Published in final edited form as:

Nat Rev Neurol. 2017 July ; 13(7): 420–433. doi:10.1038/nrneurol.2017.69.

Modulators of microglial activation and polarization after intracerebral haemorrhage

Xi Lan¹, Xiaoning Han¹, Qian Li¹, Qing-Wu Yang², and Jian Wang¹

¹Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross Building 370B, Baltimore, Maryland 21205, USA

²Department of Neurology, Xinqiao Hospital, Third Military Medical University, 183 Xinqiao Main Street, Shapingba District, Chongqing 400037, China

Abstract

Intracerebral haemorrhage (ICH) is the most lethal subtype of stroke but currently lacks effective treatment. Microglia are among the first non-neuronal cells on the scene during the innate immune response to ICH. Microglia respond to acute brain injury by becoming activated and developing classic M1-like (proinflammatory) or alternative M2-like (anti-inflammatory) phenotypes. This polarization implies as yet unrecognized actions of microglia in ICH pathology and recovery, perhaps involving microglial production of proinflammatory or anti-inflammatory cytokines and chemokines. Furthermore, alternatively activated M2-like microglia might promote phagocytosis of red blood cells and tissue debris, a major contribution to haematoma clearance. Interactions between microglia and other cells modulate microglial activation and function, and are also important in ICH pathology. This Review summarizes key studies on modulators of microglial activation and polarization after ICH, including M1-like and M2-like microglial phenotype markers, transcription factors and key signalling pathways. Microglial phagocytosis, haematoma resolution, and the potential crosstalk between microglia and T lymphocytes, neurons, astrocytes, and oligodendrocytes in the ICH brain are described. Finally, the clinical and translational implications of microglial polarization in ICH are presented, including the evidence that therapeutic approaches aimed at modulating microglial function might mitigate ICH injury and improve brain repair.

Intracerebral haemorrhage (ICH) accounts for 10–15% of all strokes, making it the second most common type of stroke^{1–3}. ICH is estimated to affect over 1 million people worldwide each year¹, and that number is expected to rise substantially as the population ages. Most patients with ICH die or become severely disabled, and supportive care is the mainstay of current therapy^{4,5}. Primary brain damage develops within the first few hours after ICH as a

Correspondence to: J.W. jwang79@jhmi.edu.

Author contributions

X.L., X.H., and Q.L., researched the data for the article. X.L., Q.-W.Y., and J. W. provided substantial contributions to discussions of the content. X.L. and J.W. wrote and revised the manuscript before submission.

Competing interests statement

The authors declare no competing interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

result of haematoma or oedema, formation and expansion of which induces mass effects and increased intracranial pressure that can lead to herniation and death. Secondary brain damage results from activation of microglia, which drive proinflammatory, oxidative and cytotoxic cascades causing cell death and functional impairment⁶.

As key innate immune cells, microglia act as guardians of the brain and are recognized to be the first non-neuronal cells to respond to various acute brain injuries⁷, including ICH⁶. Increasing evidence indicates that activated microglia are the primary source of cytokines, chemokines, prostaglandins, proteases, ferrous iron and other immunomodulatory molecules in the brain⁶. These molecules combine to initiate secondary brain injury and the subsequent brain repair processes. In fact, cell therapy with bone marrow-derived mononuclear cells or macrophages ameliorates injury and improves outcome after ischaemic stroke^{8–11}. In view of these findings, research into microglial activation and function, and on cell–cell interactions, is critical to understanding ICH pathophysiology.

To develop new and effective therapies for ICH, we also need to explore the cellular and molecular mechanisms that underlie ICH-induced brain injury and repair¹². As inflammation contributes to ICH-induced secondary brain injury⁶, modulation of the microglial phenotype might not only accelerate the absorption of haematoma and oedema, but also improve white matter integrity, brain repair and functional recovery¹³. The fact that clinical trials of open surgical haematoma evacuation have not shown positive results^{14,15} emphasizes the need to investigate endogenous mechanisms of haematoma removal. This knowledge could lead to the development of improved treatments for patients with ICH.

In this Review, we summarize key advances in our understanding of microglial function after ICH, with a particular focus on microglial activation, migration, proliferation, secretion and phagocytosis. In addition, we discuss microglial polarization, modulators and interactions with other cells. We also address the clinical and translational implications of these observations and highlight new therapeutic directions for ICH.

Microglia

Microglia are the major phagocytes of the brain. *In vitro*, activated microglia develop into either classically activated (M1, proinflammatory) or alternatively activated (M2, anti-inflammatory) phenotypes, a process termed polarization^{16,17}. Alternatively activated M2 microglia are divided into three subtypes — M2a, M2b and M2c — each with different cell surface markers and distinct biological functions¹⁸: M2a microglia mainly contribute to cell regeneration, whereas M2b and M2c cells participate in phagocytosis and removal of tissue debris.

In response to acute brain injury, microglia can dynamically and temporally change their phenotype^{19,20}. Individual classically activated and alternatively activated microglia within brain lesions can contribute to either tissue damage or repair under conditions of spinal cord injury^{21–23}, ischaemic stroke^{19,24} and traumatic brain injury (TBI)^{20,25–27}. Although the supposed dichotomy between M1 and M2 phenotypes is now recognized as an oversimplification (these two extreme activation states are only observed *in vitro*), this

classification remains useful for understanding the function of microglia in various brain diseases²⁸. In a mouse model of spinal cord injury, for example, although global M1 and M2 marker levels both increased in the acute phase²³, flow cytometry showed that microglia and macrophages had mainly developed an M1 phenotype²¹. In models of TBI^{20,29} and ischaemic stroke³⁰, an M2 to M1 shift was observed, whereas a mixed M1-like and M2-like response was reported in an animal model of epilepsy³¹. However, controversy has begun to surround the traditional concept of M1 versus M2 microglial polarization. For example, in a mouse model of experimental auto-immune encephalomyelitis³², the classic M1 marker IL-6 was induced by IL-4 and exerted an anti-inflammatory effect. Additionally, in a model of TBI, canonical markers of both polarization states were highly co-expressed by the same cell³³. Hence, whether microglia actually undergo M1 or M2 polarization has been called into question³⁴. Researchers using animal models of ICH should, therefore, pursue functional studies of microglial activities such as phagocytosis and haematoma clearance, as discussed below. The functional properties of known M1-like and M2-like markers are summarized in TABLE 1.

Changes in M1 markers

Proinflammatory cytokines—Activation of microglia usually results in production of proinflammatory cytokines. Currently, proinflammatory cytokines are thought to be produced specifically by classically activated M1 microglia, and alterations in proinflammatory cytokine profiles and levels are thought to explain the changes in microglial function after ICH.

Microglial activation can be assessed by immunohistochemical analysis of the ICH brain, using antibodies targeting cell surface markers shared by microglia and macrophages. Suitable markers include keratan sulfate (targeted by 5D4 antibody)^{35,36}, cell surface glycoprotein F4/80 (targeted by F4/80 antibody)^{35,36}, CD11b^{37,38}, Iba1 (REFS 39,40) and CD68 (REFS 41,42). However, anti-CD11b antibody cannot distinguish microglia from neutrophils and monocytes in the ICH brain: although in the intact brain CD11b is expressed only by microglia, this marker is also expressed by neutrophils and monocytes in the ICH brain^{39,43}. Myeloperoxidase, a specific marker of neutrophils, is used to discriminate microglia from neutrophils and monocytes⁴⁴. Distinguishing activated microglia from macrophages in the ICH brain by immunohistochemistry is also challenging. CD45 is helpful in this scenario because it is more highly expressed in monocytes than in microglia. Thus, CD45 expression levels can be used to separate these two cell types using flow cytometry: CD45^{Int}CD11b⁺ cells are microglia and CD45^{Hi}CD11b⁺ cells are macrophages^{45–47}.

Changes in M1-signature (proinflammatory) cytokines are prominent after ICH. In a clinical study of perihematoma brain tissue, nuclear factor- κ B (NF- κ B) was activated and migrated into the nucleus at 13–48 h after ICH, and IL-1 β and tumour necrosis factor (TNF) levels increased within 1 day after ICH^{48,49}. These findings suggest the presence of a proinflammatory state early after ICH. In both the collagenase-induced and autologous blood-induced models of ICH (TABLE 2), IL-1 β , IL-6, TNF^{50,51} and inducible nitric oxide synthase (iNOS)⁵² mRNA levels are generally elevated in the acute phase, starting to rise as

early as 3 h after ICH and peaking at 3 days^{53–54}. Changes in the corresponding protein levels follow a similar time course^{52,53,55}. In rodents, secretion of proinflammatory cytokines IL-1 β , IL-6 and TNF increased in the first 3 days⁵⁶, and then returned to within the normal range on day 7 in both colla-genase-induced³⁷ and blood-induced^{37,54} models of ICH. Interestingly, IL-1 β , but not TNF, macrophage mannose receptor 1 (CD206) or chitinase-like protein 3 (also known as Ym1), colocalized with amoeboid microglia in rats with collagenase-induced ICH⁵⁷. This colocalization pattern suggests a phagocytic and secretory phenotype that might be associated with dynamic changes in microglial morphology⁵⁷. Moreover, treatment with the TNF receptor antagonist R-7050 attenuated neurovascular injury and improved functional outcomes in mice with collagenase-induced ICH⁵⁸.

In experimental models, IFN γ levels did not change in the first 3 days (unlike IL-1 β , IL-6 and TNF levels)^{50,59,60}, but markedly increased at later stages after ICH^{50,60}. A reduction in the IFN γ :IL-4 ratio contributed to long-term recovery after ICH⁶¹. These results suggest that the ratio of IFN γ to M2-like cytokine levels might be a more reliable marker than IFN γ levels alone for the assessment of inflammatory status in the chronic phase after ICH. Our group has shown that M1 markers such as CD16, CD32 and iNOS are highly expressed on microglia on days 1 and 3 after ICH⁶², suggesting that M1-like microglial polarization occurs early in the acute phase.

Although the specific proinflammatory cytokines produced vary according to the ICH model used and time point assessed (FIG. 1), the overall direction of changes in these classic M1 markers is consistent in the acute phase after ICH. By 14 days after ICH (during the chronic phase), levels of most proinflammatory cytokines have returned to baseline⁶⁰. However, whether levels of these cytokines change during the chronic phase of ICH, and whether these changes affect brain repair processes, remains unclear.

Toll-like receptors—Increased protein expression of Toll-like receptor (TLR)2 and TLR4 is associated with poor outcomes in patients with ICH⁶³. In mice, expression of TLR2 (REFS 64,65) and TLR4 (REF. 62) proteins was upregulated (mainly in microglia) as early as 6 h after ICH and remained high throughout the first 3 days, in both collagenase-induced and blood-induced models of ICH^{54,62}. In *Tlr4*^{−/−} (REF. 54) and *Tlr2*^{−/−} (REF. 65) mice after blood-induced ICH, IL-1 β , IL-6 and TNF levels were substantially decreased compared with those in wild-type mice, a finding that supports a link between TLR2 and TLR4 activation and M1 microglia. Furthermore, TLR4 activates the NF- κ B signalling pathway, via myeloid differentiation primary response protein MYD88 and TIR domain-containing adaptor molecule 2 (also known as TRIF-related adaptor molecule)^{66,67} (FIG. 2). TLR4 also upregulates M1-signature (proinflammatory) cytokines in the blood-induced model of ICH^{54,68–70}. The same researchers demonstrated that TLR4 depletion reduced microglial autophagy, which contributes to the M1 phenotype of microglia⁷¹. In addition, treatment with a TLR4 antagonist reduced the production of proinflammatory cytokines (IL-1 β , IL-6 and TNF) and inactivated microglia⁶⁸. TLR2 exerts effects similar to those of TLR4 in the ICH brain, a fact that might be explained by the assembly of TLR2–TLR4 heterodimers, which trigger proinflammatory responses⁶⁵.

Complement C3a and C5a receptors—Complement C3a and C5a are thought to be involved in neuro-inflammation⁷², neutrophil recruitment⁷³ and blood–brain barrier disruption⁴⁵. ICH-induced microglial activation, as assessed by immunoreactivity to an anti-CD11b and anti-CD11c dual-specificity antibody (OX42), was reduced in C3-deficient mice compared with wild-type controls⁷⁴. In C5a receptor knockout mice, proinflammatory cytokine levels were decreased in the acute phase after ICH⁷⁵. Moreover, treatment with the C5a receptor antagonist PMX53 in combination with the thrombin antagonist argatroban resulted in decreased mRNA expression of the M1 markers TNF, IL-6 and iNOS in the mouse ICH brain⁷⁶. These data indicate a prominent role for activation of complement C3a and C5a receptors in the proinflammatory response to ICH.

Changes in M2 markers

Markers of M2 microglia in ICH have been considerably less well studied than M1 markers. Nevertheless, M2 microglia do have important functions in phagocytosis and toxicity clearance in other brain diseases⁷⁷, such as ischaemic stroke^{17,78}, TBI⁷⁹ and epilepsy³¹, suggesting that the function of M2 microglia in ICH should be explored.

Anti-inflammatory cytokines—IL-10 is an anti-inflammatory M2-signature cytokine⁸⁰ secreted by M2 macrophages and microglia. Treatment with IL-10 polarizes microglia and macrophages to the M2c subtype *in vitro*^{81,82}, and enhances phagocytosis by monocytes^{83–85}. IL-10 also induces production of suppressor of cytokine signalling (SOCS)1 and SOCS3, which suppress pro-inflammatory cytokine production via signal transducer and activator of transcription (STAT)3 in macrophages^{86,87}. Interestingly, IL-10 levels in blood^{88–90} and brain tissue^{90,91} are increased in patients with ICH, as well as in preclinical models of ICH^{50,92,93}. IL-10 levels are increased in patients during the early acute phase of spontaneous ICH, and this rise is associated with subsequent rebleeding⁸⁹, although this relationship is not necessarily causal.

IL-4 (REF. 94) and transforming growth factor (TGF) β 1 (REF. 60) exert anti-inflammatory effects, promote M2-like microglial responses, and improve functional recovery after ICH. Notably, the course of changes in levels of these M2 markers apparently differs between the two principal animal models of ICH. The mRNA levels of most M2 markers — such as IL-1 receptor antagonist⁵³, IL-10 (REF. 50), arginase-1, Ym1 and CD206 (REF. 94) — increased within 1 day after collagenase-induced ICH, whereas mRNA levels of TGF β ⁵³ and both mRNA and protein levels of IL-4 (REF. 56) were substantially increased on day 3 post-ICH. However, in mice with blood-induced ICH, IL-10 protein levels were unchanged and IL-4 was undetectable in the perihematoma region in the first 2 weeks⁶⁰. In the latter study, TGF β 1 protein levels increased from day 1 to day 10, and remained elevated up to day 14 after ICH; this time course was delayed compared with that of the M2 marker IL-13, protein levels of which increased from day 1 up to day 3 only⁶⁰. Furthermore, intracerebral TGF β 1 treatment decreased microglial *I/6* gene expression and improved functional outcomes in mice with blood-induced ICH⁶⁰. Interestingly, an early increase in plasma levels of TGF β 1 was associated with improved functional outcome at 90 days in patients with ICH⁶⁰. The authors of this study concluded that TGF β 1 is a potential target for ICH treatment.

Peroxisome proliferator-activated receptor- γ —Peroxisome proliferator-activated receptor- γ (PPAR γ) belongs to a superfamily of nuclear receptors that are important contributors to antioxidant and anti-inflammatory responses⁹⁵. In rats with blood-induced ICH, binding of PPAR γ to DNA in nuclear extracts was suppressed at 1 h post-ICH, and treatment with PPAR γ agonists had beneficial effects on ICH owing to their ability to reduce NF- κ B activation⁹⁶ and decrease M1 signature cytokine levels (specifically, iNOS, TNF and IL-1)⁹⁷. In mice with blood-induced ICH, treatment with a PPAR γ activator promoted microglial phagocytosis of red blood cells *in vitro* by inducing CD36 expression^{97,98}. This evidence supports a potential role for PPAR γ activation in haematoma clearance⁹⁹. The mechanisms of microglial phagocytosis and haematoma resolution after ICH are discussed in more detail below.

Mammalian target of rapamycin—Dysregulation of serine/threonine-protein kinase mTOR (mammalian target of rapamycin) activation occurs in various brain diseases, notably Alzheimer disease¹⁰⁰, Parkinson disease^{101,102} and TBI¹⁰³. In a rat model of subarachnoid haemorrhage¹⁰⁴, rapamycin and another mTOR inhibitor, AZD8055, ameliorated early brain injury by inhibiting production of IL-1 β and TNF. Rapamycin and AZD8055 also decreased the CD16:CD206 ratio in oxyhaemoglobin-treated primary microglia *in vitro*¹⁰⁴. In rats with collagenase-induced ICH, phosphorylation of mTOR was markedly increased at 30 min. Treatment with rapamycin (50–500 μ g/kg) led to a dose-dependent increase in brain levels of the M2 cytokines IL-10 and TGF β , as well as in the ratio of IL-10 to IFN γ , after ICH¹⁰⁵. In a separate study that used the same model¹⁰⁶, rapamycin treatment reduced protein levels of TNF, IL-1 β and IL-6. This evidence suggests a link between mTOR inhibition and microglial M2 polarization.

Other M2 markers—Upregulation of the M2 marker CD163 is observed in the brains of patients with ICH within 3 days of onset⁹¹. In mice with blood-induced ICH, global CD206 and Ym1 protein expression (detected by western blot) was increased at day 1 (REF. 107). Although our group showed that Ym1 expression was increased in microglia at 24 h and 72 h in both the thrombin-induced and collagenase-induced models of ICH^{62,108}, the dynamics of changes in microglial M2 markers in the collagenase-induced ICH model are still unclear. Data from the limited number of available studies reviewed here^{60,62,107} suggest that microglial M2 markers increase by day 1 — later than M1 markers, which start to increase by 3–6 h — and remain elevated longer (that is, beyond day 7) than do M1 markers (FIG. 1).

M1 to M2 transition

Very little is known about the dynamics of changes in microglial polarization after ICH^{13,59}. Two studies revealed changes over time in M1-like and M2-like markers in models of ICH^{94,107}. We⁶² and others⁶⁰ also demonstrated a similar M1 to M2 transition in both the collagenase-induced⁶² and blood-induced⁶⁰ models of ICH. In mice with collagenase-induced ICH, we observed an M1 to M2 microglial phenotype switch from day 1 to day 3 after ICH⁶², whereas in mice with blood-induced ICH, the microglial phenotype switch occurred within the first week⁶⁰. However, this transition could result from phenotypic transformation of a single microglial population, from M2-like microglial migration, or from

infiltration of M2-like circulating blood monocytes or macrophages. These possibilities deserve further study.

In summary, by about 3 days after ICH (FIG. 1), microglia polarize toward an M1-like phenotype associated with molecules involved in short-term brain damage. Indeed, M1 microglial polarization might be the main cause of microglial activation in the acute phase after ICH, whereas M2-like microglial responses and mediators might have an important role in long-term recovery, especially in aged animals (TABLE 2). Interestingly, ageing in the nondiseased brain is associated with an overall increase in the expression of microglial genes involved in neuroprotection¹⁰⁹. Data relating to the M2-like response also need to be expanded; in particular, the role of M2-like microglia in the subacute and chronic phase of ICH requires clarification. As some M2 surface markers and cytokines are not specific to microglia and macrophages, they should be assessed by double or triple immunofluorescence staining with microglia-specific and macrophage-specific markers. The mRNA and protein expression of these markers can be detected using fluorescence-activated cell sorting of microglia and macrophages. Unbiased genome-wide approaches such as transcriptomics and proteomics can also be applied.

Phagocytosis and haematoma clearance

Under physiological conditions, microglia act as phagocytes to scavenge plaques, neurofibrillary tangles and cell debris in the CNS. Haematoma clearance by microglia might be a major mechanism of recovery from ICH^{13,110–112}. We previously showed that tissue plasminogen activator in microglia assists in the clearance of haematoma³⁶.

CD36 belongs to the class B scavenger receptor family and also contributes to the phagocytic ability of microglia and macrophages^{113–116}; cells lacking phagocytic ability acquire this capacity after being transfected with CD36 (REF. 116). In both patients and animals with ICH, haematoma absorption is increased by CD36 expression^{97,98,111,115}. CD36 is also a pivotal target of PPAR γ -mediated downstream gene transcription⁹⁸. As discussed above, activation of PPAR γ increases CD36 expression and thereby promotes microglial phagocytic ability, whereas CD36 expression is downregulated by TLR4 signalling in microglia and monocytes^{117,118}. In a comparison of TLR4-deficient and wild-type mice with blood-induced ICH, perihematoma regions in the TLR4-deficient mice showed fewer activated microglia and higher expression of CD36 and fractalkine (also known as CX₃C motif chemokine 1 (CX3CL1))⁶⁹. In *CD36*^{-/-} mice with blood-induced ICH, IL-1 β and TNF mRNA expression levels were higher than those in wild-type mice. The capacity of *CD36*^{-/-} microglia from these mice to phagocytose red blood cells *in vitro* was also decreased, along with a complementary increase in IL-10 levels¹¹⁵. The authors concluded that CD36 deficiency led to TLR4 upregulation, suggesting that mediators downstream of the TLR4 pathway reduced microglial phagocytosis and slowed haematoma absorption by decreasing CD36 expression and IL-10 production¹¹⁵. In an *in vitro* study, *Tlr4*^{-/-} microglia exposed to lysed erythrocytes exhibited reductions in autophagy and inflammation compared with similarly treated wild-type microglia⁷¹. This finding suggests that TLR4-mediated autophagy contributes to microglial polarization and function.

However, whether IL-10 regulates the expression of CD36 and TLR4, thereby modulating phagocytosis of red blood cells and promoting haematoma resolution, remains unclear.

CD47 is an integrin-associated protein expressed on erythrocytes^{119,120} that regulates phagocytosis in microglia and macrophages^{121,122}. In a study that compared the effects of using blood from either wild-type mice or *Cd47*^{-/-} mice to induce ICH in wild-type mice, the mice receiving CD47-depleted blood showed increased expression of haem oxygenase-1 (HO-1) and accelerated haematoma clearance, in a manner that correlated with M2 microglial activation¹²³.

Interestingly, levels of the haemoglobin scavenger receptor CD163 increased over time in parallel with haematoma resolution in piglets with blood-induced ICH¹¹⁰, indicating a potential role for CD163 in haematoma clearance. These observations indicate that proteins capable of modulating microglial phagocytosis (for example, IL-10, CD36, CD47 and CD163) can affect haematoma resolution (FIG. 2) and deserve further research. Identification of new targets that can regulate microglial phagocytosis and haematoma clearance might lead to the development of future therapeutic interventions for ICH.

Modulators of microglial polarization

Transcription factors

Although markers of M1 and M2 microglia and macrophage polarization are readily detectable, these proposed activation states are complex and their relevance to macrophage function *in vivo* is controversial¹²⁴. Additionally, the signalling pathways that cause secondary damage after ICH are complex. Investigating the activation of transcription factor responses to brain injury is, therefore, important to our understanding of microglial M1 to M2 transition after ICH. However, very few studies have investigated transcription factors other than NF- κ B after ICH.

NF- κ B—NF- κ B is a traditional transcription factor that is activated by lipopolysaccharide and regulates the expression of most M1-signature genes (those encoding proinflammatory cytokines)¹²⁵. In human brain tissue, NF- κ B p65 expression was detected in the nuclei of glial cells by immunohistochemistry¹²⁶ and was associated with increases in IL-1 β and TNF⁴⁸. As discussed above, upregulation of TLR2 and TLR4 (which are expressed mainly in microglia) induces NF- κ B activation after ICH, and increases proinflammatory cytokine levels^{54,65}.

Signal transducer and activator of transcription proteins—The STAT family of transcription factors (STAT1–STAT6) are, like NF- κ B, activated by lipopolysaccharide. The Janus activated kinase (JAK)–STAT pathway is activated by both type I and type II interferons and thereby regulates cellular proliferation, immunity, apoptosis, and inflammation^{127,128}. However, few studies have investigated the roles of STAT proteins in ICH.

STAT family members have various roles in microglial and macrophage polarization (FIG. 2). STAT1 controls M1 macrophage polarization¹²⁹, and *in vitro*, STAT1 levels increase in

TLR3-stimulated and TLR4-stimulated microglia¹³⁰. STAT1 activation also induces expression of genes encoding IL-1 β , TNF and CXC motif chemokine 10 (CXCL10) in *Cryptococcus neoformans*-infected macrophages¹³¹. Conversely, loss of STAT1 in macrophages led to an increase in polarization to the M2 phenotype¹³¹. SOCS3 is a feedback inhibitor that limits excessive cytokine release resulting from persistent activation of STAT3; *in vitro*, SOCS3-deficient primary microglia showed increases in STAT3 activation and proinflammatory cytokine signalling, which led to their pronounced polarization to the M1 phenotype¹³². This polarization was associated with upregulation of iNOS, IL-1 β , IL-12 p40, IL-23 p9, IL-6, CC-motif chemokine 2 (CCL2), and CXCL10 (REF. 132). In mice with collagenase-induced ICH, phosphorylated STAT3 is found mainly in microglia and macrophages, and inhibition of this molecule is associated with decreases in iNOS and cyclooxygenase-2 expression¹³³. In this same model, levels of phosphorylated STAT3 and IL-6 decreased in *Tlr4*^{-/-} and *MYD88*^{-/-} mice on day 3 after induction of ICH⁶⁷, suggesting that STAT3 is an important transcription factor downstream of TLR4 that mediates M1 microglial polarization in ICH. However, STAT6 became activated in microglia challenged with IL-4, which promoted microglial M2 polarization^{134–136}. Thus, the roles of STAT proteins in ICH pathology need additional clarification.

Nuclear factor erythroid 2-related factor 2—Activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and its related signalling pathway exerts a protective effect after ICH and also contributes to haematoma clearance^{137,138}. *Nrf2*^{-/-} mice show more brain damage than do wild-type mice at 24 h after collagenase-induced ICH, although microglial activation status in these mice, as shown by immunostaining for allograft inflammatory factor-1 (AIF1, also known as Iba1), is not altered by the loss of Nrf2 (REF. 137). *In vitro*, microglial phagocytosis of red blood cells labelled with a fluorescent dye was substantially increased by Nrf2 activators such as sulforaphane¹³⁹. Moreover, mRNA level of the scavenger receptor CD36 was upregulated in microglia treated with an Nrf2 activator¹¹¹.

Interestingly, mRNA levels of HO-1, CD36, CD163, IL-10 and IL-1 β did not change appreciably in *Nrf2*^{-/-} mice compared with their levels in wild-type mice at 48 h after blood-induced ICH¹¹¹. HO-1 deletion decreased the phagocytic ability of microglia *in vitro* and delayed haematoma clearance in an *in vivo* model of subarachnoid haemorrhage¹⁴⁰. Additionally, HO-1 activity is elevated in the cerebrospinal fluid of patients with subarachnoid haemorrhage¹⁴⁰, suggesting a critical role for microglial Nrf2 and HO-1 in haematoma clearance. Moreover, induction of HO-1, one of the major downstream targets of Nrf2, polarizes macrophages to the M2 phenotype¹⁴¹. Whether Nrf2 transcriptional activation and downstream molecules also modulate microglial polarization and function after ICH deserves further investigation.

High mobility group protein 1—Though not itself a transcription factor, high mobility group protein B1 (HMG1) is a DNA-binding protein that regulates gene transcription through its interactions with nucleosomes, transcription factors and histones^{142–144}. Crucially, HMG1 is involved in proinflammatory cytokine gene transcription¹⁴³ and is secreted by haem-stimulated microglia *in vitro*¹⁴⁵. Serum levels of HMG1 are increased in

patients with ICH, and correlate with levels of IL-6 and TNF, as well as with NIH Stroke Scale scores¹⁴⁵. This evidence indicates that microglial HMG1 has a role in ICH. In addition, treatment with the nonspecific HMG1 inhibitors glycyrrhizin¹⁴⁶ and ethyl pyruvate¹⁴⁷ attenuates ICH injury and improves early outcomes in rodent models of ICH. Our group has shown that expression of HMG1 protein is substantially increased at 24 h after ICH^{39,148}. HMG1 also promotes macrophage M1 polarization via increased TLR2 and TLR4 signalling, which contributes to cardiac remodelling in mice prone to accelerated senescence¹⁴⁴. Thus, the beneficial effects of HMG1 inhibitors in rodent models of ICH might result from attenuation of microglial activation and inhibition of TLR2 and TLR4 (REF. 149).

Prostaglandin E2 receptors

Prostaglandin E2 (PGE2) is a major product of cyclo-oxygenase and prostaglandin E synthase, and is thought to be a classic proinflammatory mediator within the brain. PGE2 activates its downstream signalling pathways via the G-protein-coupled E prostanoid (EP) receptors EP1–EP4. EP1 receptors are expressed in microglia, and EP1 deletion dampens microglial activity and phagocytosis¹⁵⁰. Furthermore, treatment with the EP1 receptor antagonist SC51089 decreases microglial activation and attenuates grey and white matter injury in mouse models of ICH³⁹. Interestingly, in mice with collagenase-induced ICH, deletion of EP2 and EP3 produces opposite effects on ICH outcomes that correlate with microglial activation status¹⁴⁸. Although EP2 is expressed in neurons rather than in microglia, deletion of EP2 led to an increase in M1-like microglial activation, indicating a potential EP2-mediated interaction between neurons and microglia¹⁴⁸. By contrast, EP3 receptors are expressed in microglia¹⁰⁸, and EP3 deletion caused decreased microglial activation in mice with collagenase-induced ICH¹⁵¹. Similarly, treatment with an EP3 antagonist inhibited microglial activation in mice with thrombin-induced ICH¹⁰⁸, although this treatment increased the number of M2 microglia (as shown by CD11b and Ym1 double immunostaining)¹⁰⁸. Finally, the EP2 and EP4 dual agonist misoprostol provided protection and reduced the proinflammatory response in mice with either collagenase-induced or blood-induced ICH¹⁵². Together, these results support a potential role for PGE2 and EP receptors in modulation of microglial function after ICH.

Sphingosine-1-phosphate receptors

Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator that exerts a wide range of effects on cells via its five known receptors (S1PR1–S1PR5) in the brain^{153,154}. The S1PR agonist fingolimod was approved by the FDA in 2010 for the treatment of multiple sclerosis (MS). Microglia express all five S1PRs *in vitro*^{155,156}, and fingolimod treatment reduced proinflammatory cytokine levels in lipopolysaccharide-induced M1-like microglia^{156,157}. *In vivo*, fingolimod decreased the production of IL-1 β and TNF in animal models of status epilepticus¹⁵⁸ and neonatal hyperoxia¹⁵⁹. In an animal model of MS, microglia exhibited a reduced M1-like response and an enhanced M2-like response at day 120 with fingolimod treatment¹⁶⁰. In rodents with collagenase-induced ICH, fingolimod treatment improved functional outcomes^{161,162} and reduced levels of IFN γ after ICH¹⁶², and also inhibited M1-like responses by preventing T-lymphocyte infiltration into the brain¹⁶². Notably, fingolimod also accelerated haematoma clearance and improved clinical outcomes in patients with

ICH¹⁶³, strongly indicating the importance of S1PRs in clinical ICH. However, another group found no effects of fingolimod treatment on haematoma volume and inflammatory cells in rodents with collagenase-induced ICH¹⁶⁴. Thus, additional studies are needed to determine the role of S1PRs and whether they mediate microglial polarization after ICH. Currently, fingolimod is being investigated in a phase II clinical trial as a treatment for ischaemic and haemorrhagic stroke¹⁶⁵.

Microglial interactions with other cells

T lymphocytes

In patients with ICH, the frequency of both activated (CD3⁺CD9⁺) and regulatory (CD4⁺CD25⁺FOXP3⁺) T cells (T_{reg} cells)^{166,167} was increased in the peripheral blood of patients from day 3 to day 7 after ICH⁹⁰. In mice with blood-induced ICH, T_{reg} cells⁴⁵ and $\gamma\delta$ T cells¹⁶⁸ infiltrated the brain starting at day 4, and their levels remained elevated until day 7; furthermore, these T_{reg} cells inhibited M1 microglia *in vitro*¹⁶⁹ and *in vivo*¹⁷⁰. In a mouse model of blood-induced ICH, our group found that these T_{reg} cells promoted M2-like polarization of microglia and macrophages via the IL-10–glycogen synthase kinase-3 β signalling pathway⁴⁵. These data all support the premise that T-cell infiltration into the brain after ICH enhances M1-like responses of microglia and macrophages, whereas T_{reg} cells in the brain contribute to the microglial M1 to M2 phenotype shift.

Neurons

The signalling pathway involving soluble CX3CL1, which is expressed on neurons, and its receptor CX₃C chemokine receptor 1 (CX3CR1), which is expressed on microglia, mediates interactions between microglia and neurons, thereby modulating microglial activation^{171,172}. Mice engineered to express CX3CR1 labelled with green fluorescent protein have been widely used to study microglial activation and function^{39,62,108,173–175}. For example, after ischaemic stroke, CX3CL1 is released by neurons and participates in microglial activation via CX3CR1 (REFS 175–177), and mice deficient in CX3CL1 have a reduced susceptibility to cerebral ischaemia–reperfusion injury¹⁷⁸. However, the roles of CX3CL1 and CX3CR1 in ICH have not been well studied.

Both CX3CL1 and CX3CR1 are expressed in the brains of patients with ICH¹⁷⁹. In *Tlr4*^{−/−} mice with blood-induced ICH, mRNA levels of both CX3CL1 and CD36 were increased compared with their levels in wild-type mice⁶⁹. Moreover, mice with monocyte-specific CX3CR1 deficiency showed no defect in functional recovery after blood-induced ICH¹⁸⁰, suggesting that neuron-secreted CX3CL1 is involved in microglial phagocytosis and functional recovery after ICH, but that monocyte recruitment after ICH is independent of signalling via CX3CR1 (REF. 180). Thus, interactions between neurons and microglia, mediated by CX3CL1 binding to CX3CR1, might be involved in the dynamic regulation of microglial phenotype after ICH.

Astrocytes

The initial observation, in 2005, that microglial processes and protrusions directly contact astrocytes, suggested an important link between these two cell types in the healthy brain¹⁸¹.

In the CNS, astrocytes secrete proinflammatory (IL-6 and IL-1 β) as well as anti-inflammatory (IL-10) cytokines¹⁸². Furthermore, astrocytes are major sources of many chemokines, such as CCL2, CXCL1, CXCL10 and CXCL12 (REFS 183,184), which might have roles in microglial differentiation. Several chemokines have been implicated in macrophage activation and polarization¹⁸⁵. In patients with ICH, chemokines such as CCL20 (REF. 186), CXCL1 (REF. 186), CXCL2 (REF. 187), CXCL3 (REF. 187) and CCR1 (REF. 187) are upregulated in perihematoma brain regions, indicating a potential role for these chemokines in ICH pathology. Some chemokines and their receptors have also been studied in animal models of ICH¹⁸⁸. In rats with blood-induced ICH, gene expression microarray studies showed that, 24 h after ICH, *Cxcl2* was highly upregulated in the striatum and cortex¹⁸⁹. Moreover, the neuroprotective effect of retinoic acid receptor agonist AM80 in mice with collagenase-induced ICH is attributable to inhibition of *Cxcl2* mRNA expression⁵¹.

Few studies have assessed M1 and M2 chemokine markers in ICH. However, in mice with collagenase-induced ICH, mRNA expression of the M1 marker *Cxcl1* was increased at 6 h but not at 24 h after ICH, compared with its expression in control mice after sham ICH⁵¹. Astrocytes also participate in microglial M1 to M2 phenotype switching. In hyperammonaemic rats, sulforaphane promoted a switch in microglial polarization from the M1 to the M2 phenotype by decreasing membrane expression of sodium-dependent and chloride-dependent GABA transporter GAT3, mainly in activated astrocytes¹⁹⁰.

In an *in vitro* study, CCL2 released from primary astrocytes contributed to M1 microglial polarization¹⁹¹. In mice with collagenase-induced ICH, a lack of CCL2 or CCR2 decreased the haematoma volume early after collagenase-induced ICH but resulted in delayed recovery from ICH¹¹²; these changes correlated with decreases in microglial activation and iNOS expression¹¹². *Ccr2*^{-/-} mice with blood-induced ICH also exhibited an early improvement in motor function and fewer M1 micro-glia¹⁸⁰. A high CCL2 level at 24 h correlated with poor outcomes at day 7 in patients with ICH¹⁸⁰.

The limited research available suggests that interactions between microglia and astrocytes can be either beneficial or harmful to neurons, but the crosstalk between microglia and astrocytes has not been fully investigated. Further study of astrocytic mediators that modulate microglial polarization, phagocytosis and other functions would improve our understanding of ICH pathology and facilitate translational research.

Oligodendrocytes

During the first 2 weeks after collagenase-induced ICH in rats, oligodendrocyte precursor cells and mature oligodendrocytes proliferate and differentiate within white matter tracts of the perihematoma region¹⁹². In MS, M2 microglia promote oligodendrocyte proliferation and differentiation during the remyelination period, which is associated with functional recovery¹⁹³. After ICH induced by injection of sonicated whole blood, haptoglobin was secreted from oligodendrocytes at 24 h and protected a mixed population of brain cells (neurons, astrocytes, and microglia) from the effects of haemoglobin toxicity¹⁹⁴. However, our knowledge of how microglial polarization affects oligodendrocyte function is very limited.

Clinical and translational implications

Currently, several promising anti-ICH drugs are undergoing clinical trials (TABLE 3). Most exert neuroprotective effects by targeting M1-like microglia in the acute phase after ICH. Inhibition of M1-like microglial activation and enhancement of M2-like microglial anti-inflammatory responses are the major underlying mechanisms of these compounds. Additional drug candidates that target microglial polarization have demonstrated efficacy for ICH treatment in preclinical studies (TABLE 3). Although in this Review we have emphasized the importance of M2-like microglial responses (especially in the late recovery stage of ICH), treatment strategies for ICH that focus on modulating or enhancing M2-like microglia are still limited. As discussed above, rapamycin (at a low dose of 150 µg/kg) increased levels of M2-like anti-inflammatory cytokines IL-10 and TGFβ¹⁰⁵. Sinomenine is a dextrorotatory morphinan analogue extracted from herbs used in traditional Chinese medicine^{195,196}, and has long been used clinically for treating rheumatoid arthritis in China¹⁹⁷. *In vitro*, sinomenine reduced levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF in ICH-exposed BV-2 microglia¹⁹⁸ and increased levels of M2-like markers IL-10 and Arg1 in primary microglia exposed to erythrocyte lysate¹⁹⁹. Sinomenine increased levels of *IL10* and *Arg1* mRNA in mice with blood-induced ICH¹⁹⁹. The complicated changes in M2 phenotype microglia, as discussed above, probably make M2-like microglia a more challenging target than M1 microglia.

The effects of stem cell transplantation on ICH are also now beginning to be explored in clinical trials. Preclinical studies in rats with collagenase-induced ICH showed that transplantation of mesenchymal stem cells (MSCs) obtained from umbilical cord blood improved ICH outcomes by inhibiting the M1 markers cyclooxygenase-2 and TNF²⁰⁰. Pneumostem, a novel agent based on human MSCs obtained from umbilical cord blood, is currently under investigation in a phase I clinical trial as a treatment for premature infants with intraventricular haemorrhage²⁰¹.

Despite decades of research, effective drug treatments for ICH are still lacking. However, with the expanding investigation of microglial polarization and function in animal models of ICH, therapies that target microglial phenotype switching might soon become a clinical reality for patients affected by ICH.

Conclusions

Microglial activation has long been recognized as important in the pathology and progression of ICH^{6,202,203}. The evidence indicates that after becoming activated, microglia undergo polarization into various phenotypes that might contribute differently to neuroinflammation in models of brain disease. Although very little is known about the dynamics of microglial polarization specifically after ICH, modulation of microglial function might be expected to mitigate ICH-related brain injury, thereby promoting tissue repair and functional recovery¹³. In this regard, some promising drugs strongly inhibit M1-like microglia, whereas others are thought to enhance microglial M2-like responses. However, both M1-like and M2-like microglia have critical roles in tissue repair at different stages after brain injury, suggesting that persistence of an M2-dominant state after ICH

might impair innate immune responses and result in serious adverse effects, such as neoplasia²⁰⁴. Appropriate anti-ICH treatment, therefore, should aim to boost the correct microglial phenotype at the correct time, so as to maximize the natural processes of haematoma clearance and brain repair.

Considering the limitations of existing animal models of ICH and the numerous failed attempts to translate promising experimental treatments for ischaemic stroke into clinical use, studies of histopathological material from patients with ICH⁴⁹ are critically needed to confirm the findings from studies in rodent models. Such a step is vitally important for future translational studies. Applications of transcriptomic, epigenomic and proteomic studies, and animal models employing conditional targeting of microglial genes, are expected to move the microglial research field forward and improve our understanding of the underlying mechanisms of ICH, potentially leading to effective treatments for affected patients.

Acknowledgments

The authors' research work was supported by American Heart Association (AHA) Mid-Atlantic Affiliate Grant-in-Aid 13GRNT15730001, NIH grants R01NS078026 and R01AT007317, and a Stimulating and Advancing ACCM Research (StAAR) grant from the Department of Anesthesiology and Critical Care Medicine (ACCM), Johns Hopkins University (to J.W.), and an AHA Mid-Atlantic Affiliate Postdoctoral Fellowship Award 15POST25090114 (to X.L.). The authors thank Claire Levine, MS, ELS, for assistance with manuscript preparation.

References

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol.* 2009; 8:355–369. [PubMed: 19233729]
2. Steiner T, et al. European Stroke Organisation (ESO) guidelines for the management of spontaneous intracerebral hemorrhage. *Int J Stroke.* 2014; 9:840–855. [PubMed: 25156220]
3. Kim JY, Bae HJ. Spontaneous intracerebral hemorrhage: management. *J Stroke.* 2017; 19:28–39. [PubMed: 28178413]
4. Thabet AM, Kottapally M, Hemphill JC III. Management of intracerebral hemorrhage. *Handb Clin Neurol.* 2017; 140:177–194. [PubMed: 28187799]
5. Hemphill JC III, et al. Guidelines for the management of spontaneous intracerebral hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* 2015; 46:2032–2060. [PubMed: 26022637]
6. Wang J. Preclinical and clinical research on inflammation after intracerebral hemorrhage. *Prog Neurobiol.* 2010; 92:463–477. [PubMed: 20713126]
7. Lan X, Liu R, Sun L, Zhang T, Du G. Methyl salicylate 2-O- β -D-lactoside, a novel salicylic acid analogue, acts as an anti-inflammatory agent on microglia and astrocytes. *J Neuroinflammation.* 2011; 8:98. [PubMed: 21831328]
8. Wang J, et al. Bone marrow mononuclear cells exert long-term neuroprotection in a rat model of ischemic stroke by promoting arteriogenesis and angiogenesis. *Brain Behav Immun.* 2013; 34:56–66. [PubMed: 23891963]
9. Balami JS, Fricker RA, Chen R. Stem cell therapy for ischaemic stroke: translation from preclinical studies to clinical treatment. *CNS Neurol Disord Drug Targets.* 2013; 12:209–219. [PubMed: 23394533]
10. Larochelle A, Bellavance MA, Michaud JP, Rivest S. Bone marrow-derived macrophages and the CNS: an update on the use of experimental chimeric mouse models and bone marrow

- transplantation in neurological disorders. *Biochim Biophys Acta*. 2016; 1862:310–322. [PubMed: 26432480]
11. Jiang C, et al. Comparison of the therapeutic effects of bone marrow mononuclear cells and microglia for permanent cerebral ischemia. *Behav Brain Res*. 2013; 250:222–229. [PubMed: 23685323]
 12. Zhou Y, Wang Y, Wang J, Anne Stetler R, Yang QW. Inflammation in intracerebral hemorrhage: from mechanisms to clinical translation. *Prog Neurobiol*. 2014; 115:25–44. [PubMed: 24291544]
 13. Zhang Z, et al. Microglial polarization and inflammatory mediators after intracerebral hemorrhage. *Mol Neurobiol*. 2017; 54:1874–1886. [PubMed: 26894396]
 14. Mendelow AD, et al. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial lobar intracerebral haematomas (STICH II): a randomised trial. *Lancet*. 2013; 382:397–408. [PubMed: 23726393]
 15. Gonzales NR. Ongoing clinical trials in intracerebral hemorrhage. *Stroke*. 2013; 44:S70–S73. [PubMed: 23709737]
 16. Xiong XY, Liu L, Yang QW. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. *Prog Neurobiol*. 2016; 142:23–44. [PubMed: 27166859]
 17. Fumagalli S, Perego C, Pischutta F, Zanier ER, De Simoni MG. The ischemic environment drives microglia and macrophage function. *Front Neurol*. 2015; 6:81. [PubMed: 25904895]
 18. Chhor V, et al. Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia *in vitro*. *Brain Behav Immun*. 2013; 32:70–85. [PubMed: 23454862]
 19. Hu X, et al. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke*. 2012; 43:3063–3070. [PubMed: 22933588]
 20. Wang G, et al. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J Cereb Blood Flow Metab*. 2013; 33:1864–1874. [PubMed: 23942366]
 21. Kroner A, et al. TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. *Neuron*. 2014; 83:1098–1116. [PubMed: 25132469]
 22. Hayakawa K, et al. Lipopolysaccharide preconditioning facilitates M2 activation of resident microglia after spinal cord injury. *J Neurosci Res*. 2014; 92:1647–1658. [PubMed: 25044014]
 23. Kigerl KA, et al. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci*. 2009; 29:13435–13444. [PubMed: 19864556]
 24. Han L, et al. Rosiglitazone promotes white matter integrity and long-term functional recovery after focal cerebral ischemia. *Stroke*. 2015; 46:2628–2636. [PubMed: 26243225]
 25. Kumar A, et al. Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. *Neurobiol Aging*. 2013; 34:1397–1411. [PubMed: 23273602]
 26. Wang G, et al. HDAC inhibition prevents white matter injury by modulating microglia/macrophage polarization through the GSK3 β /PTEN/Akt axis. *Proc Natl Acad Sci USA*. 2015; 112:2853–2858. [PubMed: 25691750]
 27. Turtzo LC, et al. Macrophagic and microglial responses after focal traumatic brain injury in the female rat. *J Neuroinflammation*. 2014; 11:82. [PubMed: 24761998]
 28. Hu X, et al. Microglial and macrophage polarization — new prospects for brain repair. *Nat Rev Neurol*. 2015; 11:56–64. [PubMed: 25385337]
 29. Kumar A, Alvarez-Croda DM, Stoica BA, Faden AI, Loane DJ. Microglial/macrophage polarization dynamics following traumatic brain injury. *J Neurotrauma*. 2016; 33:1732–1750. [PubMed: 26486881]
 30. Perego C, et al. Macrophages are essential for maintaining a M2 protective response early after ischemic brain injury. *Neurobiol Dis*. 2016; 96:284–293. [PubMed: 27697537]
 31. Benson MJ, Manzanero S, Borges K. Complex alterations in microglial M1/M2 markers during the development of epilepsy in two mouse models. *Epilepsia*. 2015; 56:895–905. [PubMed: 25847097]

32. Casella G, et al. IL4 induces IL6-producing M2 macrophages associated to inhibition of neuroinflammation *in vitro* and *in vivo*. *JNeuroinflammation*. 2016; 13:139. [PubMed: 27266518]
33. Kim CC, Nakamura MC, Hsieh CL. Brain trauma elicits non-canonical macrophage activation states. *J Neuroinflammation*. 2016; 13:117. [PubMed: 27220367]
34. Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci*. 2016; 19:987–991. [PubMed: 27459405]
35. Wang J, Tsirka SE. Tuftsin fragment 1–3 is beneficial when delivered after the induction of intracerebral hemorrhage. *Stroke*. 2005; 36:613–618. [PubMed: 15692122]
36. Wang J, Rogove AD, Tsirka AE, Tsirka SE. Protective role of tuftsin fragment 1–3 in an animal model of intracerebral hemorrhage. *Ann Neurol*. 2003; 54:655–664. [PubMed: 14595655]
37. Liew HK, et al. Systemic administration of urocortin after intracerebral hemorrhage reduces neurological deficits and neuroinflammation in rats. *J Neuroinflammation*. 2012; 9:13. [PubMed: 22257737]
38. Wang J, Dore S. Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. *Brain*. 2007; 130:1643–1652. [PubMed: 17525142]
39. Zhao X, et al. Toxic role of prostaglandin E2 receptor EP1 after intracerebral hemorrhage in mice. *Brain Behav Immun*. 2015; 46:293–310. [PubMed: 25697396]
40. Jiang C, et al. Progesterone exerts neuroprotective effects and improves long-term neurologic outcome after intracerebral hemorrhage in middle-aged mice. *Neurobiol Aging*. 2016; 42:13–24. [PubMed: 27143417]
41. Tischer J, et al. Inhomogeneous distribution of Iba-1 characterizes microglial pathology in Alzheimer's disease. *Glia*. 2016; 64:1562–1572. [PubMed: 27404378]
42. Yang Y, et al. Thrombin-induced microglial activation impairs hippocampal neurogenesis and spatial memory ability in mice. *Behav Brain Funct*. 2015; 11:30. [PubMed: 26410080]
43. Hammond MD, Ai Y, Sansing LH. Gr1⁺ macrophages and dendritic cells dominate the inflammatory infiltrate 12 hours after experimental intracerebral hemorrhage. *Transl Stroke Res*. 2012; 3:s125–s131. [PubMed: 23259009]
44. Cheng T, et al. Cerebroprotection of flavanol (–)-epicatechin after traumatic brain injury via Nrf2-dependent and -independent pathways. *Free Radic Biol Med*. 2016; 92:15–28. [PubMed: 26724590]
45. Zhou K, et al. Regulatory T cells ameliorate intracerebral hemorrhage-induced inflammatory injury by modulating microglia/macrophage polarization through the IL-10/GSK3 β /PTEN axis. *J Cereb Blood Flow Metab*. 2016; 37:967–979. [PubMed: 27174997]
46. Greter M, Lelios I, Croxford AL. Microglia versus myeloid cell nomenclature during brain inflammation. *Front Immunol*. 2015; 6:249. [PubMed: 26074918]
47. Mracsko E, et al. Leukocyte invasion of the brain after experimental intracerebral hemorrhage in mice. *Stroke*. 2014; 45:2107–2114. [PubMed: 24916913]
48. Zhang Z, et al. NF- κ B activation and cell death after intracerebral hemorrhage in patients. *Neurol Sci*. 2014; 35:1097–1102. [PubMed: 24510152]
49. Wu H, et al. Dynamic changes of inflammatory markers in brain after hemorrhagic stroke in humans: a postmortem study. *Brain Res*. 2010; 1342:111–117. [PubMed: 20420814]
50. Liesz A, et al. Comparison of humoral neuroinflammation and adhesion molecule expression in two models of experimental intracerebral hemorrhage. *Exp Transl Stroke Med*. 2011; 3:11. [PubMed: 21967730]
51. Matsushita H, et al. Suppression of CXCL2 upregulation underlies the therapeutic effect of the retinoid Am80 on intracerebral hemorrhage in mice. *J Neurosci Res*. 2014; 92:1024–1034. [PubMed: 24659080]
52. Yang S, 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; 51:352–363. [PubMed: 23494638]
53. Wasserman JK, Zhu X, Schlichter LC. Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. *Brain Res*. 2007; 1180:140–154. [PubMed: 17919462]

54. Lin S, et al. Heme activates TLR4-mediated inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage. *J Neuroinflammation*. 2012; 9:46. [PubMed: 22394415]
55. Xie RX, et al. Carnosine attenuates brain oxidative stress and apoptosis after intracerebral hemorrhage in rats. *Neurochem Res*. 2016; 42:541–551. [PubMed: 27868153]
56. Zhang Y, et al. Quercetin promotes neuronal and behavioral recovery by suppressing inflammatory response and apoptosis in a rat model of intracerebral hemorrhage. *Neurochem Res*. 2015; 40:195–203. [PubMed: 25543848]
57. Yang SS, et al. High morphologic plasticity of microglia/macrophages following experimental intracerebral hemorrhage in rats. *Int J Mol Sci*. 2016; 17:E1181. [PubMed: 27455236]
58. King MD, Alleyne CH Jr, Dhandapani KM. TNF- α receptor antagonist, R-7050, improves neurological outcomes following intracerebral hemorrhage in mice. *Neurosci Lett*. 2013; 542:92–96. [PubMed: 23499961]
59. Zhao H, Garton T, Keep RF, Hua Y, Xi G. Microglia/macrophage polarization after experimental intracerebral hemorrhage. *Transl Stroke Res*. 2015; 6:407–409. [PubMed: 26446073]
60. Taylor RA, et al. TGF- β 1 modulates microglial phenotype and promotes recovery after intracerebral hemorrhage. *J Clin Invest*. 2017; 127:280–292. [PubMed: 27893460]
61. Ma L, et al. Blocking B7-1/CD28 pathway diminished long-range brain damage by regulating the immune and inflammatory responses in a mouse model of intracerebral hemorrhage. *Neurochem Res*. 2016; 41:1673–1683. [PubMed: 26980009]
62. Lan X, et al. Pinocembrin protects hemorrhagic brain primarily by inhibiting Toll-like receptor 4 and reducing M1 phenotype microglia. *Brain Behav Immun*. 2017; 61:326–339. [PubMed: 28007523]
63. Rodriguez-Yanez M, 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. [PubMed: 22498099]
64. Lively S, Schlichter LC. Age-related comparisons of evolution of the inflammatory response after intracerebral hemorrhage in rats. *Transl Stroke Res*. 2012; 3:132–146. [PubMed: 22707991]
65. Wang YC, et al. Toll-like receptor 2/4 heterodimer mediates inflammatory injury in intracerebral hemorrhage. *Ann Neurol*. 2014; 75:876–889. [PubMed: 24752976]
66. Wang Y, et al. TLR4/MD-2 activation by a synthetic agonist with no similarity to LPS. *Proc Natl Acad Sci USA*. 2016; 113:E884–E893. [PubMed: 26831104]
67. Xiong XY, et al. Toll-like receptor 4/MyD88-mediated signaling of hepcidin expression causing brain iron accumulation, oxidative injury, and cognitive impairment after intracerebral hemorrhage. *Circulation*. 2016; 134:1025–1038. [PubMed: 27576776]
68. Wang YC, et al. Toll-like receptor 4 antagonist attenuates intracerebral hemorrhage-induced brain injury. *Stroke*. 2013; 44:2545–2552. [PubMed: 23839500]
69. Sansing LH, et al. Toll-like receptor 4 contributes to poor outcome after intracerebral hemorrhage. *Ann Neurol*. 2011; 70:646–656. [PubMed: 22028224]
70. Teng W, Wang L, Xue W, Guan C. Activation of TLR4-mediated NF κ B signaling in hemorrhagic brain in rats. *Mediators Inflamm*. 2009; 2009:473276. [PubMed: 20150961]
71. Yang Z, et al. Toll-like receptor-4-mediated autophagy contributes to microglial activation and inflammatory injury in mouse models of intracerebral haemorrhage. *Neuropathol Appl Neurobiol*. 2015; 41:e95–e106. [PubMed: 25185720]
72. Lian H, et al. Astrocyte–microglia cross talk through complement activation modulates amyloid pathology in mouse models of Alzheimer’s disease. *J Neurosci*. 2016; 36:577–589. [PubMed: 26758846]
73. Garrett MC, et al. Synergistic neuroprotective effects of C3a and C5a receptor blockade following intracerebral hemorrhage. *Brain Res*. 2009; 1298:171–177. [PubMed: 19410563]
74. Yang S, et al. The role of complement C3 in intracerebral hemorrhage-induced brain injury. *J Cereb Blood Flow Metab*. 2006; 26:1490–1495. [PubMed: 16552422]
75. Yuan, B., et al. C5a/C5aR pathway plays a vital role in brain inflammatory injury via initiating Fgl-2 in intracerebral hemorrhage. *Mol Neurobiol*. 2016. <http://dx.doi.org/10.1007/s12035-016-0141-7>

76. Li G, et al. Neuroprotective effects of argatroban and C5a receptor antagonist (PMX53) following intracerebral haemorrhage. *Clin Exp Immunol.* 2014; 175:285–295. [PubMed: 24117111]
77. Chen Z, Trapp BD. Microglia and neuroprotection. *J Neurochem.* 2016; 136(Suppl 1):10–17. [PubMed: 25693054]
78. Zhao X, et al. Neuronal interleukin-4 as a modulator of microglial pathways and ischemic brain damage. *J Neurosci.* 2015; 35:11281–11291. [PubMed: 26269636]
79. Zanier ER, et al. Fractalkine receptor deficiency is associated with early protection but late worsening of outcome following brain trauma in mice. *J Neurotrauma.* 2015; 33:1060–1072. [PubMed: 26180940]
80. 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. [PubMed: 21166540]
81. Avdic S, et al. Human cytomegalovirus interleukin-10 polarizes monocytes toward a deactivated M2c phenotype to repress host immune responses. *J Virol.* 2013; 87:10273–10282. [PubMed: 23864618]
82. Koscsó B, et al. Adenosine augments IL-10-induced STAT3 signaling in M2c macrophages. *J Leukoc Biol.* 2013; 94:1309–1315. [PubMed: 23922379]
83. David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. *Nat Rev Neurosci.* 2011; 12:388–399. [PubMed: 21673720]
84. Capsoni F, et al. IL-10 up-regulates human monocyte phagocytosis in the presence of IL-4 and IFN- γ . *J Leukoc Biol.* 1995; 58:351–358. [PubMed: 7665991]
85. Leidi M, et al. M2 macrophages phagocytose rituximab-opsinized leukemic targets more efficiently than M1 cells *in vitro*. *J Immunol.* 2009; 182:4415–4422. [PubMed: 19299742]
86. Donnelly RP, Dickensheets H, Finbloom DS. The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. *J Interferon Cytokine Res.* 1999; 19:563–573. [PubMed: 10433356]
87. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol.* 2007; 7:454–465. [PubMed: 17525754]
88. Dziedzic T, et al. Intracerebral hemorrhage triggers interleukin-6 and interleukin-10 release in blood. *Stroke.* 2002; 33:2334–2335. [PubMed: 12215608]
89. Wang KW, et al. Molecular biomarker of inflammatory response is associated with rebleeding in spontaneous intracerebral hemorrhage. *Eur Neurol.* 2011; 66:322–327. [PubMed: 22075847]
90. Shi L, et al. Increased frequency of circulating regulatory T cells in patients with acute cerebral hemorrhage. *Neurosci Lett.* 2015; 591:115–120. [PubMed: 25703227]
91. Liu B, et al. CD163/hemoglobin oxygenase-1 pathway regulates inflammation in hematoma surrounding tissues after intracerebral hemorrhage. *J Stroke Cerebrovasc Dis.* 2015; 24:2800–2809. [PubMed: 26403367]
92. Ewen 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; 66:337–346. [PubMed: 23090788]
93. Gao L, et al. Transplanted neural stem cells modulate regulatory T, $\gamma\delta$ T cells and corresponding cytokines after intracerebral hemorrhage in rats. *Int J Mol Sci.* 2014; 15:4431–4441. [PubMed: 24633197]
94. Yang J, et al. Interleukin-4 ameliorates the functional recovery of intracerebral hemorrhage through the alternative activation of microglia/macrophage. *Front Neurosci.* 2016; 10:61. [PubMed: 27013935]
95. Neve BP, Fruchart JC, Staels B. Role of the peroxisome proliferator-activated receptors (PPAR) in atherosclerosis. *Biochem Pharmacol.* 2000; 60:1245–1250. [PubMed: 11007963]
96. Zhao X, Zhang Y, Strong R, Grotta JC, Aronowski J. 15D-prostaglandin J2 activates peroxisome proliferator-activated receptor- γ , promotes expression of catalase, and reduces inflammation, behavioral dysfunction, and neuronal loss after intracerebral hemorrhage in rats. *J Cereb Blood Flow Metab.* 2006; 26:811–820. [PubMed: 16208315]

97. Zhao X, et al. Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor γ in microglia/macrophages. *Ann Neurol*. 2007; 61:352–362. [PubMed: 17457822]
98. Zhao X, Grotta J, Gonzales N, Aronowski J. Hematoma resolution as a therapeutic target: the role of microglia/macrophages. *Stroke*. 2009; 40:S92–S94. [PubMed: 19064796]
99. Chang CF, et al. Alternative activation-skewed microglia/macrophages promote hematoma resolution in experimental intracerebral hemorrhage. *Neurobiol Dis*. 2017; 103:54–69. [PubMed: 28365213]
100. An WL, et al. Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. *Am J Pathol*. 2003; 163:591–607. [PubMed: 12875979]
101. Pan T, et al. Neuroprotection of rapamycin in lactacystin-induced neurodegeneration via autophagy enhancement. *Neurobiol Dis*. 2008; 32:16–25. [PubMed: 18640276]
102. Malagelada C, Jin ZH, Jackson-Lewis V, Przedborski S, Greene LA. Rapamycin protects against neuron death in *in vitro* and *in vivo* models of Parkinson's disease. *J Neurosci*. 2010; 30:1166–1175. [PubMed: 20089925]
103. Chen S, et al. Alterations in mammalian target of rapamycin signaling pathways after traumatic brain injury. *J Cereb Blood Flow Metab*. 2007; 27:939–949. [PubMed: 16955078]
104. You W, et al. Inhibition of mammalian target of rapamycin attenuates early brain injury through modulating microglial polarization after experimental subarachnoid hemorrhage in rats. *J Neurol Sci*. 2016; 367:224–231. [PubMed: 27423593]
105. Lu Q, 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. [PubMed: 24602288]
106. Wang JP, Zhang MY. Role for target of rapamycin (mTOR) signal pathway in regulating neuronal injury after intracerebral hemorrhage. *Cell Physiol Biochem*. 2017; 41:145–153. [PubMed: 28214828]
107. Wan S, et al. Microglia activation and polarization after intracerebral hemorrhage in mice: the role of protease-activated receptor-1. *Transl Stroke Res*. 2016; 7:478–487. [PubMed: 27206851]
108. Han X, et al. Inhibition of prostaglandin E2 receptor EP3 mitigates thrombin-induced brain injury. *J Cereb Blood Flow Metab*. 2016; 36:1059–1074. [PubMed: 26661165]
109. Hickman SE, et al. The microglial sensome revealed by direct RNA sequencing. *Nat Neurosci*. 2013; 16:1896–1905. [PubMed: 24162652]
110. Cao S, et al. Hematoma changes during clot resolution after experimental intracerebral hemorrhage. *Stroke*. 2016; 47:1626–1631. [PubMed: 27125525]
111. Zhao X, et al. Dimethyl fumarate protects brain from damage produced by intracerebral hemorrhage by mechanism involving Nrf2. *Stroke*. 2015; 46:1923–1928. [PubMed: 25977275]
112. Yao Y, Tsirka SE. The CCL2–CCR2 system affects the progression and clearance of intracerebral hemorrhage. *Glia*. 2012; 60:908–918. [PubMed: 22419223]
113. McGilvray ID, Serghides L, Kapus A, Rotstein OD, Kain KC. Nonopsonic monocyte/macrophage phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes: a role for CD36 in malarial clearance. *Blood*. 2000; 96:3231–3240. [PubMed: 11050008]
114. Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia*. 2002; 40:195–205. [PubMed: 12379907]
115. Fang H, et al. CD36-mediated hematoma absorption following intracerebral hemorrhage: negative regulation by TLR4 signaling. *J Immunol*. 2014; 192:5984–5992. [PubMed: 24808360]
116. Ren Y, Silverstein RL, Allen J, Savill J. CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. *J Exp Med*. 1995; 181:1857–1862. [PubMed: 7536797]
117. Li X, et al. Prostaglandin E2 receptor subtype 2 regulation of scavenger receptor CD36 modulates microglial A β 42 phagocytosis. *Am J Pathol*. 2015; 185:230–239. [PubMed: 25452117]
118. Zamora C, et al. Functional consequences of CD36 downregulation by TLR signals. *Cytokine*. 2012; 60:257–265. [PubMed: 22795952]

119. Olsson M, Nilsson A, Oldenborg PA. Target cell CD47 regulates macrophage activation and erythrophagocytosis. *Transfus Clin Biol.* 2006; 13:39–43. [PubMed: 16564725]
120. Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47–SIRP α signalling pathway. *Trends Cell Biol.* 2009; 19:72–80. [PubMed: 19144521]
121. Olsson M, Nilsson A, Oldenborg PA. Dose-dependent inhibitory effect of CD47 in macrophage uptake of IgG-opsonized murine erythrocytes. *Biochem Biophys Res Commun.* 2007; 352:193–197. [PubMed: 17112468]
122. Jaiswal S, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell.* 2009; 138:271–285. [PubMed: 19632178]
123. Ni W, Mao S, Xi G, Keep RF, Hua Y. Role of erythrocyte CD47 in intracerebral hematoma clearance. *Stroke.* 2016; 47:505–511. [PubMed: 26732568]
124. Hume DA. The many alternative faces of macrophage activation. *Front Immunol.* 2015; 6:370. [PubMed: 26257737]
125. Taetzsch T, et al. Redox regulation of NF- κ B p50 and M1 polarization in microglia. *Glia.* 2015; 63:423–440. [PubMed: 25331559]
126. Zhang ZL, et al. Nuclear factor- κ B activation in perihematomal brain tissue correlates with outcome in patients with intracerebral hemorrhage. *J Neuroinflammation.* 2015; 12:53. [PubMed: 25889503]
127. Arimoto KI, et al. STAT2 is an essential adaptor in USP18-mediated suppression of type I interferon signaling. *Nat Struct Mol Biol.* 2017; 24:279–289. [PubMed: 28165510]
128. Jeon SB, et al. Galectin-3 exerts cytokine-like regulatory actions through the JAK–STAT pathway. *J Immunol.* 2010; 185:7037–7046. [PubMed: 20980634]
129. Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat Rev Immunol.* 2011; 11:750–761. [PubMed: 22025054]
130. Das A, et al. Transcriptome sequencing of microglial cells stimulated with TLR3 and TLR4 ligands. *BMC Genomics.* 2015; 16:517. [PubMed: 26159724]
131. Leopold Wager CM, et al. STAT1 signaling within macrophages is required for antifungal activity against *Cryptococcus neoformans*. *Infect Immun.* 2015; 83:4513–4527. [PubMed: 26351277]
132. Qin H, et al. Signal transducer and activator of transcription-3/suppressor of cytokine signaling-3 (STAT3/SOCS3) axis in myeloid cells regulates neuroinflammation. *Proc Natl Acad Sci USA.* 2012; 109:5004–5009. [PubMed: 22411837]
133. Kim CK, et al. Detrimental effects of leptin on intracerebral hemorrhage via the STAT3 signal pathway. *J Cereb Blood Flow Metab.* 2013; 33:944–953. [PubMed: 23462572]
134. Freilich RW, Woodbury ME, Ikezu T. Integrated expression profiles of mRNA and miRNA in polarized primary murine microglia. *PLoS ONE.* 2013; 8:e79416. [PubMed: 24244499]
135. Uhlemann R, et al. Actin dynamics shape microglia effector functions. *Brain Struct Funct.* 2016; 221:2717–2734. [PubMed: 25989853]
136. Ferreira R, Lively S, Schlichter LC. IL-4 type 1 receptor signaling up-regulates KCNN4 expression, and increases the KCa3.1 current and its contribution to migration of alternative-activated microglia. *Front Cell Neurosci.* 2014; 8:183. [PubMed: 25071444]
137. Wang J, et al. Role of Nrf2 in protection against intracerebral hemorrhage injury in mice. *Free Radic Biol Med.* 2007; 43:408–414. [PubMed: 17602956]
138. Shang H, et al. Time course of Keap1–Nrf2 pathway expression after experimental intracerebral haemorrhage: correlation with brain oedema and neurological deficit. *Free Radic Res.* 2013; 47:368–375. [PubMed: 23438812]
139. Zhao X, et al. Cleaning up after ICH: the role of Nrf2 in modulating microglia function and hematoma clearance. *J Neurochem.* 2015; 133:144–152. [PubMed: 25328080]
140. Schallner N, et al. Microglia regulate blood clearance in subarachnoid hemorrhage by heme oxygenase-1. *J Clin Invest.* 2015; 125:2609–2625. [PubMed: 26011640]
141. Naito Y, Takagi T, Higashimura Y. Heme oxygenase-1 and anti-inflammatory M2 macrophages. *Arch Biochem Biophys.* 2014; 564:83–88. [PubMed: 25241054]
142. Lee S, et al. A small molecule binding HMGB1 and HMGB2 inhibits microglia-mediated neuroinflammation. *Nat Chem Biol.* 2014; 10:1055–1060. [PubMed: 25306442]

143. Kim JB, et al. HMGB1, a novel cytokine-like mediator linking acute neuronal death and delayed neuroinflammation in the postischemic brain. *J Neurosci*. 2006; 26:6413–6421. [PubMed: 16775128]
144. Karuppagounder V, et al. Modulation of macrophage polarization and HMGB1–TLR2/TLR4 cascade plays a crucial role for cardiac remodeling in senescence-accelerated prone mice. *PLoS ONE*. 2016; 11:e0152922. [PubMed: 27070323]
145. Zhou Y, et al. Elevation of high-mobility group protein box-1 in serum correlates with severity of acute intracerebral hemorrhage. *Mediators Inflamm*. 2010; 2010:142458. [PubMed: 20936104]
146. Ohnishi M, et al. HMGB1 inhibitor glycyrrhizin attenuates intracerebral hemorrhage-induced injury in rats. *Neuropharmacology*. 2011; 61:975–980. [PubMed: 21752338]
147. Lei C, et al. High-mobility group box 1 protein promotes neuroinflammation after intracerebral hemorrhage in rats. *Neuroscience*. 2013; 228:190–199. [PubMed: 23085216]
148. Wu H, et al. Cerebroprotection by the neuronal PGE2 receptor EP2 after intracerebral hemorrhage in middle-aged mice. *J Cereb Blood Flow Metab*. 2017; 37:39–51. [PubMed: 26746866]
149. Li D, et al. Blockade of high mobility group box-1 signaling via the receptor for advanced glycation end-products ameliorates inflammatory damage after acute intracerebral hemorrhage. *Neurosci Lett*. 2015; 609:109–119. [PubMed: 26483322]
150. Singh N, et al. Role of PGE2 EP1 receptor in intracerebral hemorrhage-induced brain injury. *Neurotox Res*. 2013; 24:549–559. [PubMed: 23824501]
151. Leclerc JL, Lampert AS, Diller MA, Doré S. Genetic deletion of the prostaglandin E2 E prostanoid receptor subtype 3 improves anatomical and functional outcomes after intracerebral hemorrhage. *Eur J Neurosci*. 2015; 41:1381–1391. [PubMed: 25847406]
152. Wu H, et al. PGE2 receptor agonist misoprostol protects brain against intracerebral hemorrhage in mice. *Neurobiol Aging*. 2015; 36:1439–1450. [PubMed: 25623334]
153. van Echten-Deckert G, Hagen-Euteneuer N, Karaca I, Walter J. Sphingosine-1-phosphate: boon and bane for the brain. *Cell Physiol Biochem*. 2014; 34:148–157. [PubMed: 24977488]
154. Yung YC, Stoddard NC, Mirendil H, Chun J. Lysophosphatidic acid signaling in the nervous system. *Neuron*. 2015; 85:669–682. [PubMed: 25695267]
155. Tham CS, Lin FF, Rao TS, Yu N, Webb M. Microglial activation state and lysophospholipid acid receptor expression. *Int J Dev Neurosci*. 2003; 21:431–443. [PubMed: 14659994]
156. Noda H, Takeuchi H, Mizuno T, Suzumura A. Fingolimod phosphate promotes the neuroprotective effects of microglia. *J Neuroimmunol*. 2013; 256:13–18. [PubMed: 23290828]
157. Marfia G, et al. The adipose mesenchymal stem cell secretome inhibits inflammatory responses of microglia: evidence for an involvement of sphingosine-1-phosphate signalling. *Stem Cells Dev*. 2016; 25:1095–1107. [PubMed: 27217090]
158. Gao F, et al. Fingolimod (FTY720) inhibits neuroinflammation and attenuates spontaneous convulsions in lithium-pilocarpine induced status epilepticus in rat model. *Pharmacol Biochem Behav*. 2012; 103:187–196. [PubMed: 22960129]
159. Serdar M, et al. Fingolimod protects against neonatal white matter damage and long-term cognitive deficits caused by hyperoxia. *Brain Behav Immun*. 2016; 52:106–119. [PubMed: 26456693]
160. Rothhammer V, et al. Sphingosine 1-phosphate receptor modulation suppresses pathogenic astrocyte activation and chronic progressive CNS inflammation. *Proc Natl Acad Sci USA*. 2017; 114:2012–2017. [PubMed: 28167760]
161. Rolland WB II, et al. FTY720 is neuroprotective and improves functional outcomes after intracerebral hemorrhage in mice. *Acta Neurochir Suppl*. 2011; 111:213–217. [PubMed: 21725758]
162. Rolland WB, et al. Fingolimod reduces cerebral lymphocyte infiltration in experimental models of rodent intracerebral hemorrhage. *Exp Neurol*. 2013; 241:45–55. [PubMed: 23261767]
163. Fu Y, et al. Fingolimod for the treatment of intracerebral hemorrhage: a 2-arm proof-of-concept study. *JAMA Neurol*. 2014; 71:1092–1101. [PubMed: 25003359]
164. Lu L, et al. Fingolimod exerts neuroprotective effects in a mouse model of intracerebral hemorrhage. *Brain Res*. 2014; 1555:89–96. [PubMed: 24502984]

165. US National Library of Medicine. ClinicalTrials.gov. 2014. <https://clinicaltrials.gov/ct2/show/NCT02002390>
166. Chen S, et al. Regulatory T cell in stroke: a new paradigm for immune regulation. *Clin Dev Immunol.* 2013; 2013:689827. [PubMed: 23983771]
167. Gong G, et al. Phosphoantigen-activated V γ 2V δ 2 T cells antagonize IL-2-induced CD4⁺CD25⁺Foxp3⁺ T regulatory cells in mycobacterial infection. *Blood.* 2009; 113:837–845. [PubMed: 18981295]
168. Zhong Q, et al. Interleukin-23 secreted by activated macrophages drives $\gamma\delta$ T cell production of interleukin-17 to aggravate secondary injury after intracerebral hemorrhage. *J Am Heart Assoc.* 2016; 5:e004340. [PubMed: 27729335]
169. Yang Z, et al. Regulatory T cells inhibit microglia activation and protect against inflammatory injury in intracerebral hemorrhage. *Int Immunopharmacol.* 2014; 22:522–525. [PubMed: 25000335]
170. Mao, LL., et al. Adoptive regulatory T-cell therapy attenuates perihematomal inflammation in a mouse model of experimental intracerebral hemorrhage. *Cell Mol Neurobiol.* 2016. <http://dx.doi.org/10.1007/s10571-016-0429-1>
171. Paolicelli RC, Bisht K, Tremblay ME. Fractalkine regulation of microglial physiology and consequences on the brain and behavior. *Front Cell Neurosci.* 2014; 8:129. [PubMed: 24860431]
172. Limatola C, Ransohoff RM. Modulating neurotoxicity through CX3CL1/CX3CR1 signaling. *Front Cell Neurosci.* 2014; 8:229. [PubMed: 25152714]
173. Wolf Y, Yona S, Kim KW, Jung S. Microglia, seen from the CX3CR1 angle. *Front Cell Neurosci.* 2013; 7:26. [PubMed: 23507975]
174. Zabel MK, et al. Microglial phagocytosis and activation underlying photoreceptor degeneration is regulated by CX3CL1–CX3CR1 signaling in a mouse model of retinitis pigmentosa. *Glia.* 2016; 64:1479–1491. [PubMed: 27314452]
175. Denes A, Ferenczi S, Halasz J, Kornyei Z, Kovacs KJ. Role of CX3CR1 (fractalkine receptor) in brain damage and inflammation induced by focal cerebral ischemia in mouse. *J Cereb Blood Flow Metab.* 2008; 28:1707–1721. [PubMed: 18575457]
176. Tarozzo G, Campanella M, Ghiani M, Bulfone A, Beltramo M. Expression of fractalkine and its receptor, CX3CR1, in response to ischaemia–reperfusion brain injury in the rat. *Eur J Neurosci.* 2002; 15:1663–1668. [PubMed: 12059974]
177. Zhu J, Zhou Z, Liu Y, Zheng J. Fractalkine and CX3CR1 are involved in the migration of intravenously grafted human bone marrow stromal cells toward ischemic brain lesion in rats. *Brain Res.* 2009; 1287:173–183. [PubMed: 19563789]
178. Soriano SG, et al. Mice deficient in fractalkine are less susceptible to cerebral ischemia–reperfusion injury. *J Neuroimmunol.* 2002; 125:59–65. [PubMed: 11960641]
179. Gaetani P, et al. Immunohistochemical expression of the chemokine fractalkine and its receptor in the human brain cortex after severe traumatic brain injury and brain hemorrhage. *J Neurosurg Sci.* 2013; 57:55–62. [PubMed: 23584220]
180. Taylor RA, Hammond MD, Ai Y, Sansing LH. CX3CR1 signaling on monocytes is dispensable after intracerebral hemorrhage. *PLoS ONE.* 2014; 9:e114472. [PubMed: 25469644]
181. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science.* 2005; 308:1314–1318. [PubMed: 15831717]
182. Nash B, et al. Functional duality of astrocytes in myelination. *J Neurosci.* 2011; 31:13028–13038. [PubMed: 21917786]
183. McKimmie CS, Graham GJ. Astrocytes modulate the chemokine network in a pathogen-specific manner. *Biochem Biophys Res Commun.* 2010; 394:1006–1011. [PubMed: 20331977]
184. Allaman I, Belanger M, Magistretti PJ. Astrocyte–neuron metabolic relationships: for better and for worse. *Trends Neurosci.* 2011; 34:76–87. [PubMed: 21236501]
185. Mantovani A, et al. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 2004; 25:677–686. [PubMed: 15530839]
186. Rosell A, et al. Brain perihematoma genomic profile following spontaneous human intracerebral hemorrhage. *PLoS ONE.* 2011; 6:e16750. [PubMed: 21311749]

187. Carmichael ST, et al. Genomic profiles of damage and protection in human intracerebral hemorrhage. *J Cereb Blood Flow Metab.* 2008; 28:1860–1875. [PubMed: 18628781]
188. Yao Y, Tsirka SE. Chemokines and their receptors in intracerebral hemorrhage. *Transl Stroke Res.* 2012; 3:70–79. [PubMed: 24323863]
189. Lu A, et al. Brain genomics of intracerebral hemorrhage. *J Cereb Blood Flow Metab.* 2006; 26:230–252. [PubMed: 16034371]
190. Hernandez-Rabaza V, et al. Neuroinflammation increases GABAergic tone and impairs cognitive and motor function in hyperammonemia by increasing GAT-3 membrane expression. Reversal by sulforaphane by promoting M2 polarization of microglia. *J Neuroinflammation.* 2016; 13:83. [PubMed: 27090509]
191. He M, et al. Astrocyte-derived CCL2 is associated with M1 activation and recruitment of cultured microglial cells. *Cell Physiol Biochem.* 2016; 38:859–870. [PubMed: 26910882]
192. Joseph MJ, Caliaperumal J, Schlichter LC. After intracerebral hemorrhage, oligodendrocyte precursors proliferate and differentiate inside white-matter tracts in the rat striatum. *Transl Stroke Res.* 2016; 7:192–208. [PubMed: 26743212]
193. Miron VE, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci.* 2013; 16:1211–1218. [PubMed: 23872599]
194. Zhao X, et al. Neuroprotective role of haptoglobin after intracerebral hemorrhage. *J Neurosci.* 2009; 29:15819–15827. [PubMed: 20016097]
195. Shukla SM, Sharma SK. Sinomenine inhibits microglial activation by A β and confers neuroprotection. *J Neuroinflammation.* 2011; 8:117. [PubMed: 21917137]
196. Qian L, et al. Sinomenine, a natural dextrorotatory morphinan analog, is anti-inflammatory and neuroprotective through inhibition of microglial NADPH oxidase. *J Neuroinflammation.* 2007; 4:23. [PubMed: 17880684]
197. Xu M, Liu L, Qi C, Deng B, Cai X. Sinomenine versus NSAIDs for the treatment of rheumatoid arthritis: a systematic review and meta-analysis. *Planta Med.* 2008; 74:1423–1429. [PubMed: 18683125]
198. Yang Z, et al. Sinomenine inhibits microglia activation and attenuates brain injury in intracerebral hemorrhage. *Mol Immunol.* 2014; 60:109–114. [PubMed: 24815539]
199. Shi H, et al. Sinomenine enhances microglia M2 polarization and attenuates inflammatory injury in intracerebral hemorrhage. *J Neuroimmunol.* 2016; 299:28–34. [PubMed: 27725118]
200. Kim K, et al. The effect of human umbilical cord blood-derived mesenchymal stem cells in a collagenase-induced intracerebral hemorrhage rat model. *Exp Neurobiol.* 2015; 24:146–155. [PubMed: 26113794]
201. US National Library of Medicine. ClinicalTrials.gov. 2017. <https://clinicaltrials.gov/ct2/show/NCT02274428>
202. Wang J, Dore S. Inflammation after intracerebral hemorrhage. *J Cereb Blood Flow Metab.* 2007; 27:894–908. [PubMed: 17033693]
203. Wang J, Tsirka SE. Contribution of extracellular proteolysis and microglia to intracerebral hemorrhage. *Neurocrit Care.* 2005; 3:77–85. [PubMed: 16159103]
204. Komohara Y, Jinushi M, Takeya M. Clinical significance of macrophage heterogeneity in human malignant tumors. *Cancer Sci.* 2014; 105:1–8. [PubMed: 24168081]
205. Siddiqui TA, Lively S, Schlichter LC. Complex molecular and functional outcomes of single versus sequential cytokine stimulation of rat microglia. *J Neuroinflammation.* 2016; 13:66. [PubMed: 27009332]
206. Vogel DY, et al. Macrophages migrate in an activation-dependent manner to chemokines involved in neuroinflammation. *J Neuroinflammation.* 2014; 11:23. [PubMed: 24485070]
207. Guedj K, et al. M1 macrophages act as LT β R-independent lymphoid tissue inducer cells during atherosclerosis-related lymphoid neogenesis. *Cardiovasc Res.* 2014; 101:434–443. [PubMed: 24272771]
208. Zhong TY, et al. Hemocyanins stimulate innate immunity by inducing different temporal patterns of proinflammatory cytokine expression in macrophages. *J Immunol.* 2016; 196:4650–4662. [PubMed: 27183578]

209. Tarassishin L, Suh HS, Lee SC. Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3K/Akt pathway. *J Neuroinflammation*. 2011; 8:187. [PubMed: 22208359]
210. Chong BF, et al. A subset of CD163⁺ macrophages displays mixed polarizations in discoid lupus skin. *Arthritis Res Ther*. 2015; 17:324. [PubMed: 26568320]
211. Hennessy E, Griffin EW, Cunningham C. Astrocytes are primed by chronic neurodegeneration to produce exaggerated chemokine and cell infiltration responses to acute stimulation with the cytokines IL-1 β and TNF- α . *J Neurosci*. 2015; 35:8411–8422. [PubMed: 26041910]
212. Sielska M, et al. Distinct roles of CSF family cytokines in macrophage infiltration and activation in glioma progression and injury response. *J Pathol*. 2013; 230:310–321. [PubMed: 23520016]
213. Suenaga J, et al. White matter injury and microglia/macrophage polarization are strongly linked with age-related long-term deficits in neurological function after stroke. *Exp Neurol*. 2015; 272:109–119. [PubMed: 25836044]
214. Wachholz S, et al. Microglia activation is associated with IFN- α induced depressive-like behavior. *Brain Behav Immun*. 2015; 55:105–113. [PubMed: 26408795]
215. Okuneva O, et al. Abnormal microglial activation in the *Cstb*^{-/-} mouse, a model for progressive myoclonus epilepsy, EPM1. *Glia*. 2015; 63:400–411. [PubMed: 25327891]
216. Stojakovic, A., et al. Role of the IL-1 pathway in dopaminergic neurodegeneration and decreased voluntary movement. *Mol Neurobiol*. 2016. <http://dx.doi.org/10.1007/s12035-016-9988-x>
217. Liu X, et al. Interleukin-4 is essential for microglia/macrophage M2 polarization and long-term recovery after cerebral ischemia. *Stroke*. 2016; 47:498–504. [PubMed: 26732561]
218. Peferoen LA, et al. Activation status of human microglia is dependent on lesion formation stage and remyelination in multiple sclerosis. *J Neuropathol Exp Neurol*. 2015; 74:48–63. [PubMed: 25470347]
219. Yamashita U, Kuroda E. Regulation of macrophage-derived chemokine (MDC, CCL22) production. *Crit Rev Immunol*. 2002; 22:105–114. [PubMed: 12433129]
220. Evans BJ, Haskard DO, Sempowski G, Landis RC. Evolution of the macrophage CD163 phenotype and cytokine profiles in a human model of resolving inflammation. *Int J Inflam*. 2013; 2013:780502. [PubMed: 23738227]
221. Munder M, et al. Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. *Blood*. 2005; 105:2549–2556. [PubMed: 15546957]
222. Pesce JT, et al. Arginase-1-expressing macrophages suppress T_H2 cytokine-driven inflammation and fibrosis. *PLoS Pathog*. 2009; 5:e1000371. [PubMed: 19360123]
223. Nair MG, Cochrane DW, Allen JE. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of Ym1 and Fizz1 that can be partly replicated *in vitro*. *Immunol Lett*. 2003; 85:173–180. [PubMed: 12527225]
224. Raes G, et al. Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. *J Leukoc Biol*. 2002; 71:597–602. [PubMed: 11927645]
225. Durafour BA, et al. Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia*. 2012; 60:717–727. [PubMed: 22290798]
226. 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; 75:1058–1071.
227. Roberts AW. G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors*. 2005; 23:33–41. [PubMed: 16019425]
228. Babu R, Bagley JH, Di C, Friedman AH, Adamson C. Thrombin and hemin as central factors in the mechanisms of intracerebral hemorrhage-induced secondary brain injury and as potential targets for intervention. *Neurosurg Focus*. 2012; 32:E8.
229. Yang J, et al. Multimodality MRI assessment of grey and white matter injury and blood–brain barrier disruption after intracerebral haemorrhage in mice. *Sci Rep*. 2017; 7:40358. [PubMed: 28084426]
230. Lei B, et al. Intrastriatal injection of autologous blood or clostridial collagenase as murine models of intracerebral hemorrhage. *J Vis Exp*. 2014; 89:51439.

231. Bao L, et al. Thrombin-induced apoptosis in neurons through activation of c-Jun-N-terminal kinase. *Toxicol Mech Methods*. 2017; 27:18–23. [PubMed: 27841083]
232. Hu S, et al. Thrombin-induced autophagy: a potential role in intracerebral hemorrhage. *Brain Res*. 2011; 1424:60–66. [PubMed: 22015349]
233. Kirkman MA, Allan SM, Parry-Jones AR. Experimental intracerebral hemorrhage: avoiding pitfalls in translational research. *J Cereb Blood Flow Metab*. 2011; 31:2135–2151. [PubMed: 21863040]
234. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012; 32:612–627. [PubMed: 22293989]
235. Kleinig TJ, et al. Hemoglobin crystals: a pro-inflammatory potential confounder of rat experimental intracerebral hemorrhage. *Brain Res*. 2009; 1287:164–172. [PubMed: 19576188]
236. MacLellan CL, Davies LM, Fingas MS, Colbourne F. The influence of hypothermia on outcome after intracerebral hemorrhage in rats. *Stroke*. 2006; 37:1266–1270. [PubMed: 16574928]
237. MacLellan CL, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008; 28:516–525. [PubMed: 17726491]
238. Manaenko A, Chen H, Zhang JH, Tang J. Comparison of different preclinical models of intracerebral hemorrhage. *Acta Neurochir Suppl*. 2011; 111:9–14. [PubMed: 21725724]
239. Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: effects of aging on brain edema and neurological deficits. *Stroke*. 2004; 35:2571–2575. [PubMed: 15472083]
240. Wasserman JK, Schlichter LC. White matter injury in young and aged rats after intracerebral hemorrhage. *Exp Neurol*. 2008; 214:266–275. [PubMed: 18848934]
241. Lee JC, Cho GS, Choi BO, Kim HC, Kim WK. Aging exacerbates intracerebral hemorrhage-induced brain injury. *J Neurotrauma*. 2009; 26:1567–1576. [PubMed: 19473060]
242. Okauchi M, et al. Deferoxamine treatment for intracerebral hemorrhage in aged rats: therapeutic time window and optimal duration. *Stroke*. 2010; 41:375–382. [PubMed: 20044521]
243. MacLellan CL, Silasi G, Auriat AM, Colbourne F. Rodent models of intracerebral hemorrhage. *Stroke*. 2010; 41:S95–S98. [PubMed: 20876518]
244. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014; 255:49–55. [PubMed: 24582611]
245. US National Library of Medicine. ClinicalTrials.gov. 2017. <https://clinicaltrials.gov/ct2/show/NCT02175225>
246. US National Library of Medicine. ClinicalTrials.gov. 2016. <https://clinicaltrials.gov/ct2/show/NCT01805895>
247. Kata D, et al. Rosuvastatin enhances anti-inflammatory and inhibits pro-inflammatory functions in cultured microglial cells. *Neuroscience*. 2016; 314:47–63. [PubMed: 26633263]
248. US National Library of Medicine. ClinicalTrials.gov. 2009. <https://clinicaltrials.gov/ct2/show/NCT00364559>
249. Yu Z, et al. Erythropoietin reduces brain injury after intracerebral hemorrhagic stroke in rats. *Mol Med Rep*. 2013; 8:1315–1322. [PubMed: 24008820]
250. Chau M, Chen D, Wei L. Erythropoietin attenuates inflammatory factors and cell death in neonatal rats with intracerebral hemorrhage. *Acta Neurochir Suppl*. 2011; 111:299–305. [PubMed: 21725772]
251. US National Library of Medicine. ClinicalTrials.gov. 2015. <https://clinicaltrials.gov/ct2/show/NCT00413946>
252. 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. [PubMed: 26657742]

Key points

- Microglial polarization after intracerebral haemorrhage (ICH) modulates microglial phagocytic function and might affect haematoma clearance
- Activation of microglia to an M1-like phenotype occurs mainly in the acute phase after ICH
- M2-like microglial responses occur in the subacute and chronic phase and might contribute to phagocytosis of cell debris and haematoma clearance
- Microglial polarization can be regulated by transcription factors, chemokines, receptors and their signalling pathways, and interactions between microglia and other cells in the brain (T lymphocytes, neurons, astrocytes and oligodendrocytes)
- Data from clinical trials and preclinical studies suggest that targeting of microglial phenotype switching represents a new research direction for ICH treatment

Figure 1. Dynamic changes in microglial marker levels and profiles over time after intracerebral haemorrhage

Top panel, dashed red curve: microglial M1-like response exhibits a decreasing trend in the first 14 days. Top panel, dashed blue curve: microglial M2 like response exhibits an increasing trend in the first 14 days. Bottom panel: microglia exhibit an M1-like response as early as 6 h after intracerebral haemorrhage (ICH), as shown by upregulation of proinflammatory cytokines such as IL-1 β , IL-6, tumour necrosis factor (TNF) and inducible nitric oxide synthase (iNOS). M2 markers, such as arginase-1 (Arg1), chitinase-like protein 3 (also known as Ym1), CD206, CD163 and IL-10, start to increase on day 1 after ICH. Transforming growth factor- β (TGF β) is upregulated from day 7 until day 14 post-ICH. Although a mixed M1-like and M2-like microglial phenotype is evident during days 1 to 3, the balance of evidence supports an M1 to M2 phenotype switch in the first 7 days. IFN γ levels increase on day 7 after ICH, and levels of most proinflammatory cytokines return to baseline on day 14.

Figure 2. Modulators of microglial polarization and phagocytosis after intracerebral haemorrhage

Several transcription factors modulate microglial polarization towards an M1-like (left panel) or an M2-like (centre panel) phenotype. Activation of high-mobility group protein 1 (HMG1) and Toll-like receptor (TLR)2 or TLR4 promotes microglial M1-like responses. Signal transducer and activator of transcription (STAT)1 is activated by TLRs and IFN γ , promoting microglial polarization to an M1 phenotype. After intracerebral haemorrhage (ICH), TLRs modulate STAT3 phosphorylation, which increases M1-like polarization. G-protein-coupled E prostanoïd (EP) receptors EP1 and EP3 are mainly expressed on microglia and drive neuronal toxicity mediated by M1 upregulation after ICH. STAT6 accumulates in response to IL-4 and is responsible for the transcription of M2-related genes. Sphingosine-1-phosphate (S1P) receptor signalling contributes to the downregulation of proinflammatory cytokines and enhances M2-like responses after ICH. Although EP2 receptors are not expressed in microglia after ICH, EP2 deletion results in an increased microglial proinflammatory response. Other modulators regulate microglial phagocytosis (right panel). Activation of the transcription factor nuclear factor erythroid 2 related factor 2 (Nrf2) is associated with microglial phagocytosis after ICH and increases CD36 and haem oxygenase-1 (HO-1) expression. The *CD36* gene is a target of peroxisome proliferator-activated receptor- γ (PPAR γ), which belongs to the nuclear receptor family. CD47 expression in red blood cells (RBC) can decrease their phagocytosis by microglia after ICH. Although CD36 expression is increased in the ICH brain, TLR4 activation might negatively regulate its expression and delay haematoma clearance. IFNGR, IFN γ receptor; NF- κ B, nuclear factor- κ B; MAL, Toll/IL-1 receptor domain-containing adaptor protein (also known as MYD88 adapter-like protein); MYD88, myeloid differentiation primary response protein

MYD88; SOCS3, suppressor of cytokine signalling 3; TRAM, TIR domain-containing adaptor molecule 2 (also known as TRIF-related adaptor molecule); TRIF, TIR domain-containing adaptor molecule 1.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1**Markers of M1 and M2 microglia**

Marker	Type	Function and characteristic
M1		
IL-1 β	Cytokine	Proinflammatory
IL-6	Cytokine	Proinflammatory
IL-12 p70	Cytokine	Proinflammatory
TNF	Cytokine	Proinflammatory
IFN γ ²⁰⁵	Cytokine	Proinflammatory, M1 microglia and macrophage inducer
CCL5 (REF. 206)	Chemokine	Recruits immune cells
CCL20 (REF. 207)	Chemokine	Recruits immune cells
CXCL1 (REFS 208,209)	Chemokine	Recruits immune cells
CXCL10 (REFS 210,211)	Chemokine	Recruits immune cells
GM-CSF ²¹²	Chemokine	Recruits immune cells, induces M1 microglia and macrophages
CD16 (REF. 213)	Immunoglobulin Fc receptor	Induces proinflammatory signalling
CD32 (REF. 213)	Immunoglobulin Fc receptor	Induces proinflammatory signalling
CD86 (REF. 214)	Surface receptor	Classic M1 microglia and macrophage marker
MHC-II ²¹⁵	Surface receptor	Mediates T cell differentiation to T _H 1
iNOS	Metabolic enzyme	Mediates nitric oxide synthesis
M2a		
IL-1Ra ²¹⁶	Cytokine	IL-1 receptor antagonist
IL-4 (REFS 78,217)	Cytokine	Anti-inflammatory, increases microglia and macrophage phagocytosis
TGF β	Cytokine	Anti-inflammatory
CCL22 (REFS 218,219)	Chemokine	Recruits dendritic cells, T _H 2 cells and regulatory T cells
CD206 (REF. 213)	Mannose receptor	Mediates endocytosis and phagocytosis in response to microglia and macrophage activation
M2b, M2c		
CD163 (REF. 220)	Scavenger receptor	Haemoglobin clearance
Arg1 (REFS 221,222)	Cytosolic enzyme	Suppresses inflammation; upregulated by IL-4 and IL-13
Ym1 (REFS 223,224)	Secretory protein	Anti-inflammatory; induction depends on IL-4 and IL-13
FIZZ1 (REF. 224)	Secretory protein	Anti-inflammatory; induction depends on IL-4 and IL-13
IL-10 (REF. 225)	Cytokine	Anti-inflammatory, mediates microglia and macrophage phagocytosis
IL-4Ra ²²⁶	Cytokine	Binds to IL-4 and IL-13
G-CSF ²²⁷	Cytokine	Mediates microglia and macrophage survival, proliferation and differentiation

Arg1, arginase 1; CCL, chemokine (CC motif) ligand; CXCL, chemokine (CXC motif) ligand; FIZZ1, resistin-like molecule α 1; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; IL-1Ra, IL-1 receptor antagonist; IL-4Ra, IL-4 receptor- α ; iNOS, inducible nitric oxide synthase; MHC-II, major histocompatibility complex-II; TGF β , transforming growth factor β ; T_H, T-helper; TNF, tumour necrosis factor; Ym1, chitinase-like protein 3.

Table 2

Animal models of ICH

Collagenase-induced model	Blood-induced model	Thrombin-induced model
Method		
Collagenase injected into the brain breaks down small blood vessels and initiates bleeding	Autologous blood injected into the brain	Thrombin injected into the brain
Clinical features mimicked		
Acute cerebrovascular rupture with blood–brain barrier breakdown	Blood toxicity and the resulting inflammation	Thrombin toxicity (thrombin released from haematoma is a main contributor to secondary brain damage in acute ICH ²²⁸)
Advantages		
<ul style="list-style-type: none"> • Haematoma expansion and increased intracranial pressure resembles that in clinical ICH²²⁹ • Brain infiltration by systemic immune cells is similar to that in blood-induced models when the blood volume is matched⁴⁷ • Simple procedure (requires less time than blood injection) 	<ul style="list-style-type: none"> • Haematoma size is easier to control than in collagenase-induced ICH²³⁰ • Brain infiltration of systemic immune cells is similar to that in collagenase-induced models when the blood volume is matched⁴⁷ 	<ul style="list-style-type: none"> • Used for investigating mechanisms of thrombin toxicity that cause neuroinflammation²²⁸ and cell death^{231,232} • Simple procedure
Limitations		
Excessive inflammation claimed by some (not all) researchers ^{6,233,234}	<ul style="list-style-type: none"> • Excessive inflammation claimed by some (not all) researchers^{6,50,233,235} • Limited blood–brain barrier disruption and rapid haematoma resolution^{236,237} • Blood reflux • No small vessel rupture • Not suitable for assessing long-term functional outcomes^{233,237,238} 	No uses other than in thrombin toxicity
Adverse effects of ageing		
<ul style="list-style-type: none"> • Axonal damage in white matter before day 3 worse in aged versus young animals, despite similar grey matter losses^{239–241} • Functional outcomes worse in aged versus young rats by day 3, attributed to increased microglial activation^{239–241} • Increases in M1 markers (IL-1β, TNF, IL-6, iNOS) are delayed and higher in old versus young rats: levels increase within 24 h in young rats, but not until days 3–7 in aged rats⁶⁴ • Increases in M2 markers (arginase-1, CD163) delayed in old versus young rats; IL-4 increased only in aged rats⁶⁴ 	<ul style="list-style-type: none"> • Brain injury and swelling on day 3 worse in old versus young rats, attributed to increased microglial activation and suppression of reactive astrocytes^{130,132} • Neurological deficits worse in old versus young rats on day 1 and throughout the study¹³⁰ • Increased expression of IL-1β and reduced expression of GFAP and ciliary neurotrophic factor in aged versus young rats¹³² 	No data

Collagenase-induced model	Blood-induced model	Thrombin-induced model
<ul style="list-style-type: none">• TGFβ upregulated on day 3 in young and aged rats⁶⁴		

In translational medicine, parallel testing in different but related animal models is strongly encouraged^{6,233,234}. The two principal animal models of ICH are the collagenase-induced and blood-induced models, both of which are used to study its pathophysiology, test potential drug targets, and assess functional recovery^{237,242–244}. GFAP, glial fibrillary acidic protein; ICH, intracerebral haemorrhage; iNOS, inducible nitric oxide synthase; TGF β , transforming growth factor- β ; TNF, tumour necrosis factor.

Table 3

Promising anti-ICH drugs that target microglial polarization

Drug	Mode of action	Effects on microglia	Development phase
Fingolimod	S1PR activator	Decreases M1-like microglial responses ^{156,157,162}	Phase II ¹⁶⁵
Deferoxamine	Iron chelation and haematoma clearance	Decreases M1-like microglial responses ^{121,200}	Phase II ²⁴⁵
Minocycline	Iron chelation and haematoma clearance	Decreases M1-like microglial responses ⁵³	Phase I and II ²⁴⁶
Rosuvastatin	Lipid metabolism	Promotes M1-to-M2 phenotype shift ²⁴⁷	Phase II ²⁴⁸
Erythropoietin	Anti-inflammatory response	Decreases M1-like microglial responses ^{249,250}	Phase II ²⁵¹
Pinocembrin	TLR4 inhibitor	Decreases M1-like microglial responses ⁶²	Preclinical
Resatorvid	TLR4 inhibitor	Decreases M1-like microglial responses ^{68,252}	Preclinical
Sinomenine	Anti-inflammatory response	Enhances M2-like microglial responses ¹⁹⁹	Preclinical
Rapamycin	mTOR activator	Enhances M2-like microglial responses ¹⁰⁵	Preclinical

mTOR, mammalian target of rapamycin; S1PR, sphingosine-1-phosphate receptor; TLR4, Toll-like receptor 4.