ACCSCIENCE PUBLISHING

Gene & Protein in Disease

REVIEW ARTICLE

Hematoma clearance by reactive microglia after intracerebral hemorrhage

Zhenhua Zhang[1†], Wei Xu[1,2†], Honghui Sheng[1], Leo Huang[3], Jiaxin Zhang[4], Lanting Zhang[5], Limin Wang[6], Junmin Wang[1], Xiuhua Ren[1*], Chao Jiang[7*], and Jian Wang[1*]

[1]Department of Human Anatomy, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou, Henan Province, China

[2]School of Public Health, Zhengzhou University, Zhengzhou, Henan Province, China

[3]Department of Psychology, University of Toronto, Toronto, Ontario M5S 1A1, Canada

[4]Saint John Paul the Great Catholic High School, Dumfries, VA 20026, USA

[5]High School Division of Zhengzhou Middle School, Zhengzhou, Henan Province, China

[6]Department of Neurology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangdong Neuroscience Institute, Guangzhou, China

[7]Department of Neurology, Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China

Abstract

Intracerebral hemorrhage (ICH) is a subtype of stroke with high incidence rate and mortality. The pathogenesis of ICH involves primary brain injury and secondary brain injury. Unfortunately, no approved treatment options and therapies targeting them have shown satisfactory outcomes. Microglia are resident innate immune cells with phagocytic function in the central nervous system that rapidly respond to brain injury. Recent research has indicated that reactive microglia with enhanced phagocytosis reprogrammed by the interleukin 10 (IL-10) signaling pathway are critical for endogenous hematoma clearance. In this review, we first summarize the progress of microglial activation and function after ICH, focusing on specific microglial markers, pro- and anti-inflammatory molecules, as well as phenotypic and functional changes. The available evidence supports that microglia play a dual role after ICH. Second, we summarize the results of previous studies on hematoma clearance, focusing on reactive microglia in clearing hematoma through endogenous pathways reprogrammed by IL-10 or other molecules and necessitating the prospect of further research in this field. This review will help us better understand the role of reactive microglia in hematoma clearance and identify potential therapeutic targets to facilitate translational research in this direction.

Keywords: Intracerebral hemorrhage; Microglial/microphage; Inflammation; Anti-inflammation; Interleukin 10; Phagocytosis

†These authors contributed equally to this work.

***Corresponding authors:**
Xiuhua Ren
(renxiuhua@zzu.edu.cn)
Chao Jiang
(chaojzzu@126.com)
Jian Wang
(jianwang2020@outlook.com)

Citation: Zhang Z, Xu W, Sheng H, *et al.*, 2023, Hematoma clearance by reactive microglia after intracerebral hemorrhage. *Gene Protein Dis*.
https://doi.org/10.36922/gpd.336

Received: January 26, 2023
Accepted: March 20, 2023
Published Online: March 30, 2023

1. Introduction

Intracerebral hemorrhage (ICH) is caused by blood leaking into the brain parenchyma due to rupture of the small cerebral artery or the cerebral arterial[1]. For 10 – 15% of stroke

cases worldwide, ICH affects approximately 2 million people annually, and more than 50% of spontaneous ICH cases occur in the basal ganglia[2-5]. With around 40% of patients dying within 30 days after initial ictus, ICH has the highest mortality among all strokes, resulting in enormous disease and social burden[6,7]. The treatment of ICH mainly focuses on supporting therapy and managing both symptoms and complications. Unfortunately, there is still no approved treatment for ICH[8,9].

ICH leads to primary and secondary brain injury[10]. Within the first hour following blood vessel rupture, hematoma forms, and expands with a resulting mass effect, leading to primary brain injury[8,11]. In contrast, the blood degradation products released from the hematoma, microglial/macrophage activation with the release of pro-inflammatory molecules, and infiltrated blood immunocytes all contribute to secondary brain injury after ICH[12-14]. Consequently, hematoma removal is a potential treatment option to prevent secondary injury cascades. However, surgical removal of the hematoma has not shown to significantly increase the survival rate of patients or promote the neurological recovery of survivors[15].

Treatment with potential neuroprotective agents identified to protect against secondary injury has not produced satisfactory functional recovery in patients with ICH[16]. We aim to summarize the progress of microglial activation and function after ICH by highlighting the dual role of microglia and microglial-specific markers since it can be targeted to mitigate secondary brain injury and improve brain repair and recovery. Furthermore, we outline the time course of changes in the molecular markers of microglia activation after ICH and emphasize the phagocytic function of microglia. Finally, we discuss the underlying mechanism of hematoma clearance by microglia after ICH. This review will help us better understand the role of microglia in hematoma clearance after ICH and identify potential therapeutic targets to facilitate translational research in this direction.

2. Literature search strategy and selection criteria

For this review, we searched PubMed for articles published between April 19, 2016, and January 6, 2023, using the terms "ICH," "inflammation," "hematoma clearance," "Microglial/macrophage," "Anti-inflammation," "IL-10," "phagocytosis," and "molecular markers." We used English search words and included papers published with English abstracts. We selected articles describing microglial activation and function as well as hematoma clearance mechanisms after ICH.

3. Microglia and macrophage activation and function after ICH

Microglia, as resident innate immune cells in the central nervous system (CNS) with the ability to phagocytose, are the first responders to brain injury, and they constitute 5%–20% of all glial cells in the adult human brain[17]. In the past, activated microglia and macrophages were commonly grouped as microglia/macrophages (MMΦ) as it was difficult to differentiate them by immunohistology[18]. However, in recent years, with flow cytometry, microglia can be identified by the $CD11b^{+}CD45^{int}$ phenotype, while monocyte-derived macrophages can be identified by the $CD11b^{+}CD45^{high}F4/80^{+}Ly6c^{-}$ phenotype[19-21]. Microglia are amoeba-like cells that can migrate to the damaged site and activate when pathological changes disrupt brain homeostasis[22,23]. After microglial activation, monocyte-derived macrophages infiltrate the perihematomal region[20,24,25]. Established anti-inflammatory markers increase on day 1, with a significant increase on day 3, and a further increase on day 7[19]. Hemorrhagic injury to the brain contributes to prominent microglial activation, characterized by increased ionized calcium-binding adaptor molecule 1 (Iba1) and CD11b expression as well as thick and short cell processes[26].

Microglia are highly diverse and have dual effect on the brain during inflammation. Through transcriptome analysis, surveillant microglia, homeostasis-associated microglia, axon-associated microglia, highly activated microglia, synapse-associated microglia, and virus-associated microglia have been identified in the CNS[27-29]. Clinical and preclinical studies have shown that there is microglial heterogeneity across specific brain regions in terms of morphology, density, gene expression, and proliferation. Although it is not fully understood, evidence has indicated that microglia play a unique role in different brain regions[30]. However, two microglial activation states, which include the pro- and anti-inflammatory phenotypes, still predominate in stroke and ICH[26]. Although recent studies have shown that microglial activation states are much more complex than these two phenotypes, we can use these two terms for convenience of description[31]. However, we suggest focusing more on microglial function than on phenotype alone.

The molecular markers identifying the pro-inflammatory phenotype include inducible nitric oxide synthase (iNOS), CD68, CD16/32, and CD86, while arginase 1 (ARG1), CD206, CD163, FIZZ1, YM1, and CD36 are the molecular markers that characterize the anti-inflammatory phenotype after ICH[26]. Very recent research has shown that transmembrane protein 119 (TMEM119), Fc receptor-like S (FCRLS), olfactomedin-

like 3 (OLFML3), sialic acid-binding immunoglobulin-like lectin H (Siglec-H), and G protein-coupled receptor 34 (GPR34) are specific markers of stably expressed microglia[32-34]. CD163 and CD44 are general markers of monocyte-derived macrophages that infiltrate the brain, contributing to functional recovery after ICH[34-36]. Through transmission electron microscopy, reactive phagocytic microglia are identified in the ICH brain, which can phagocytose erythrocytes and necrotic neurons[37]. Microglial phagocytosis markers include CD68, triggering receptor expressed on myeloid cells 2 (TREM2), galectin-3 (GAL-3), and CD11c[38]. More research on these markers and their role in the pathogenesis of ICH can generate exciting new findings, such as ICH outcome improvement by inhibiting the expression of pro-inflammatory markers[26].

Depending on the different phenotypes, microglia play a role in brain injury or repair after ICH[26]. The nuclear factor kappa B signaling pathway regulates the expression of pro-inflammatory mediators in activated microglia[22,25,39]. Pro-inflammatory microglia produce pro-inflammatory cytokines, such as IL-12, IL-1β, IL-6, tumor necrosis factor alpha (TNF-α), nitric oxide (NO), and reactive oxygen species (ROS), that contribute to blood-brain barrier (BBB) rupture and edema formation after ICH[10,26,40,41]. In contrast, signal transducer and activator of transcription 6 (STAT6) accumulates in response to IL-4 and promotes anti-inflammatory microglial phenotypic transformation[26]. Microglia of the anti-inflammatory phenotype produce anti-inflammatory cytokines, such as transforming growth factor beta (TGF-β), IL-4, IL-10, IL-13, extracellular matrix proteins, glucocorticoids, and growth factors[22]. Since these cytokines exhibit anti-inflammatory and neuroprotective properties, they protect the BBB and promote brain repair. Due to their ability to switch between pro-inflammatory and anti-inflammatory phenotypes, activated microglia can regulate the immune response according to the microenvironment[23]. Research has shown that microglial activation occurs within 1 h after the onset of ICH[42], with upregulated expression of pro-inflammatory cytokines, such as iNOS, IL-6, TNF, and IL-1 β, in the first 3 days, followed by a rapid decrease[7]. Furthermore, markers of the anti-inflammatory phenotype, such as IL-10, ARG1, CD206, CD163, and YM1, begin to rise on day 1 after ICH, with a change in phenotype from pro-inflammatory to anti-inflammatory in the first 7 days. From days 7–14, the anti-inflammatory cytokine TGF-β is upregulated, while the levels of most pro-inflammatory cytokines return to baseline on day 14 (Table 1)[26]. In summary, there is a change in microglial phenotype from the pro-inflammatory phenotype in the acute stage to a mixed phenotype in the subacute stage of ICH. Eventually, anti-inflammatory markers predominate at the injury site in the chronic stage of ICH (Figure 1)[43].

In early 2003, we reported for the first time that microglial activation contributes to endogenous hematoma clearance after ICH[44]. Our recent study has demonstrated that improving microglial and macrophage alternative activation contributes to hematoma absorption and better neurological function, as demonstrated in the hindlimb adduction test, the corner turn test, and the forelimb placing test[8]. In addition, microglia promote cell debris and hematoma clearance and reduce harmful cytokine production during activation through phenotypic changes in the subacute and chronic stages to reduce brain injury and promote functional recovery after ICH[11,18]. Furthermore, the cytokines TNF- α, IL-4, IL-6, and IL-10 can reprogram microglia toward a phagocytic phenotype with improved phagocytosis for hematoma clearance[26,38,45].

4. Hematoma clearance by reactive microglia: Role of IL-10-mediated pathway

Previous research has identified certain drugs or molecules that can promote the clearance of hematoma, including Vitamin D, which can stimulate microglia/macrophage proliferation and differentiation; IL-4, which improves STAT6 activation; low-density lipoprotein receptor-1 (LRP1), which promotes heme scavenging through binding to hemopexin-heme complexes; and IL-10, peroxisome proliferator-activated receptor gamma (PPAR-γ), and nuclear factor-erythroid 2 p45-related factor 2 (NRF2), which upregulate CD36 expression[46-49]. Furthermore, fractalkine (FKN) promotes hematoma absorption through the PPAR-γ/CD163/hemeoxygenase-1 (HO-1) signaling pathway in microglia, while PPAR-γ plays a protective role through the haptoglobin (HP)-hemoglobin (Hb)-CD163 pathway[50,51]. Other strategies that may also be promising for hematoma clearance include the CD47-blocking antibody, pulsed electromagnetic field (PEMF) therapy, intranasal IL-4 treatment, exogenous sulforaphane administration that can activate NRF2, and treatment with plasma kallikrein inhibitor (aprotinin) and PPAR-γ agonist, such as monascin, rosiglitazone, and pioglitazone[46,49,51-54]. The phase 2 clinical trial on pioglitazone for hematoma resolution (ClinicalTrials.gov Identifier: NCT00827892) was completed in 2013 but has not released the final report to date, probably due to negative result. Hence, researchers are now focusing on signaling pathways other than those involving PPAR-γ, such as IL-10, a critical anti-inflammatory cytokine that could contribute to hematoma clearance. Therefore, studying the role and mechanism of action of microglial IL-10 signaling can help identify new and promising therapeutic strategies for hematoma clearance after ICH.

Table 1. Time course of changes in microglia and macrophage activation markers after intracerebral hemorrhage

Type	Specific markers	Flow cytometry phenotype	Immunostaining phenotype	Molecular markers	Cytokines released in serum	Time course with activation status
Microglia	TMEM119 FCRLS OLFML3 Siglec-H GPR34[32-34]	$CD11b^{+}CD45^{int}$[19,20]	Pro-inflammatory phenotype	CD16/32 CD86 iNOS	Pro-inflammatory cytokines: TNF-α, IL-6, IL-12, IL1-β, and NO	Microglia are activated within 1 h[42]. Multiple pro-inflammatory factors are released in the first 3 days and decrease after 3 days[24]. Most pro-inflammatory cytokines return to baseline level on day 14.
			Anti-inflammatory phenotype	CD206 ARG1 CD36 YM1 FIZZ1	Anti-inflammatory cytokines: TGF-β, IL-4, IL-10, IL-13, and various growth factors	Anti-inflammatory markers increase on day 1. The pro-inflammatory phenotype changes to the anti-inflammatory phenotype in the first 7 days. Predominance: alternative activation phenotype[43].
Macrophages	CD163 CD44[34-36]	$CD11b^{+}CD45^{high}F4/80^{+}Ly6c$[19-21]	Pro-inflammatory phenotype		Pro-inflammatory cytokines: TNF-α, IL-1, IL-6, IL-8, and IL-12	Infiltration of the perihematomal and hematoma regions after microglia activation[24].
			Anti-inflammatory phenotype		Anti-inflammatory cytokines: TGF-β and IL-10	Polarization markers increase on day 1 with a significant increase on day 3 and a further increase on day 7[19].

ARG1: Arginase 1; FCRLS: Fc receptor-like S; GPR34: G protein-coupled receptor 34; ICH: Intracerebral hemorrhage; IL-10: Interleukin 10; iNOS: inducible nitric oxide synthase; MMΦ: Microglia/macrophages; NO: Nitric oxide; OLFML3: Olfactomedin-like 3; Siglec-H: Sialic acid binding immunoglobulin-like lectin H; TGF-β: Transforming growth factor beta; TMEM119: Transmembrane protein 119; TNF-α: Tumor necrosis factor alpha

IL-10, a critical modulator of glial activation, is secreted by monocyte-derived macrophages, resident microglia, and regulatory T cells (Tregs) in the CNS. The IL-10 secreted by macrophages inhibits the activation of adjacent macrophages and the production of pro-inflammatory cytokines. Tregs control immune homeostasis, suppress pro-inflammatory function, and promote their own immune activity through IL-10 production[55]. Our recent research focuses on the IL-10 released by microglia, which maintains the balance between pro- and anti-inflammatory cytokine levels[11,23]. In a mouse brain affected by ICH, IL-10 increased on day 1, peaked on day 7, and returned to baseline 14 days after ictus[45]. IL-10-induced alternative microglial activation is a primary protective mechanism that modulates microglial phagocytosis and accelerates hematoma clearance[8,26].

Furthermore, our research has demonstrated that IL-10 increases the level and accumulation of phosphorylated STAT3 in the nucleus of Hb-treated primary microglia[45]. The IL-10 signaling pathway activates STAT3, promotes CD36 transcription and translation, and strengthens microglial phagocytosis, leading to enhanced hematoma clearance[45]. However, we should identify the downstream target molecules of CD36 in this signaling pathway.

Exogenous administered IL-10 can inhibit apoptosis in brain tissues around the hematoma after ICH by inhibiting the expression of nerve growth factor precursor (proNGF) and $p75^{NTR}$, increasing the level of B-cell lymphoma 2 (BCL2), and decreasing the level of BCL-2-associated X protein (BAX), thus exerting neuroprotective effects[56]. However, we have used transmission electron microscopy and revealed that ferroptosis, rather than apoptosis, is the primary form of cell death after ICH[37,57]. Therefore, whether endogenous and exogenous IL-10 can inhibit ferroptosis after ICH remains unknown from a scientific standpoint.

In our recent study, we investigated the role of IL-10 in phagocytosis and hematoma clearance using hippocampal slice cultures and a mouse ICH model, respectively[45]. Our data showed that in slice cultures, exposure to hemoglobin increases the level of IL-10 in microglia and improves phagocytosis dependent on the expression of CD36 regulated by IL-10. After ICH, IL-10 knockout mice showed increased iron deposition, inflammation, brain edema, and

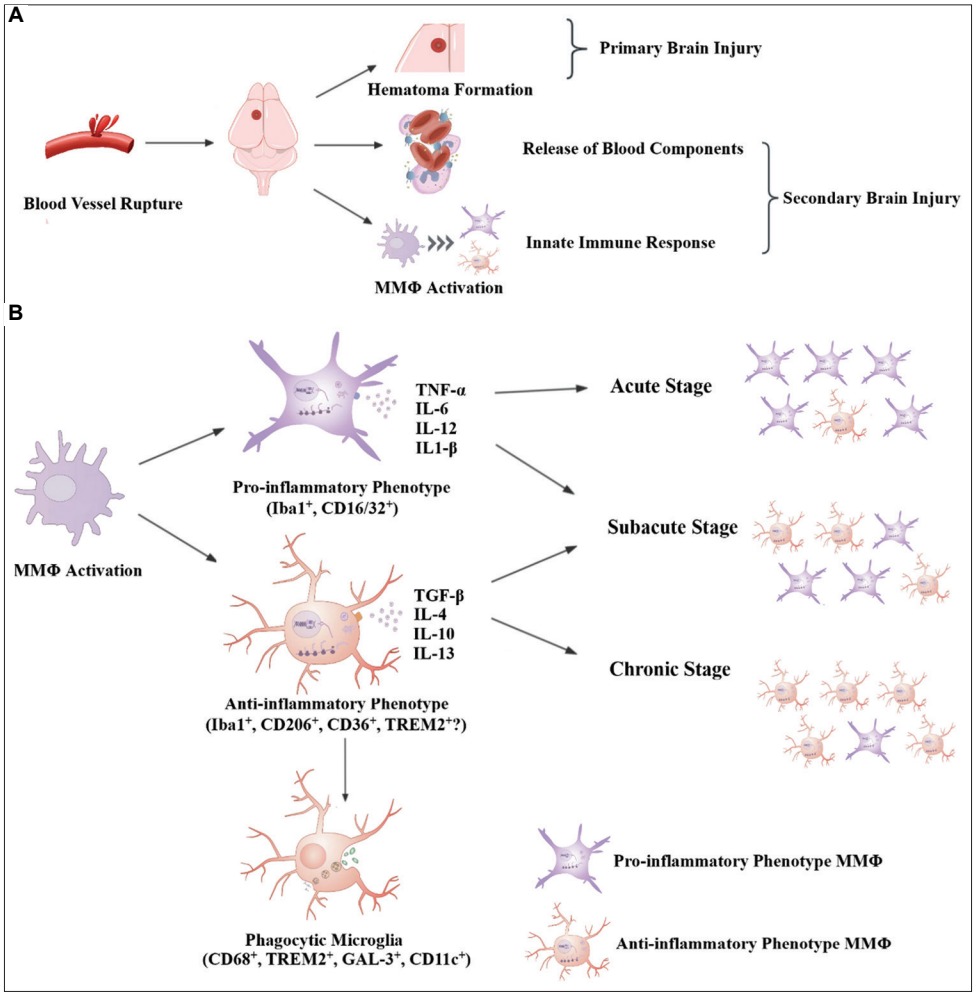

Figure 1. Intracerebral hemorrhage (ICH)-induced brain injury and microglial and macrophage activation. (A) ICH is caused by the rupture of small arterial. The subsequent formation and expansion of hematoma and the mass effect lead to primary brain injury, which initiates the release of blood components and innate immune responses, resulting in secondary brain injury. (B) There are two activation states of microglia/macrophages (MMΦ): pro-inflammatory phenotype (ionized calcium-binding adaptor molecule 1 [Iba1$^+$] and CD16/32$^+$) and anti-inflammatory phenotype (Iba1$^+$, CD206$^+$, CD36$^+$, and triggering receptor expressed on myeloid cells 2 [TREM2$^+$]). Pro-inflammatory microglia release cytokines such as tumor necrosis factor alpha, interleukin 6 (IL-6), IL-12, and IL-1β, while anti-inflammatory microglia release transforming growth factor beta, IL-4, IL-10, and IL-13. Although CD68$^+$, TREM2$^+$, galectin-3 [GAL-3$^+$], and CD11c$^+$ have been found to be expressed by reactive phagocytic microglia in the aged brain, only a few studies have explored phagocytic microglial markers in the ICH brain. After ICH, there is a change in microglial phenotype from the pro-inflammatory phenotype in the acute phase to a broad spectrum of phenotypes in the subacute stage. Ultimately, the anti-inflammatory phenotype predominates at the injury site in the chronic stage of ICH (TREM2$^+$?: the role of TREM2 in hematoma clearance is unknown).

neurologic deficits related to delayed hematoma clearance. However, 2 h after ICH, daily intranasal administration of mouse recombinant IL-10 improved hematoma clearance and mitigated neurologic deficits. In addition, IL-10-deficient mice had decreased phagocytic capacity due to low microglial CD36 levels, and IL-10 deficiency markedly increased monocyte-derived macrophage infiltration and brain inflammation. From these results, we concluded that IL-10 improves microglial phagocytosis and monocyte-derived macrophage infiltration and CD36 plays a crucial role as a phagocytosis effector regulated by IL-10 after ICH[45].

IL-10 may enhance microglial phagocytosis by upregulating the expression of CD68, GAL-3, CD11, and microglial phagocytic receptors, including TREM2, in the aged brain[38,58]. However, no studies have explored their role in ICH models, particularly in clearing hematoma. However, a clinical study has shown that, compared to patients with unfavorable outcome, patients with favorable outcome had significantly higher serum and hematoma IL-10 levels. At the same time, binary logistic analysis has revealed that increased IL-10 levels in serum and hematoma were associated with favorable outcome in patients with ICH on day 90[59]. Combined with evidence

from other translational studies on brain injury[58], increasing IL-10 signaling may be a promising therapeutic strategy to optimize the microenvironment, promote hematoma clearance, and improve neurological outcomes.

5. Conclusions and perspectives

Rapid microglia activation occurs following ICH, exhibiting predominantly pro-inflammatory, anti-inflammatory, or mixed phenotypes at different stages of the pathological process. The different phenotypes of microglia have distinct biological functions and molecular markers and can release various cytokines/chemokines, which play a dual role in ICH brain. Due to the heterogeneity of microglia, future research should focus on function rather than phenotype.

In microglia, IL-10 signaling promotes their transition from pro-inflammatory to anti-inflammatory phenotype, upregulates CD36 expression, increases phagocytosis, enhances hematoma clearance, and inhibits cell death in brain tissues around the hematoma, all of which are conducive to brain repair. Our review outlines the activation and function of microglia, specific markers, and the drugs or molecules that can enhance hematoma clearance as well as summarizes the results of recent research on signaling pathways that promote hematoma clearance, in particular, the IL-10-mediated pathway. However, knowledge gaps remain to be filled, such as whether CD68, TREM2, GAL-3, and CD11c play a role in hematoma clearance, whether IL-10 can inhibit ferroptosis after ICH, *etc*. Although increasing IL-10 signaling may be a promising therapeutic strategy for hematoma clearance, we need additional data from both preclinical and clinical studies to support the potential of its clinical translation.

Acknowledgments

The authors thank Chengkang Qu for his assistance with figure preparation.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Xiuhua Ren, Chao Jiang, Jian Wang
Visualization: Zhenhua Zhang, Wei Xu, Honghui Sheng, Lanting Zhang
Writing – original draft: Zhenhua Zhang, Wei Xu, Honghui Sheng, Leo Huang, Jiaxin Zhang, Limin Wang, Junmin Wang, Xiuhua Ren, Chao Jiang
Writing – review & editing: Wei Xu, Leo Huang, Chao Jiang, Jian Wang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Wang J, 2010, Preclinical and clinical research on inflammation after intracerebral hemorrhage. *Prog Neurobiol*, 92(4): 463–477.

https://doi.org/10.1016/j.pneurobio.2010.08.001

2. Wan J, Ren H, Wang J, 2019, Iron toxicity, lipid peroxidation and ferroptosis after intracerebral haemorrhage. *Stroke Vasc Neurol*, 4(2): 93–95.

https://doi.org/10.1136/svn-2018-000205

3. An SJ, Kim TJ, Yoon BW, 2017, Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*, 19(1): 3–10.

https://doi.org/10.5853/jos.2016.00864

4. Murphy SJ, Werring DJ, 2020, Stroke: Causes and clinical features. *Med* (*Abingdon*), 48(9): 561–566.

https://doi.org/10.1016/j.mpmed.2020.06.002

5. Krishnamurthi RV, Feigin VL, Forouzanfar MH, *et al.*, 2013, Global and regional burden of first-ever ischaemic and haemorrhagic stroke during 1990-2010: Findings from the Global Burden of Disease Study 2010. *Lancet Glob Health*, 1(5): e259–e281

https://doi.org/10.1016/S2214-109X(13)70089-5

6. Feigin VL, Stark BA, Johnson CO, *et al.*, 2021, Global, regional, and national burden of stroke and its risk factors, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurology*, 20(10): 795–820.

https://doi.org/10.1016/S1474-4422(21)00252-0

7. Shi X, Bai H, Wang J, *et al.*, 2021, Behavioral assessment of sensory, motor, emotion, and cognition in rodent models of intracerebral hemorrhage. *Front Neurol*, 12: 667511.

https://doi.org/10.3389/fneur.2021.667511

8. Chang CF, Wan J, Li Q, *et al.*, 2017, Alternative activation-skewed microglia/macrophages promote hematoma resolution in experimental intracerebral hemorrhage. *Neurobiol Dis*, 103: 54–69.

https://doi.org/10.1016/j.nbd.2017.03.016

9. Al-Kawaz MN, Hanley DF, Ziai W, 2020, Advances in therapeutic approaches for spontaneous intracerebral hemorrhage. *Neurotherapeutics*, 17(4): 1757–1767.

https://doi.org/10.1007/s13311-020-00902-w

10. Jiang C, Guo H, Zhang Z, *et al.*, 2022, Molecular, pathological, clinical, and therapeutic aspects of perihematomal edema in different stages of intracerebral hemorrhage. *Oxid Med Cell Longev*, 2022: 3948921.

https://doi.org/10.1155/2022/3948921

11. Bai Q, Xue M, Yong VW, 2020, Microglia and macrophage phenotypes in intracerebral haemorrhage injury: Therapeutic opportunities. *Brain*, 143(5): 1297–1314.

https://doi.org/10.1093/brain/awz393

12. Wang J, Dore S, 2007, Inflammation after intracerebral hemorrhage. *J Cereb Blood Flow Metab*, 27(5): 894–908.

https://doi.org/10.1038/sj.jcbfm.9600403

13. Wang M, Ye X, Hu J, *et al.*, 2020, NOD1/RIP2 signalling enhances the microglia-driven inflammatory response and undergoes crosstalk with inflammatory cytokines to exacerbate brain damage following intracerebral haemorrhage in mice. *J Neuroinflammation*, 17(1): 364.

https://doi.org/10.1186/s12974-020-02015-9

14. Song D, Yeh CT, Wang J, *et al.*, 2022, Perspectives on the mechanism of pyroptosis after intracerebral hemorrhage. *Front Immunol*, 13: 989503.

https://doi.org/10.3389/fimmu.2022.989503

15. Hanley DF, Thompson RE, Rosenblum M, *et al.*, 2019, Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): A randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*, 393(10175): 1021–1032.

https://doi.org/10.1016/s0140-6736(19)30195-3

16. Dastur CK, Yu WG, 2017, Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*, 2(1): e000047.

https://doi.org/10.1136/svn-2016-000047

17. Thion M, Ginhoux F, Garel S, 2018, Microglia and early brain development: An intimate journey. *Science*, 362(6411): 185–189.

https://doi.org/10.1126/science.aat0474

18. Zhang Z, Zhang Z, Lu H, *et al.*, 2017, Microglial polarization and inflammatory mediators after intracerebral hemorrhage. *Mol Neurobiol*, 54(3): 1874–1886.

https://doi.org/10.1007/s12035-016-9785-6

19. Min H, Jang YH, Cho IH, *et al.*, 2016, Alternatively activated brain-infiltrating macrophages facilitate recovery from collagenase-induced intracerebral hemorrhage. *Mol Brain*, 9: 42.

https://doi.org/10.1186/s13041-016-0225-3

20. Li Q, Lan X, Han X, *et al.*, 2018, Expression of Tmem119/Sall1 and Ccr2/CD69 in FACS-sorted microglia-and monocyte/macrophage-enriched cell populations after intracerebral hemorrhage. *Front Cell Neurosci*, 12: 520.

https://doi.org/10.3389/fncel.2018.00520

21. Silvin A, Uderhardt S, Piot C, *et al.*, 2022, Dual ontogeny of disease-associated microglia and disease inflammatory macrophages in aging and neurodegeneration. *Immunity*, 55(8): 1448–1465.e6.

https://doi.org/10.1016/j.immuni.2022.07.004

22. Liu J, Liu L, Wang X, *et al.*, 2021, Microglia: A double-edged sword in intracerebral hemorrhage from basic mechanisms to clinical research. *Front Immunol*, 12: 675660.

https://doi.org/10.3389/fimmu.2021.675660

23. Laffer B, Bauer D, Wasmuth S, *et al.*, 2019, Loss of IL-10 promotes differentiation of microglia to a M1 phenotype. *Front Cell Neurosci*, 13: 430.

https://doi.org/10.3389/fncel.2019.00430

24. Ren H, Han R, Chen X, *et al.*, 2020, Potential therapeutic targets for intracerebral hemorrhage-associated inflammation: An update. *J Cereb Blood Flow Metab*, 40(9): 1752–1768.

https://doi.org/10.1177/0271678X20923551

25. Lan X, Han X, Liu X, *et al.*, 2019, Inflammatory responses after intracerebral hemorrhage: From cellular function to therapeutic targets. *J Cereb Blood Flow Metab*, 39(1): 184–186.

https://doi.org/10.1177/0271678X18805675

26. Lan X, Han X, Li Q, *et al.*, 2017, Modulators of microglial activation and polarization after intracerebral haemorrhage. *Nat Rev Neurol*, 13(7): 420–433.

https://doi.org/10.1038/nrneurol.2017.69

27. Sideris-Lampretsas G, Malcangio M, 2021, Microglial heterogeneity in chronic pain. *Brain Behav Immun*, 96: 279–289.

https://doi.org/10.1016/j.bbi.2021.06.005

28. Silva NJ, Dorman LC, Vainchtein ID, *et al.*, 2021, *In situ* and transcriptomic identification of microglia in synapse-rich regions of the developing *Zebrafish* brain. *Nat Commun*, 12(1): 5916.

https://doi.org/10.1038/s41467-021-26206-x

29. Huarte OU, Kyriakis D, Heurtaux T, *et al.*, 2021, Single-cell transcriptomics and *in situ* morphological analyses reveal microglia heterogeneity across the nigrostriatal pathway. *Front Immunol*, 12: 639613.

https://doi.org/10.3389/fimmu.2021.639613

30. Barko K, Shelton M, Xue X, *et al.*, 2022, Brain region-and sex-specific transcriptional profiles of microglia. *Front Psychiatry*, 13: 945548.

https://doi.org/10.3389/fpsyt.2022.945548

31. Paolicelli RC, Sierra A, Stevens B, *et al.*, 2022, Microglia states and nomenclature: A field at its crossroads. *Neuron*,

110(21): 3458–3483.

https://doi.org/10.1016/j.neuron.2022.10.020

32. Spittau B, Dokalis N, Prinz M, 2020, The role of TGFβ signaling in microglia maturation and activation. *Trends Immunol*, 41(9): 836–848.

https://doi.org/10.1016/j.it.2020.07.003

33. Grassivaro F, Martino G, Farina C, 2021, The phenotypic convergence between microglia and peripheral macrophages during development and neuroinflammation paves the way for new therapeutic perspectives. *Neural Regen Res*, 16(4): 635–637.

https://doi.org/10.4103/1673-5374.295272

34. Jurga AM, Paleczna M, Kuter KZ, 2020, Overview of general and discriminating markers of differential microglia phenotypes. *Front Cell Neurosci*, 14: 198.

https://doi.org/10.3389/fncel.2020.00198

35. Skytthe MK, Graversen JH, Moestrup SK, 2020, Targeting of CD163$^+$ macrophages in inflammatory and malignant diseases. *Int J Mol Sci*, 21(15): 5497.

https://doi.org/10.3390/ijms21155497

36. Gantzel RH, Kjaer MB, Laursen TL, *et al.*, 2020, Macrophage activation markers, soluble CD163 and mannose receptor, in liver fibrosis. *Front Med (Lausanne)*, 7: 615599.

https://doi.org/10.3389/fmed.2020.615599

37. Li Q, Weiland A, Chen X, *et al.*, 2018, Ultrastructural characteristics of neuronal death and white matter injury in mouse brain tissues after intracerebral hemorrhage: Coexistence of ferroptosis, autophagy, and necrosis. *Front Neurol*, 9: 581.

https://doi.org/10.3389/fneur.2018.00581

38. Sanchez-Molina P, Almolda B, Benseny-Cases N, *et al.*, 2021, Specific microglial phagocytic phenotype and decrease of lipid oxidation in white matter areas during aging: Implications of different microenvironments. *Neurobiol Aging*, 105: 280–295.

https://doi.org/10.1016/j.neurobiolaging.2021.03.015

39. Lan X, Han X, Li Q, *et al.*, 2017, Pinocembrin protects hemorrhagic brain primarily by inhibiting toll-like receptor 4 and reducing M1 phenotype microglia. *Brain Behav Immun*, 61: 326–339.

https://doi.org/10.1016/j.bbi.2016.12.012

40. Zhu H, Wang Z, Yu J, *et al.*, 2019, Role and mechanisms of cytokines in the secondary brain injury after intracerebral hemorrhage. *Prog Neurobiol*, 178: 101610.

https://doi.org/10.1016/j.pneurobio.2019.03.003

41. Hua W, Chen X, Wang J, *et al.*, 2020, Mechanisms and potential therapeutic targets for spontaneous intracerebral hemorrhage. *Brain Hemorrhages*, 1(2): 99–104.

https://doi.org/10.1016/j.hest.2020.02.002

42. Wang J, Doré S, 2007, Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. *Brain*, 130(Pt 6): 1643–1652.

https://doi.org/10.1093/brain/awm095

43. Taylor RA, Chang CF, Goods BA, *et al.*, 2017, TGF-β1 modulates microglial phenotype and promotes recovery after intracerebral hemorrhage. *J Clin Invest*, 127(1): 280–292.

https://doi.org/10.1172/JCI88647

44. Wang J, Rogove AD, Tsirka AE, *et al.*, 2003, Protective role of tuftsin fragment 1-3 in an animal model of intracerebral hemorrhage. *Ann Neurol*, 54(5): 655–664.

https://doi.org/10.1002/ana.10750

45. Li Q, Lan X, Han X, *et al.*, 2021, Microglia-derived interleukin-10 accelerates post-intracerebral hemorrhage hematoma clearance by regulating CD36. *Brain Behav Immun*, 94: 437–457.

https://doi.org/10.1016/j.bbi.2021.02.001

46. Xu J, Chen Z, Yu F, *et al.*, 2020, IL-4/STAT6 signaling facilitates innate hematoma resolution and neurological recovery after hemorrhagic stroke in mice. *Proc Natl Acad Sci U S A*, 117(51): 32679–32690.

https://doi.org/10.1073/pnas.2018497117

47. Liu J, Li N, Zhu Z, *et al.*, 2022, Vitamin D enhances hematoma clearance and neurologic recovery in intracerebral hemorrhage. *Stroke*, 53(6): 2058–2068.

https://doi.org/10.1161/strokeaha.121.037769

48. Wilkinson DA, Keep RF, Hua Y, *et al.*, 2018, Hematoma clearance as a therapeutic target in intracerebral hemorrhage: From macro to micro. *J Cereb Blood Flow Metab*, 38(4): 741–745.

https://doi.org/10.1177/0271678X17753590

49. Zhao X, Sun G, Ting SM, *et al.*, 2015, Cleaning up after ICH: The role of Nrf2 in modulating microglia function and hematoma clearance. *J Neurochem*, 133(1): 144–152.

https://doi.org/10.1111/jnc.12974

50. You M, Long C, Wan Y, *et al.*, 2022, Neuron derived fractalkine promotes microglia to absorb hematoma via CD163/HO-1 after intracerebral hemorrhage. *Cell Mol Life Sci*, 79(5): 224.

https://doi.org/10.1007/s00018-022-04212-6

51. Wang G, Li T, Duan SN, *et al.*, 2018, PPAR-γ promotes hematoma clearance through haptoglobin-hemoglobin-CD163 in a rat model of intracerebral hemorrhage. *Behav Neurol*, 2018: 7646104.

https://doi.org/10.1155/2018/7646104

52. Tao C, Keep RF, Xi G, *et al.*, 2020, CD47 blocking antibody accelerates hematoma clearance after intracerebral

hemorrhage in aged rats. *Transl Stroke Res*, 11(3): 541–551.

https://doi.org/10.1007/s12975-019-00745-4

53. Yang Y, Wang P, Liu A, *et al.*, 2022, Pulsed electromagnetic field protects against brain injury after intracerebral hemorrhage: Involvement of anti-inflammatory processes and hematoma clearance via CD36. *J Mol Neurosci*, 72(10): 2150–2161.

https://doi.org/10.1007/s12031-022-02063-1

54. Siaw-Debrah F, Nyanzu M, Ni H, *et al.*, 2017, Preclinical studies and translational applications of intracerebral hemorrhage. *Biomed Res Int*, 2017: 5135429.

https://doi.org/10.1155/2017/5135429

55. Bedke T, Muscate F, Soukou S, *et al.*, 2019, Title: IL-10-producing T cells and their dual functions. *Semin Immunol*, 44: 101335.

https://doi.org/10.1016/j.smim.2019.101335

56. Song L, Xu LF, Pu ZX, *et al.*, 2019, IL-10 inhibits apoptosis in brain tissue around the hematoma after ICH by inhibiting proNGF. *Eur Rev Med Pharmacol Sci*, 23(7): 3005–3011.

https://doi.org/10.26355/eurrev_201904_17582

57. Li Q, Han X, Lan X, *et al.*, 2017, Inhibition of neuronal ferroptosis protects hemorrhagic brain. *JCI Insight*, 2(7): e90777.

https://doi.org/10.1172/jci.insight.90777

58. Yi S, Jiang X, Tang X, *et al.*, 2020, IL-4 and IL-10 promotes phagocytic activity of microglia by up-regulation of TREM2. *Cytotechnology*, 72(4): 589–602.

https://doi.org/10.1007/s10616-020-00409-4

59. Jiang C, Wang Y, Hu Q, *et al.*, 2020, Immune changes in peripheral blood and hematoma of patients with intracerebral hemorrhage. *FASEB J*, 34(2): 2774–2791.

https://doi.org/10.1096/fj.201902478R