002; Power *et al.*, 2003; Yong, 2005; Kim *et al.*, 2005; Wells *et al.*, 2005; Xue *et al.*, 2006, 2009; Xue and Yong, 2006, 2008; Zhao *et al.*, 2006
Toll-like receptors	
TLR2, TLR4	Lin *et al.*, 2012; Rodriguez-Yanez *et al.*, 2012; Liu *et al.*, 2016
Others	
NF-κB, iNOS, IFNγ, HMGB1	Benveniste, 1998; Wagner, 2007; Ohnishi *et al.*, 2011; Yang *et al.*, 2013; Zhang *et al.*, 2014; Lan *et al.*, 2017*a*; Wang *et al.*, 2017*a*
Complement C3a, C5a	Yang *et al.*, 2006; Garrett *et al.*, 2009; Li *et al.*, 2014; Yuan *et al.*, 2017
G-protein-coupled E prostanoidreceptors (EPR)1, EPR3	Singh *et al.*, 2013; Zhao *et al.*, 2015*c*; Han *et al.*, 2016
T cell immunoglobulin and mucin domain-3 (Tim-3)	Xu *et al.*, 2013
Heme oxygenase 1 (HO-1)	Wang and Dore, 2007; Zhang *et al.*, 2017; Li *et al.*, 2018
Nod-like receptor pyrin domain-containing(NLRP) 3/6	Yuan *et al.*, 2015; Wang *et al.*, 2017*b*; Yao *et al.*, 2017; Ye *et al.*, 2017
Soluble epoxide hydrolase (sEH)	Wu *et al.*, 2017*a*
Adiponectin receptor 1 (AdipoR1)	Zhao *et al.*, 2018
Regulatory phenotypes	
Anti-inflammatory cytokines	
IL-1Ra, IL-4, IL-4Ra, IL-10	Ouyang *et al.*, 2011; Avdic *et al.*, 2013; Shi *et al.*, 2015; Lively *et al.*, 2016; Yang *et al.*, 2016
TGFβ1	Lively and Schlichter, 2012; Shi *et al.*, 2015; Taylor *et al.*, 2017
Others	
Dopamine D1 receptor (DRD1), DRD2	Zhang *et al.*, 2015; Wang *et al.*, 2018*a*, *b*
Nuclear factor-erythroid 2 p45-related factor 2 (Nrf2)	Zhao *et al.*, 2015*a*, *b*
Cannabinoid receptor-2 (CB2R)	Li *et al.*, 2017
Melanocortin receptor 4 (MC4R)	Chen *et al.*, 2018
Peroxisome proliferator-activated receptor γ (PPARγ)	Zhao *et al.*, 2007, 2009
Sphingosine-1-phosphate (S1P)	Noda *et al.*, 2013; Rolland *et al.*, 2013; Fu *et al.*, 2014; Yang *et al.*, 2019
Regulatory T lymphocytes (Tregs)	Mao *et al.*, 2017; Zhou *et al.*, 2017

may have to be considered in a time-dependent sequence, with prompt administration of medications to suppress pro-inflammatory M/M, while those that elevate regulatory properties may be more suitable at later periods after the insult. Alternately, the medications that polarize M/M from pro-inflammatory into a regulatory state may be applicable immediately after ICH, in order to redress the imbalance of M/M neuroinflammation right after haemorrhage onset. The possibility also exists of applying medications in combination to suppress pro-inflammatory while elevating regulatory features of M/M from the beginning of the injury. While much remains to be done to define how best to foster recovery from ICH, it is clear that to have optimal improvement, the attendant over-representation of M/M in brain haemorrhage must be addressed.

There remain many challenges, opportunities and future directions in modulating M/M to improve the prognosis of ICH. First, while M/M of pro-inflammatory and regulatory phenotypes can be documented, many features of one often can be found in the other, complicating the distinction between the subsets. Second, many of the medications described herein are tested in a reductionist approach, using cells in culture or for short periods in an animal model, with focus on a limited number of targets; a much more comprehensive investigation in preclinical studies would be needed, and translation to human clinical trials would be necessary to evaluate the utility of particular drugs in clinical subjects after an ICH injury. In these studies, highly advanced flow cytometry and other multiplex assays should be applied to capture the response of

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circulating monocytes to drug treatment; if possible, PET scans that seek to evaluate brain M/M activity and their phenotype could be included.

Another challenge in ICH is that many medications of interest to ICH lack a compelling corroborative dataset from other groups and much more research would need to be conducted to establish a drug's utility in the condition. The exception, as noted above, are minocycline, statins and S1PR agonists, and it is notable that there are clinical efforts with these drugs. It would also be reasonable to consider using these drugs in combination after ICH, although that would substantially elevate the complexity of the clinical trial design. Moreover, preclinical studies at least with minocycline have suggested that the best therapeutic response is through fast intervention, at high doses, and targeting the lesion as locally as possible with medication (Xue *et al.*, 2010).

In summary, numerous mechanisms lead to the generation of pro-inflammatory M/M following ICH (Fig. 2). Whether several of these act in concert or in a cascade, and whether there is a precise temporal sequence by which each is expressed, remains to be addressed. Moreover, whether particular mechanisms are more dominant than others in activating pro-inflammatory M/M is worthy of investigation, as a predominant hierarchical target could lead to a more effective therapy. Finally, while medications listed in Fig. 4 affect M/M, it should be noted that their therapeutic effect when used in whole animals or humans could be due to additional targets, such as the reduction of leucocyte migration into lesions. In this light, it is reasonable to use medications in ICH that have a broad spectrum of activity, including on M/M, so as to overcome the many pathophysiological mechanisms of ICH. The therapy of ICH will remain complicated for some time to come, but the pathophysiological processes are increasingly being targeted, and medications with effects on redressing the phenotype of M/M represent one step in the right direction to improve prognosis from devastating ICH.

Funding

The authors acknowledge operating grant support from the National Natural Science Foundation of China (grants: 81870942, 81471174 and 81520108011), National Key Research and Development Program of China (grant: 2018YFC1312200), and Innovation Scientists and Technicians Troop Constructions Projects of Henan Province of China (for M.X.); and from the Canadian Institutes of Health Sciences (V.W.Y.).

Competing interests

The authors report no competing interests.

References

Altumbabic M, Peeling J, Del Bigio MR. Intracerebral hemorrhage in the rat. Effects of haematoma aspiration. Stroke 1998; 29: 1917–22.

An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: an update. J Stroke 2017; 19: 3–10.

Arvin B, Neville LF, Barone FC, Feuerstein GZ. The role of inflammation and cytokines in brain injury. Neurosci Biobehav Rev 1996; 20: 445–52.

Avdic S, Cao JZ, McSharry BP, Clancy LE, Brown R, Steain M, et al. Human cytomegalovirus interleukin-10 polarizes monocytes toward a deactivated M2c phenotype to repress host immune responses. J Virol 2013; 87: 10273–82.

Beaino W, Janssen B, Kooij G, van der Pol SMA, van Het Hof B, van Horssen J, et al. Purinergic receptors P2Y12R and P2X7R: potential targets for PET imaging of microglia phenotypes in multiple sclerosis. J Neuroinflammation 2017; 14: 259.

Benveniste EN. Cytokine actions in the central nervous system. Cytokine Growth Factor Rev 1998; 9: 259–75.

Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: effect on regional cerebral blood flow. Surg Neurol 1988; 29: 101–7.

Bullock R, Mendelow AD, Teasdale GM, Graham DI. Intracranial hemorrhage induced at arterial pressure in the rat. Part 1: description of technique, ICP changes and neuropathologic findings. Neurol Res 1984; 6: 184–8.

Casha S, Zygun D, McGowan D, Baines I, Yong VW, Hurlbert RJ. Results of a phase II placebo-controlled randomized trial of minocycline in acute spinal cord injury. Brain 2012; 135: 1224–36.

Chang CF, Wan J, Li Q, Renfroe SC, Heller NM, Wang J. Alternative activation-skewed microglia/macrophages promote haematoma resolution in experimental intracerebral hemorrhage. Neurobiol Dis 2017; 103: 54–69.

Chen S, Zhao L, Sherchan P, Ding Y, Yu J, Nowrangi D, et al. Activation of melanocortin receptor 4 with RO27-3225 attenuates neuroinflammation through AMPK/JNK/p38 MAPK pathway after intracerebral hemorrhage in mice. J Neuroinflammation 2018; 15: 106.

Cordonnier C, Demchuk A, Ziai W, Anderson CS. Intracerebral haemorrhage: current approaches to acute management. Lancet (London, England) 2018; 392: 1257–68.

Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161–74.

Ewen T, Qiuting L, Chaogang T, Tao T, Jun W, Liming T, et al. Neuroprotective effect of atorvastatin involves suppression of TNF-α and upregulation of IL-10 in a rat model of intracerebral hemorrhage. Cell Biochem Biophys 2013; 66: 337–46.

Fouda AY, Newsome AS, Spellicy S, Waller JL, Zhi W, Hess DC, et al. Minocycline in acute cerebral hemorrhage: an early phase randomized trial. Stroke 2017; 48: 2885–7.

Fu Y, Hao J, Zhang N, Ren L, Sun N, Li YJ, et al. Fingolimod for the treatment of intracerebral hemorrhage: a 2-arm proof-of-concept study. JAMA Neurol 2014; 71: 1092–101.

Garau A, Bertini R, Colotta F, Casilli F, Bigini P, Cagnotto A, et al. Neuroprotection with the CXCL8 inhibitor repertaxin in transient brain ischemia. Cytokine 2005; 30: 125–31.

Garcia JH, Ho KL, Caccamo DV, Intracerebral hemorrhage: pathology of selected topics. In: Kase CS, Caplan LR, editors. Intracerebral hemorrhage. Boston: Butterworth-Heinemann; 1994. p. 45–72.

Garrett MC, Otten ML, Starke RM, Komotar RJ, Magotti P, Lambris JD, et al. Synergistic neuroprotective effects of C3a and C5a receptor blockade following intracerebral hemorrhage. Brain Res 2009; 1298: 171–7.

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Gingrich MB, Junge CE, Lyuboslavsky P, Traynelis SF. Potentiation of NMDA receptor function by the serine protease thrombin. J Neurosci 2000; 20: 4582–95.
Gingrich MB, Traynelis SF. Serine proteases and brain damage - is there a link? Trends Neurosci 2000; 23: 399–407.
Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, et al. S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science 2002; 297: 1186–90.
Hammond MD, Taylor RA, Mullen MT, Ai Y, Aguila HL, Mack M, et al. CCR2+ Ly6C(hi) inflammatory monocyte recruitment exacerbates acute disability following intracerebral hemorrhage. J Neurosci 2014; 34: 3901–9.
Han L, Cai W, Mao L, Liu J, Li P, Leak RK, et al. Rosiglitazone promotes white matter integrity and long-term functional recovery after focal cerebral ischemia. Stroke 2015; 46: 2628–36.
Han X, Lan X, Li Q, Gao Y, Zhu W, Cheng T, et al. Inhibition of prostaglandin E2 receptor EP3 mitigates thrombin-induced brain injury. J Cereb Blood Flow Metab 2016; 36: 1059–74.
Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke 2004; 35: 1744–9.
Kim YS, Kim SS, Cho JJ, Choi DH, Hwang O, Shin DH, et al. Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. J Neurosci 2005; 25: 3701–11.
Kingman TA, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral mass: time-related effects on local cerebral blood flow. J Neurosurg 1987; 67: 732–8.
Klebe D, McBride D, Flores JJ, Zhang JH, Tang J. Modulating the immune response towards a neuroregenerative peri-injury milieu after cerebral hemorrhage. J Neuroimmune Pharmacol 2015; 10: 576–86.
Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, et al. Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis 2013; 4: e525.
Kohler E, Prentice DA, Bates TR, Hankey GJ, Claxton A, van Heerden J, et al. Intravenous minocycline in acute stroke: a randomized, controlled pilot study and meta-analysis. Stroke 2013; 44: 2493–9.
Lan X, Han X, Li Q, Li Q, Gao Y, Cheng T, et al. Pinocembrin protects hemorrhagic brain primarily by inhibiting toll-like receptor 4 and reducing M1 phenotype microglia. Brain Behav Immun 2017b; 61: 326–39.
Lan X, Han X, Li Q, Yang Q-W, Wang J. Modulators of microglial activation and polarization after intracerebral haemorrhage. Nat Rev Neurol 2017a; 13: 420–33.
Lei C, Wu B, Cao T, Liu M, Hao Z. Brain recovery mediated by toll-like receptor 4 in rats after intracerebral hemorrhage. Brain Res 2016; 1632: 1–8.
Li G, Fan RM, Chen JL, Wang CM, Zeng YC, Han C, et al. Neuroprotective effects of argatroban and C5a receptor antagonist (PMX53) following intracerebral haemorrhage. Clin Exp Immunol 2014; 175: 285–95.
Li QQ, Li LJ, Wang XY, Sun YY, Wu J. Research progress in understanding the relationship between heme oxygenase-1 and intracerebral hemorrhage. Front Neurol 2018; 9: 682.
Li L, Tao Y, Feng Z, Niu Y, Tan Q, Zheng H, et al. Inflammatory regulations by driving microglial M2 polarization: neuroprotective effects of cannabinoid receptor-2 activation in intracerebral hemorrhage. Front Immunol 2017; 14: 112.
Lin S, Yin Q, Zhong Q, Lv FL, Zhou Y, Li JQ, et al. Heme activates TLR4-mediated inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage. J Neuroinflammation 2012; 9: 46.
Liu D-L, Zhao L-X, Zhang S, Du J-R. Peroxiredoxin 1-mediated activation of TLR4/NF-kappa B pathway contributes to neuroinflammatory injury in intracerebral hemorrhage. Int Immunopharmacol 2016; 41: 82–9.
Lively S, Hutchings S, Schlichter LC. Molecular and cellular responses to interleukin-4 treatment in a rat model of transient ischemia. J Neuropathol Exp Neurol 2016; 75: 1058–71.
Lively S, Lam D, Wong R, Schlichter LC. Comparing effects of transforming growth factor beta 1 on microglia from rat and mouse: transcriptional profiles and potassium channels. Front Cell Neurosci 2018; 3: 115.
Lively S, Schlichter LC. Age-related comparisons of evolution of the inflammatory response after intracerebral hemorrhage in rats. Transl Stroke Res 2012; 3: 132–46.
Lu Q, Gao L, Huang L, Ruan L, Yang J, Huang W, et al. Inhibition of mammalian target of rapamycin improves neurobehavioral deficit and modulates immune response after intracerebral hemorrhage in rat. J Neuroinflammation 2014; 11: 44.
Lu Y, Xiao G, Luo W. Minocycline suppresses NLRP3 inflammasome activation in experimental ischemic stroke. Neuroimmunomodulation 2016; 23: 230–8.
Mao L, Li P, Zhu W, Cai W, Liu Z, Wang Y, et al. Regulatory T cells ameliorate tissue plasminogen activator-induced brain haemorrhage after stroke. Brain 2017; 140: 1914–31.
Mayne M, Ni W, Yan HJ, Xue M, Johnston JB, Del Bigio MR, et al. Antisense oligodeoxynucleotide inhibition of tumor necrosis factor-alpha expression is neuroprotective after intracerebral hemorrhage. Stroke 2001; 32: 240–8.
McCawley L, Matrisian L. Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol 2001; 13: 534–40.
Michell-Robinson MA, Touil H, Healy LM, Owen DR, Durafourt BA, Bar-Or A, et al. Roles of microglia in brain development, tissue maintenance and repair. Brain 2015; 138: 1138–59.
Mishra MK, Yong VW. Myeloid cells: targets of medications in multiple sclerosis. Nat Rev Neurol 2016; 12: 539–51.
Moller T, Hanisch UK, Ransom BR. Thrombin-induced activation of cultured rodent microglia. J Neurochem 2000; 75: 1539–47.
Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8: 958–69.
Mracko E, Veltkamp R. Neuroinflammation after intracerebral hemorrhage. Front Cell Neurosci 2018; 8: 1–13.
Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM. Early hemodynamic changes in experimental intracerebral hemorrhage. J Neurosurg 1986; 65: 697–703.
Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005; 308: 1314–8.
Nishino A, Suzuki M, Ohtani H, Motohashi O, Umezawa K, Nagura H, et al. Thrombin may contribute to the pathophysiology of central nervous system injury. J Neurotrauma 1993; 10: 167–79.
Noda H, Takeuchi H, Mizuno T, Suzumura A. Fingolimod phosphate promotes the neuroprotective effects of microglia. J Neuroimmunol 2013; 256: 13–8.
Noorbakhsh F, Vergnolle N, Hollenberg MD, Power C. Proteinase-activated receptors in the nervous system. Nat Rev Neurosci 2003; 4: 981–90.
Ohnishi M, Katsuki H, Fukutomi C, Takahashi M, Motomura M, Fukunaga M, et al. HMGB1 inhibitor glycyrrhizin attenuates intracerebral hemorrhage-induced injury in rats. Neuropharmacology 2011; 61: 975–80.
Ohnishi M, Monda A, Takemoto R, Matsuoka Y, Kitamura C, Ohashi K, et al. Sesamin suppresses activation of microglia and p44/42 MAPK pathway, which confers neuroprotection in rat intracerebral hemorrhage. Neuroscience 2013; 232: 45–52.
Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 2011; 29: 71–109.
Poon CC, Sarkar S, Yong VW, Kelly J. Glioblastoma-associated microglia and macrophages: targets for therapies to improve prognosis. Brain 2017; 140: 1548–60.

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Power C, Henry S, Del Bigio MR, Larsen PH, Corbett D, Imai Y, et al. Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. Ann Neurol 2003; 53: 731–42.
Prinz M, Erny D, Hagemeyer N. Ontogeny and homeostasis of CNS myeloid cells. Nat Immunol 2017; 18: 385–92.
Qin C, Fan WH, Liu Q, Shang K, Murugan M, Wu LJ, et al. Fingolimod protects against ischemic white matter damage by modulating microglia toward M2 polarization via STAT3 pathway. Stroke 2017; 48: 3336–46.
Qureshi AI, Suri MF, Ling GS, Khan J, Guterman LR, Hopkins LN. Absence of early proinflammatory cytokine expression in experimental intracerebral hemorrhage. Neurosurgery 2001; 49: 416–20.
Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996; 98: 2572–9.
Ribe AR, Vestergaard CH, Vestergaard M, Fenger-Grøn M, Pedersen HS, Lietzen LW, et al. Statins and risk of intracerebral haemorrhage in a stroke-free population: a Nationwide Danish Propensity Score Matched Cohort Study. EClin Med 2019; 8: 78–84.
Rodriguez-Yanez M, Brea D, Arias S, Blanco M, Pumar JM, Castillo J, et al. Increased expression of Toll-like receptors 2 and 4 is associated with poor outcome in intracerebral hemorrhage. J Neuroimmunol 2012; 247: 75–80.
Rolland WB, Lekic T, Krafft PR, Hasegawa Y, Altay O, Hartman R, et al. Fingolimod reduces cerebral lymphocyte infiltration in experimental models of rodent intracerebral hemorrhage. Exp Neurol 2013; 241: 45–55.
Romanic AM, White RF, Arleth AJ, Ohlstein EH, Barone FC. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats-inhibition of matrix metalloproteinase-9 reduces infarct size. Stroke 1998; 29: 1020–30.
Rosenberg GA. Matrix metalloproteinases in neuroinflammation. Glia 2002; 39: 279–91.
Rosenberg GA, Cunningham LA, Wallace J, Alexander S, Estrada EY, Grossetete M, et al. Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. Brain Res 2001; 893: 104–12.
Rosenberg GA, Estrada EY, Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke 1998; 29: 2189–95.
Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. Stroke 1990; 21: 801–7.
Rosenberg GA, Navratil M. Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. Neurology 1997; 48: 921–6.
Sanchez Mejia RO, Ona VO, Li M, Friedlander RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. Neurosurgery 2001; 48: 1393–9.
Shao A, Zhu Z, Li L, Zhang S, Zhang J. Emerging therapeutic targets associated with the immune system in patients with intracerebral haemorrhage (ICH): from mechanisms to translation. EBioMedicine 2019.
Sheng Z, Liu Y, Li H, Zheng W, Xia B, Zhang X, et al. Efficacy of minocycline in acute ischemic stroke: a systematic review and meta-analysis of rodent and clinical studies. Front Neurol 2018; 9: 1103.
Shi H, Zheng K, Su Z, Su H, Zhong M, He X, et al. Sinomenine enhances microglia M2 polarization and attenuates inflammatory injury in intracerebral hemorrhage. J Neuroimmunol 2016; 299: 28–34.
Shi L, Qin J, Song B, Wang QM, Zhang R, Liu X, et al. Increased frequency of circulating regulatory T cells in patients with acute cerebral hemorrhage. Neurosci Lett 2015; 591: 115–20.
Shi K, Tian DC, Li ZG, Ducruet AF, Lawton MT, Shi FD. Global brain inflammation in stroke. Lancet Neurol 2019.
Singh N, Ma B, Leonardo CC, Ahmad AS, Narumiya S, Dore S. Role of PGE(2) EP1 receptor in intracerebral hemorrhage-induced brain injury. Neurotox Res 2013; 24: 549–59.
Sinn DI, Lee ST, Chu K, Jung KH, Kim EH, Kim JM, et al. Proteasomal inhibition in intracerebral hemorrhage: neuroprotective and anti-inflammatory effects of bortezomib. Neurosci Res 2007; 58: 12–8.
Stamatovic SM, Shakui P, Keep RF, Moore BB, Kunkel SL, Van Rooijen N, et al. Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. J Cereb Blood Flow Metab 2005; 25: 593–606.
Striggow F, Riek M, Breder J, Henrich Noack P, Reymann KG, Reiser G. The protease thrombin is an endogenous mediator of hippocampal neuroprotection against ischemia at low concentrations but causes degeneration at high concentrations. Proc Natl Acad Sci USA 2000; 97: 2264–9.
Sun N, Shen Y, Han W, Shi K, Wood K, Fu Y, et al. Selective sphingosine-1-phosphate receptor 1 modulation attenuates experimental intracerebral hemorrhage. Stroke 2016; 47: 1899–906.
Suo Z, Wu M, Citron BA, Gao C, Festoff BW. Persistent protease-activated receptor 4 signaling mediates thrombin-induced microglial activation. J Biol Chem 2003; 278: 31177–83.
Szymanska A, Biernaskie J, Laidley D, Granter-Button S, Corbett D. Minocycline and intracerebral hemorrhage: influence of injury severity and delay to treatment. Exp Neurol 2006; 197: 189–96.
Tapia-Perez H, Sanchez-Aguilar M, Torres-Corzo JG, Rodriguez-Leyva I, Gonzalez-Aguirre D, Gordillo-Moscoso A, et al. Use of statins for the treatment of spontaneous intracerebral hemorrhage: results of a pilot study. Cen Eur Neurosurg 2009; 70: 15–20.
Taylor RA, Chang CF, Goods BA, Hammond MD, Mac Grory B, Ai Y, et al. TGF-beta1 modulates microglial phenotype and promotes recovery after intracerebral hemorrhage. J Clin Invest 2017; 127: 280–92.
Taylor RA, Hammond MD, Ai Y, Sansing LH. CX3CR1 signaling on monocytes is dispensable after intracerebral hemorrhage. PLoS One 2014; 9: e114472.
Wagner KR. Modeling intracerebral hemorrhage: glutamate, nuclear factor-kappa B signaling and cytokines. Stroke 2007; 38: 753–8.
Wagner KR, Xi GH, Hua Y, Zuccarello M, de Courten-Myers GM, Broderick JP, et al. Ultra-early clot aspiration after lysis with tissue plasminogen activator in a porcine model of intracerebral hemorrhage: edema reduction and blood-brain barrier protection. J Neurosurg 1999; 90: 491–8.
Walsh KB, Campos B, Hart K, Thakar C, Adeoye O. M2 monocyte microparticles are increased in intracerebral hemorrhage. J Stroke Cerebrovasc Dis 2017; 26: 2369–75.
Wan S, Cheng Y, Jin H, Guo D, Hua Y, Keep RF, et al. Microglia activation and polarization after intracerebral hemorrhage in mice: the role of protease-activated receptor-1. Transl Stroke Res 2016; 7: 478–87.
Wang Y, Chen Q, Tan Q, Feng Z, He Z, Tang J, et al. Simvastatin accelerates haematoma resolution after intracerebral hemorrhage in a PPAR gamma-dependent manner. Neuropharmacology 2018b; 128: 244–54.
Wang J, Dore S. Heme oxygenase-I exacerbates early brain injury after intracerebral haemorrhage. Brain 2007; 130: 1643–52.
Wang PF, Li ZG, Zhang Y, Ju XH, Liu XW, Zhou AM, et al. NLRP6 inflammasome ameliorates brain injury after intracerebral hemorrhage. Front Cell Neurosci 2017b; 11: 206.
Wang D, Liu K, Wake H, Teshigawara K, Mori S, Nishibori M. Anti-high mobility group box-1 (HMGB1) antibody inhibits hemorrhage-induced brain injury and improved neurological deficits in rats. Sci Rep 2017a; 7: 46243.
Wang T, Nowrangi D, Yu L, Lu T, Tang J, Han B, et al. Activation of dopamine D1 receptor decreased NLRP3-mediated inflammation in intracerebral hemorrhage mice. J Neuroinflammation 2018a; 15: 2.

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Wang J, Tsirka SE. Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. Brain 2005; 128: 1622–33.
Wang JP, Zhang MY. Role for target of rapamycin (mTOR) signal pathway in regulating neuronal injury after intracerebral hemorrhage. Cell Physiol Biochem 2017; 41: 145–53.
Wasserman JK, Schlichter LC. Minocycline protects the blood-brain barrier and reduces edema following intracerebral hemorrhage in the rat. Exp Neurol 2007; 207: 227–37.
Wells JE, Biernaskie J, Szymanska A, Larsen PH, Yong VW, Corbett D. Matrix metalloproteinase (MMP)-12 expression has a negative impact on sensorimotor function following intracerebral haemorrhage in mice. Eur J Neurosci 2005; 21: 187–96.
Wells JE, Hurlbert RJ, Fehlings MG, Yong VW. Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. Brain 2003; 126: 1628–37.
Wolf Y, Yona S, Kim KW, Jung S. Microglia, seen from the CX3CR1 angle. Front Cell Neurosci 2013; 7: 26.
Wu C-H, Shyue S-K, Hung T-H, Wen S, Lin C-C, Chang C-F, et al. Genetic deletion or pharmacological inhibition of soluble epoxide hydrolase reduces brain damage and attenuates neuroinflammation after intracerebral hemorrhage. J Neuroinflammation 2017; 14: 230.
Wu G, Wu J, Jiao Y, Wang L, Wang F, Zhang Y. Rosiglitazone infusion therapy following minimally invasive surgery for intracerebral hemorrhage evacuation decreases matrix metalloproteinase-9 and blood-brain barrier disruption in rabbits. BMC Neurol 2015; 17: 37.
Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 2016; 44: 450–62.
Xu C, Wang T, Cheng S, Liu Y. Increased expression of T cell immunoglobulin and mucin domain 3 aggravates brain inflammation via regulation of the function of M/M after intracerebral hemorrhage in mice. J Neuroinflammation 2013; 10: 141.
Xue M, Balasubramaniam J, Buist R, Peeling J, Del Bigio MR. Periventricular/intraventricular hemorrhage in neonatal mouse cerebrum. J Neuropathol Exp Neurol 2003a; 62: 1154–65.
Xue M, Balasubramaniam J, Del Bigio MR. Brain inflammation following intracerebral hemorrhage. Curr Neuropharmacol 2003b; 1: 325–32.
Xue M, Del Bigio MR. Intracerebral injection of autologous whole blood in rats: time course of inflammation and cell death. Neurosci Lett 2000; 283: 230–2.
Xue M, Del Bigio MR. Acute tissue damage after injections of thrombin and plasmin into rat striatum. Stroke 2001; 32: 2164–9.
Xue M, Del Bigio MR. Immune pre-activation exacerbates hemorrhagic brain injury in immature mouse brain. J Neuroimmunol 2005; 165: 75–82.
Xue M, Fan Y, Liu S, Zygun DA, Demchuk A, Yong VW. Contributions of multiple proteases to neurotoxicity in a mouse model of intracerebral haemorrhage. Brain 2009; 132: 26–36.
Xue M, Hollenberg MD, Yong VW. Combination of thrombin and matrix metalloproteinase-9 exacerbates neurotoxicity in cell culture and intracerebral hemorrhage in mice. J Neurosci 2006; 26: 10281–91.
Xue M, Mikliaeva EI, Casha S, Zygun D, Demchuk A, Yong VW. Improving outcomes of neuroprotection by minocycline. Guides from cell culture and intracerebral hemorrhage in mice. Am J Pathol 2010; 176: 1193–202.
Xue M, Yong VW. Matrix metalloproteinases in intracerebral hemorrhage. Neurol Res 2008; 30: 775–82.
Yang S, Chen Y, Deng X, Jiang W, Li B, Fu Z, et al. Hemoglobin-induced nitric oxide synthase overexpression and nitric oxide production contribute to blood-brain barrier disruption in the rat. J Mol Neurosci 2013; 51: 352–63.
Yang J, Ding S, Huang W, Hu J, Huang S, Zhang Y, et al. Interleukin-4 ameliorates the functional recovery of intracerebral hemorrhage through the alternative activation of microglia/macrophage. Front Neurosci 2016; 10: 61.
Yang Z, Dong S, Zheng Q, Zhang L, Tan X, Zou J, et al. FTY720 attenuates iron deposition and glial responses in improving delayed lesion and long-term outcomes of collagenase-induced intracerebral hemorrhage. Brain Res 2019; 1718: 91–102.
Yang Z, Liu Y, Yuan F, Li Z, Huang S, Shen H, et al. Sinomenine inhibits microglia activation and attenuates brain injury in intracerebral hemorrhage. Mol Immunol 2014; 60: 109–14.
Yang S, Nakamura T, Hua Y, Keep RF, Younger JG, He Y, et al. The role of complement C3 in intracerebral hemorrhage-induced brain injury. J Cereb Blood Flow Metab 2006; 26: 1490–5.
Yang X, Sun J, Kim TJ, Kim YJ, Ko SB, Kim CK, et al. Pretreatment with low-dose fimasartan ameliorates NLRP3 inflammasome-mediated neuroinflammation and brain injury after intracerebral hemorrhage. Exp Neurol 2018; 310: 22–32.
Yang L, Tang J, Chen Q, Jiang B, Zhang B, Tao Y, et al. Hyperbaric oxygen preconditioning attenuates neuroinflammation after intracerebral hemorrhage in rats by regulating microglia characteristics. Brain Res 2015a; 1627: 21–30.
Yang Y, Zhang M, Kang X, Jiang C, Zhang H, Wang P, et al. Thrombin-induced microglial activation impairs hippocampal neurogenesis and spatial memory ability in mice. Behav Brain Funct 2015b; 11: 30.
Yao ST, Cao F, Chen JL, Chen W, Fan RM, Li G, et al. NLRP3 is required for complement-mediated caspase-1 and IL-1beta activation in ICH. J Mol Neurosci 2017; 61: 385–95.
Yao S, Li L, Sun X, Hua J, Zhang K, Hao L, et al. FTY720 inhibits MPP + induced microglial activation by affecting NLRP3 inflammasome activation. J Neuroimmune Pharmacol 2019; 14: 478–92.
Ye X, Zuo D, Yu L, Zhang L, Tang J, Cui C, et al. ROS/TXNIP pathway contributes to thrombin induced NLRP3 inflammasome activation and cell apoptosis in microglia. Biochem Biophys Res Commun 2017; 485: 499–505.
Yong VW. Metalloproteinases: mediators of pathology and regeneration in the CNS. Nat Rev Neurosci 2005; 6: 931–44.
Yong VW, Power C, Forsyth P, Edwards DR. Metalloproteinases in biology and pathology of the nervous system. Nat Rev Neurosci 2001; 2: 502–11.
Yong HYF, Rawji KS, Ghorbani S, Xue M, Yong VW. The benefits of neuroinflammation for the repair of the injured central nervous system. Cell Mol Immunol 2019; 16: 540–6.
Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. Lancet Neurol 2004; 3: 744–51.
Yu A, Duan H, Zhang T, Pan Y, Kou Z, Zhang X, et al. IL-17A promotes microglial activation and neuroinflammation in mouse models of intracerebral haemorrhage. Mol Immunol 2016; 73: 151–7.
Yuan B, Fu F, Huang S, Lin C, Yang G, Ma K, et al. C5a/C5aR pathway plays a vital role in brain inflammatory injury via initiating Fgl-2 in intracerebral hemorrhage. Mol Neurobiol 2017; 54: 6187–97.
Yuan B, Shen H, Lin L, Su T, Zhong S, Yang Z. Recombinant adenovirus encoding NLRP3 RNAi attenuate inflammation and brain injury after intracerebral hemorrhage. J Neuroimmunol 2015; 287: 71–5.
Zhang Y, Chen Y, Wu J, Manaenko A, Yang P, Tang J, et al. Activation of dopamine D2 receptor suppresses neuroinflammation through alpha B-crystalline by inhibition of NF-kappa B nuclear translocation in experimental ICH mice model. Stroke 2015; 46: 2637–46.
Zhang Z, Liu Y, Huang Q, Su Y, Zhang Y, Wang G, et al. NF-kappaB activation and cell death after intracerebral hemorrhage in patients. Neurol Sci 2014; 35: 1097–102.
Zhang Z, Song Y, Zhang Z, Li D, Zhu H, Liang R, et al. Distinct role of heme oxygenase-1 in early- and late-stage intracerebral hemorrhage in 12-month-old mice. J Cereb Blood Flow Metab 2017; 37: 25–38.

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Zhao L, Chen S, Sherchan P, Ding Y, Zhao W, Guo Z, et al. Recombinant CTRP9 administration attenuates neuroinflammation via activating adiponectin receptor 1 after intracerebral hemorrhage in mice. J Neuroinflammation 2018; 15: 215.

Zhao X, Grotta J, Gonzales N, Aronowski J. Haematoma resolution as a therapeutic target. The role of microglia/macrophages. Stroke 2009; 40: S92–4.

Zhao X, Sun G, Ting SM, Song S, Zhang J, Edwards NJ, et al. Cleaning up after ICH: the role of Nrf2 in modulating microglia function and haematoma clearance. J Neurochem 2015a; 133: 144–52.

Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, et al. Haematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. Ann Neurol 2007; 61: 352–62.

Zhao X, Sun G, Zhang J, Ting SM, Gonzales N, Aronowski J. Dimethyl fumarate protects brain from damage produced by intracerebral hemorrhage by mechanism involving Nrf2. Stroke 2015b; 46: 1923–8.

Zhao BQ, Wang S, Kim HY, Storrie H, Rosen BR, Mooney DJ, et al. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006; 12: 441–5.

Zhao X, Wu T, Chang CF, Wu H, Han X, Li Q, et al. Toxic role of prostaglandin E2 receptor EP1 after intracerebral hemorrhage in mice. Brain Behav Immun 2015c; 46: 293–310.

Zhou K, Zhong Q, Wang Y-C, Xiong X-Y, Meng Z-Y, Zhao T, et al. Regulatory T cells ameliorate intracerebral hemorrhage-induced inflammatory injury by modulating microglia/macrophage polarization through the IL-10/GSK3 beta/PTEN axis. J Cereb Blood Flow Metab 2017; 37: 967–79.

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