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MDPI

Review

Therapeutic Role of Microglia/Macrophage Polarization in Intracerebral Hemorrhage

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Abstract: Intracerebral hemorrhage (ICH) is a significant health problem with high mortality and morbidity rates, partly due to limited treatment options. Hematoma after ICH causes neurological deficits due to the mass effect. Hemorrhage catalyzes secondary damage, resulting in increased neurological damage, poor prognosis, and treatment problems. This review evaluates the role of immunotherapeutic approaches in ICH based on original full-text and review articles on the pathophysiology and immunotherapy of ICH, with emphasis on the modulation of microglia/macrophage polarization to the M2 subtype. In this review, we concluded that the pathophysiology of injury progression after ICH is complex and multifaceted. Inflammation plays a dominant role in secondary injuries. Furthermore, cells involved in the inflammatory process have dual roles in pro-inflammatory/destructive and anti-inflammatory/healing. While the role of inflammation in the pathophysiology makes the immune system a therapeutic target in ICH, the dual role of cells makes them a therapeutic target that can modulate anti-inflammatory/healing. Resident microglia (and even macrophages migrating from a peripheral source) are important therapeutic targets for modulation because of their role in the initiation phase and in shaping immunity. Although clinical results remain poor, experimental and clinical trial data seem promising for deciphering the pathophysiology of ICH and providing treatment options.

Keywords: intracerebral hemorrhage; immune mechanisms; immunotherapy; M1/M2 macrophage polarization; macrophages; microglia

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1. Introduction

Intracerebral hemorrhage (ICH) is one of the most dangerous types of strokes [1]. Approximately 15 million strokes are reported worldwide annually. Despite accounting for up to 20% of all stroke cases (over 2 million worldwide), ICH is associated with a higher mortality rate than ischemic stroke or subarachnoid hemorrhage, with mortality rates reaching 54% after the index event [2]. More than half of the fatal events occur in the first 2 days [3]. Among those who survive, only approximately 20% regain functional independence [4]. In addition to being associated with high mortality and morbidity, ICH also has fewer treatment options than ischemic stroke. The incidence increases significantly with advanced age, and the majority of patients are over 65 years of age [3,4]. In the United States alone, ICH is estimated to incur an annual healthcare cost of USD 12.7 billion [2]. Therefore, it is a significant burden not only in terms of health but also in terms of social and economic aspects [5].

Hematoma after ICH can catalyze primary damage caused by the mass effect and secondary damage caused by the inflammatory reaction, resulting in poor prognosis and treatment problems for patients [6]. The treatment of ICH remains a major challenge.

Current medical treatments are based on lowering the blood and intracranial pressure and/or hemostatic therapy [3,7,8]. In addition to having the highest mortality rate, ICH is the least treatable subtype of stroke [3].

The severity, location, and extent of the hemorrhage affect both the prognosis and management of the disease. A larger initial blood volume and progressive growth in the hematoma volume are associated with worse functional outcomes and increased mortality rate [9]. The middle cerebral artery (MCA), part of the circle of the Willis anastomotic system that supplies blood to a wide area of brain tissue, is highly vulnerable and is associated with the majority of stroke-related disability [10,11].

It is important to understand the pathophysiological mechanisms associated with the occurrence and progression of ICH to choose the best treatment [12]. Following the initial injury, secondary injuries occur in which complex mechanisms involving many cells and molecules play a role. Furthermore, crosstalk between the cells involved in this process and their mutual influence on each other's phenotype and function is noteworthy for secondary injury in ICH [13].

Disruption of the blood–brain barrier (BBB) is a key role in the progression of ICH pathophysiology. Disruption of the BBB results in the uncontrolled flow of blood-borne cells, macromolecules, and fluid into the brain, resulting in devastating malignant brain edema and life-threatening hemorrhagic transformation (Figure 1). Rapid activation of blood-borne immune cells further enhances impaired BBB damage, as they cause microvascular dysregulation and increase BBB permeability by secreting inflammation-associated molecules. There in an extensive inflammatory response in the brain after ICH, involving both the proliferation of resident microglia (Mig) and the recruitment of circulating leukocytes such as monocytes, neutrophils, lymphocytes, and macrophages (Møs) [14]. The production of specific chemokines within a few hours at the site of injury facilitates the selective recruitment, adhesion, and migration of leukocytes through interactions with chemokine receptors expressed in circulating immune cells. This contributes to the development of secondary brain damage [15].

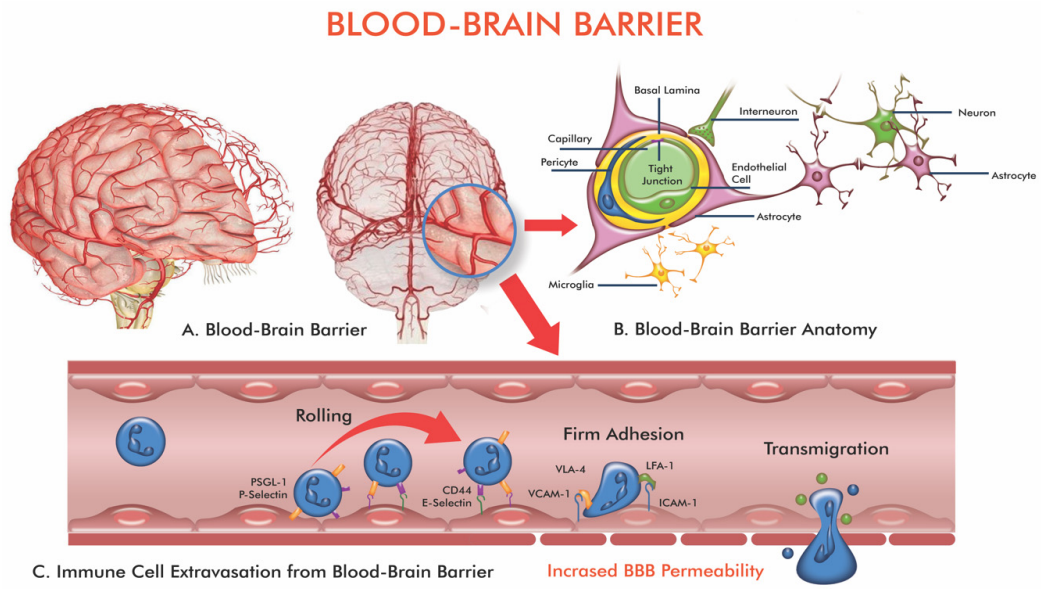

Figure 1. Illustration of the anatomical structure of the BBB and immune cell migration as a result of permeability disruption. (**A**) brain vessel, (**B**) anatomical structure of the BBB, (**C**) migration of immune cells through the damaged barrier in the brain vessel.

The weakness of current therapeutic approaches and the knowledge that the basic pathophysiological mechanisms underlying poor prognosis are immunologically initiated

have made attempts to find effective immunotherapeutic targets for ICH a hot research topic [12]. The purpose of this study was to evaluate the role of an immunotherapeutic approach in ICH, mainly emphasizing the modulation of microglia/macrophage polarization to the M2 subtype, in light of recent data. For these reasons, important immunological mechanisms and therapeutic strategies have been summarized.

2. Inflammatory Response to Intracerebral Hemorrhage (ICH)

ICH is a common form of hemorrhagic stroke with poor prognosis and limited therapeutic options. Primary and secondary brain damage occur because of bleeding. Primary damage develops as a result of the mass effect and physical disruption of the brain parenchyma. The fundamental factors for secondary damage are neuroinflammatory reactions and the release of clot components. Infiltrating white blood cells, including resident microglia and peripheral leukocytes, has been suggested [16].

Hematoma formation after ICH usually stimulates an inflammatory reaction via activated microglia/macrophages (Mig/Møs), which induces inflammatory signaling pathways. Therefore, these cells contribute to the activation of the immune cascade and secretion of pro-inflammatory cytokines [12,17]. The onset of ICH disrupts the integrity of the BBB and exposes the brain parenchyma to neurotoxic substances and immune cells, ultimately increasing the risk of secondary brain injury. Subsequently, the secretion of cytokines triggers innate immunity. The movement of circulating immune cells, especially neutrophils, into the brain parenchyma and meninges further damages brain cells by secreting numerous destructive substances, such as cytokines, reactive oxygen species (ROS), matrix metalloproteinases (MMPs), and neutrophil extracellular traps (NETs). In the later period, the activation of acquired immunity triggered by the spread of dead cell-derived antigens exacerbates the systemic immune response, eventually leading to the emergence of systemic negative effects, such as systemic immune response syndrome and cardiac disfunction [18].

The inflammatory response following cerebral hemorrhage is particularly deleterious in the acute phase because the surrounding tissue is destroyed by proteases (such as matrix metalloproteinases, primarily MMP-2, 3, and 9) and ROS secreted by infiltrating leukocytes [1,19]. The accumulation of microglia, macrophages, and T lymphocytes occurs later, significantly affecting the immune response. These cells have subtypes that lead to a pro-inflammatory (destructive) or anti-inflammatory (regenerative) state [3]. The BBB is a unique anatomical interface between the blood and central nervous system (CNS) formed by endothelial cells (ECs), the end legs of astrocytes, and pericytes embedded in the capillary basement membrane. Extravasation of peripheral immune cells (PICs) occurs by binding to adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1), expressed by EC and pericytes. Although these adhesion molecules are expressed at extremely low levels under resting conditions, their levels increase exponentially after any damage signal [1,19]. In addition to these molecules, many other immunological or non-immunological molecules play a vital role in cell migration, signaling pathways, shaping the immune mechanism, and crosstalk between immune cells [1,15,18].

3. Immune Cells in ICH

The immune cells involved in brain hemorrhage normally fall into two categories: The first category is neuroglia, including microglia and astrocytes, which occur naturally in the brain tissue and are involved in the innate immune response. The other category is leukocytes, which includes neutrophils, monocytes, macrophages, and lymphocytes. Leukocytes migrate from the bloodstream, are recruited at the site of cerebral hemorrhage,

and play an important role in the acquired immune reaction [20,21]. The different roles of cells involved in the pathophysiology of ICH are summarized in Table 1.

Table 1. Different roles of cells involved in the pathophysiology of ICH.

Immune Cell	Phenotype	Essential Function	Role in ICH	Reference
Microglia/Macrophage	M1	Pro-inflammatory	- Recruit PICs by secreting chemotactic substances - Neuronal apoptosis - Disruption of the BBB	[12]
	M2	Anti-inflammatory	- Clearance of cell debris by phagocytosis - Tissue repair	[12]
Astrocyte	A1	Pro-inflammatory	- Neuronal death - Perihematomal edema	[12]
	A2	Resolution of inflammation	- Hematoma clearance - Tissue repair	[12]
Neutrophil	N1	Pro-inflammatory	- Aggravate brain damage	[17]
	N2	Resolution of inflammation	- Contributing to resolution of inflammation by inducing TGFβ	[17]
Lymphocyte	Th1	Support cell-mediated immunity and phagocytotic protective responses	- Exacerbating brain damage by secreting pro-inflammatory cytokines including IL-2, IL-12, and IFN-γ	[12,20]
	Th2	Support humoral immunity	- Exhibiting neuroprotective effects for injured brain by secreting anti-inflammatory cytokines including IL-4, IL-10	[12,20]
	Th17	Creation of inflammation	- Exacerbating ischemic damage by IL-17 production	[20,21]
	Treg	Maintenance of peripheral tolerance	- Promoting neuroprotection and repair	[20,21]

Abbreviations: PICs: peripheral immune cells; BBB: blood–brain barrier; TGFβ: Transforming growth factor-β.

3.1. Local Immune Cells

3.1.1. Microglia

Microglia constitute the largest population of immune cells in the brain. Their highly ramified structures allow them to constantly scan the surrounding environment [3,16]. In experimental models, microglia are activated within minutes after brain injury, while peripheral macrophages infiltrate 1–2 days later [1,22,23]. It is difficult to distinguish between activated microglial cells and macrophages, which have high phagocytic properties. Furthermore, both expressed similar cellular surface markers, including CD11b and Iba-1 [24]. Both microglia and macrophages are divided into M1 (pro-inflammatory, classically activated) and M2 (anti-inflammatory, alternatively activated) [12,25]. The M1 subtypes are generally active in the early stages of damage, while the M2 subtypes are usually effective in the later resolution period, usually after day three, and gradually reach their peak after day seven [12].

M1 microglia, whose activation is typically induced by IFN-γ and lipopolysaccharide (LPS), produce inflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-12, and chemokines such as CC-2 [26]. These cells also produce enzymes such as NADPH oxidase (ROS producing) and inducible nitric oxide synthase (iNOS) (NO producing), MHC-II molecules, integrins (such as CD11b and CD11c), co-stimulatory molecules (such as CD36, CD45, and CD47), and Fc receptors, which contribute to neurological damage [27]. M2 microglia, which are typically induced by cytokines such as IL-4 and IL-13, express cytokines such as IL-10 and TGF-β, and growth factors such as insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), colony stimulating factor (CSF) -1, and neurotrophic growth factors (NGFs) (Figure 2) [26,28].

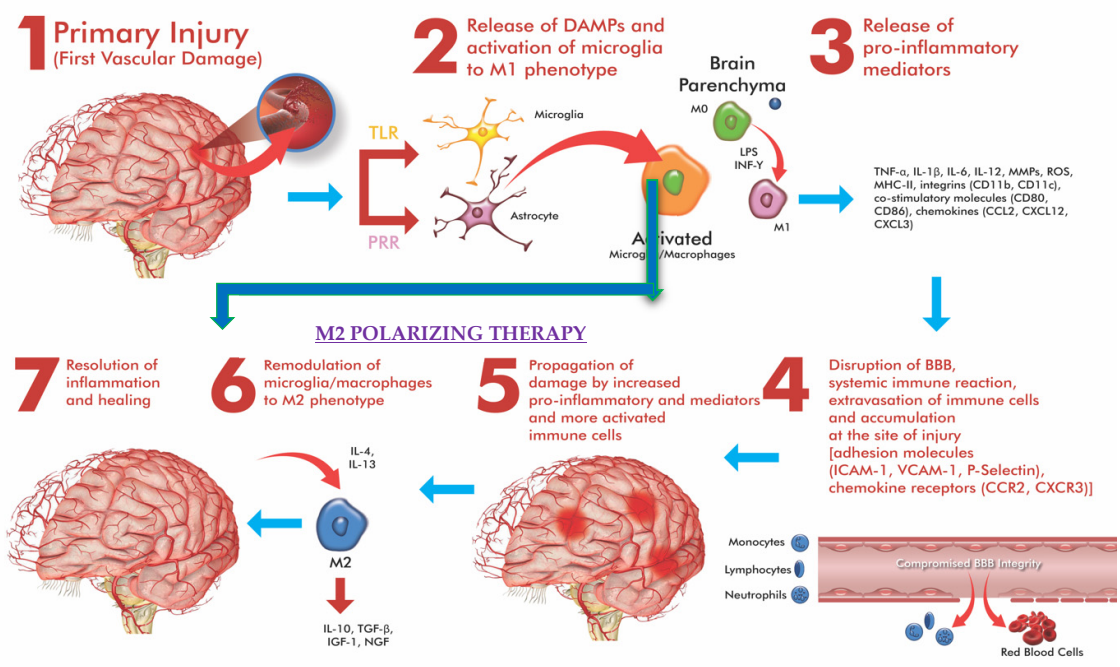

Figure 2. Schematic representation of the injury mechanisms after intracerebral hemorrhage and the treatment strategy polarizing microglia/macrophage to the M2 subtype.

I. Pathophysiological process: (Step 2 to 6): After brain injury, vascular rupture of the brain parenchyma contributes to the formation of hematoma, which causes primary brain damage (Step-1). Simultaneously, within hours to days, hematoma-derived toxic products initiate secondary brain damage by also inducing the inflammatory response. In the first stage of this step, microglia are activated and polarized into the pro-inflammatory M1 subtype (Step-2). M1 polarization of microglia results in the release of pro-inflammatory factors such as TNF-α and IL-6 and the aggravation of the inflammatory response (Step-3). The increase in inflammatory response causes disruption of the blood–brain barrier (BBB), increased number of cell adhesion molecules, and migration of immune cells, including monocyte-derived macrophages, from peripheral blood to the site of cerebral hemorrhage (Step-4). Finally, brain damage is further exacerbated by the release of certain substances (such as reactive oxygen species), cytokines (such as IFN-γ and TNF-α), and proteases (not shown in the figure) (Step-5). During the subsequent resolution period, microglia/macrophages polarize to anti-inflammatory M2 subtypes (Step-6). This anti-inflammatory process contributes to the resolution of inflammation and healing brain damage (Step-7).

II. Polarization therapy process: (Dark blue arrow shifting from Step 2 to 6, entitled "M2 polarizing therapy"): Targeted polarization of microglia/macrophage polarizers to

anti-inflammatory M2 subtypes supports the healing process by reducing the inflammatory reaction and preventing BBB damage.

M2 microglia promote the phagocytosis of cell debris and misfolded proteins, promote the reconstruction and repair of the extracellular matrix, and promote neuron survival through neurotrophic factors [29,30].

Although microglia/macrophage are generally considered M1 and M2 subtypes, the M2 subtypes can further be divided into sub-phenotypes: M2a (wound healing/anti-inflammatory), M2b (immune-mediated/pro-inflammatory), M2c (regulatory/anti-inflammatory), and M2d (tumor-associated/proangiogenic) [31]. Furthermore, several macrophage subtypes have been defined, such as tumor-associated macrophages (TAMs), lipid-associated microglia/macrophages (LAMs), disease-associated microglia (DAM), and scar-associated macrophages (SAMs), each representing specialized contexts related to defining spatial localization, origin, and functional pathways [32]. Recently, it has been observed that the LAM subset is stroke-associated myeloid cells. Certain genes, such as Spp1, that affect post-stroke outcome are upregulated [33].

The bone marrow and spleen are well-known tissues that provide monocytes with ICH-induced inflammation. However, skull bone marrow-derived monocytes/macrophages (SBMs) have recently been discovered to be another local source of brain damage [34]. The proximity of the SBM to the injury site allows rapid influx to the injury site, especially in the acute phase after stroke [34,35]. Another subtype is border-associated macrophages (BAMs), which are clearly confined to the anatomical boundaries between the periphery and CNS parenchyma. They are believed to act as regulators of CNS homeostasis within the local niches. Subpopulations of BAMs include perivascular macrophages (pvMs), meningeal macrophages (mMs), and choroid plexus macrophages (cpMs) [35].

Unfortunately, few studies have investigated the relationship between sub-phenotypes and ICH, and studies have generally evaluated the M1 and M2 phenotypes broadly, here representing a critical direction moving forward.

3.1.2. Astrocytes

Another type of cerebral immune cell is astrocytes, which express significant amounts of receptors that enable the recognition of pathogens, primarily pattern recognition receptors (PRRs) and Toll-like receptors (TLRs). Astrocytes also secrete pro-inflammatory mediators, such as cytokines, chemokines, adhesion molecules, proteases, and iNOS, when they sense danger signals [36]. While A1 astrocytes increase pro-inflammatory cytokines (such as IL-1β, IL-6, and TNF-α) and chemokines (such as CCL2 and CCL5), the other astrocyte subgroup A2 produces IL-2, IL-10, and TGF-β, which accelerate the resolution of inflammation [1].

Other cells: it is thought that oligodendrocytes may play a neuroprotective role in ICH by promoting remyelination and helping the repair process after injury, while pericyte cover in blood vessels contributes to preserving vascular integrity [37,38].

3.2. Systemic Immune Cells

The release of chemoattractant substances at the injury site leads to endothelial activation, which disrupts the BBB. These processes facilitate the infiltration and accumulation of innate and adaptive immune cells at the injury site [35].

3.2.1. Innate Immune Cells

Neutrophils

Neutrophils, one of the most abundant immune effector cells, are the first cells to respond to any injury. Their counts increase within a few hours after the injury and remain high for a week [17]. Neutrophils are known for their destructive role in BBB

disruption following brain injury. A high circulating neutrophil-to-lymphocyte ratio (NLR) is associated with symptomatic intracranial hemorrhage and poor functional outcome [39]. In ICH, neutrophils (N1 subtype) migrate from the peripheral blood to the site of cerebral hemorrhage and further exacerbate brain damage by secreting substances (such as ROS), cytokines (such as IFN-γ and TNF-α), proteases (particularly MMP-2, 3, and 9), and NET. In animal models, the association between NETs and tissue plasminogen activator (tPA) exacerbates cerebral hemorrhage [40]. In addition to the N1 subtype, neutrophils can also be polarized to the N2 subtype. The N2 subtype, induced by transforming growth factor-β (TGFβ), contributes to the resolution of inflammation and promotes macrophage clearance [38].

Monocytes/Macrophages

In fact, microglia residing in the brain tissue and macrophages in the periphery have similar functions [18]. However, microglia participate in this process faster in the ICH region than monocyte-derived macrophages (MDMs) in the periphery [1,19,22]. Monocytes and MDMs are not found in the brain parenchyma under physiological conditions [1]. However, in addition to the local response, ICH triggers systemic responses and the activation of PICs [35]. A higher number of circulating monocytes is associated with worse outcomes [38].

Monocyte recruitment is primarily mediated by chemoattractants such as CCL2 and CXCL12 released from infarct tissue and receptors such as CCR2 and CXCR2 expressed on monocytes [35,38]. Some substances produced by neutrophils, such as ROS and IL-6, also attract monocytes and macrophages to the site of injury [1].

Increasing the expression of cell adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), also facilitates monocyte migration [37]. Twelve hours after the onset of ICH, monocytes outnumber neutrophils in the perihematomal area. As the levels of inflammatory factors increase, tissue damage increases further [38].

Like microglia, monocytes can also polarize into classically activated macrophages (M1 macrophages, $CD14^{++}CD16^{-}$) or alternatively activated macrophages (M2 macrophages, $CD14^{+}CD16^{++}$) [18]. M1 macrophages secrete pro-inflammatory mediators including tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL12, chemokine (C-C motif) ligand-2, and nitric oxide (NO). This subtype regulates the activation of other immune cells and their recruitment to injury sites. In contrast, M2 macrophages triggered by IL-4 and IL-13 typically secrete anti-inflammatory cytokines, mainly IL-10 and transforming growth factor (TGF)-β, and contribute to tissue repair [35,37]. The M2 phenotype tends to be more abundant in the late stages of ICH and has a longer life [1].

Other cells: Additionally, other non-phagocytic cells, such as dendritic cells (DCs), mast cells, natural killer cells (NKs), and Th17, are also involved in the secondary injury [18,41–43]. However, these cell types are not the focus of this review.

3.2.2. Adaptive Immune Cells

Lymphocytes

Under normal physiological conditions, leukocytes, including lymphocytes, rarely enter the brain parenchyma. However, they do not remain in the CNS unless they encounter antigens and become activated [2,44]. Increasing evidence shows that both innate and adaptive immunity, which also means T lymphocytes, play an important role in the progression of brain damage in ICH [43]. However, the role of lymphocytes in ICH has not been well defined [2]. Establishing an antigen-specific immune response against any antigen usually requires several (5–7) days. However, mouse models showed that they begin

to be activated earlier (within 3–4 days) during ICH events (Figure 3). The predominant subtypes are $CD4^+$ T cells (T helper cells) rather than $CD8^+$ T cells (cytotoxic T cells) [45].

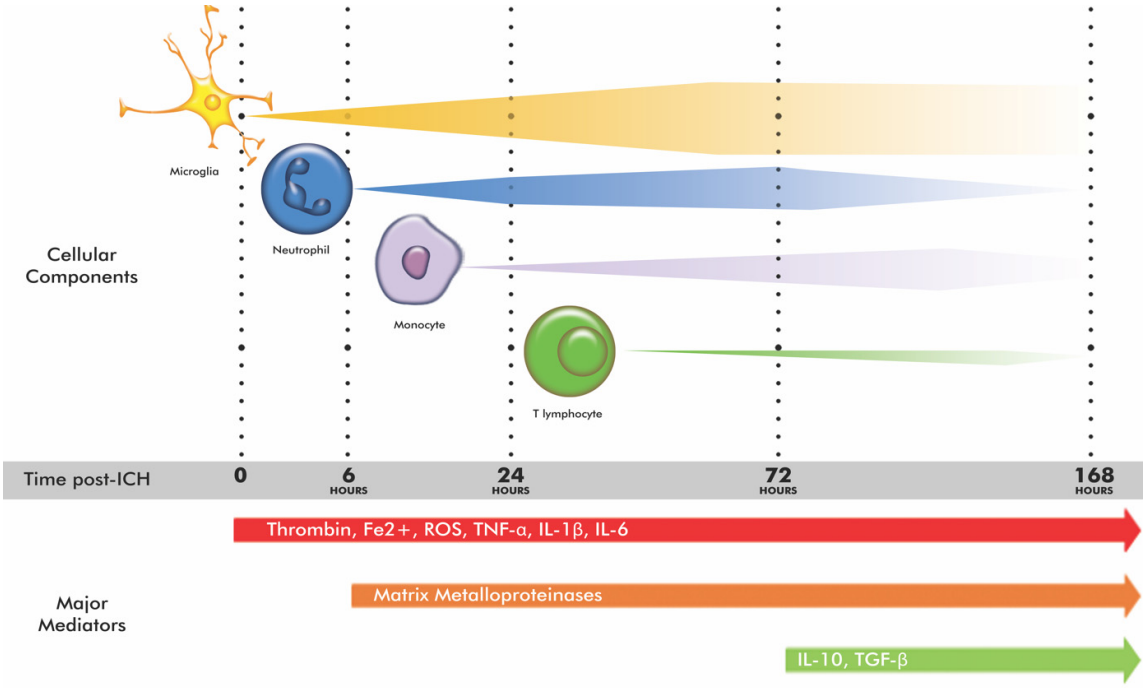

Figure 3. Timeline of inflammatory cell activation and differential factor expression after intracerebral hemorrhage.

Another set of lymphocytes involved in the inflammatory response in ICH are Th17 and Treg cells [2]. Th17 and Treg cells are functionally opposite to each other in the normal immunological response. However, the actual role of Th17 cells in the pathophysiology of neuroinflammation after ICH is still unknown. It is believed that the disruption of the BBB occurs by binding to IL-17 and IL-22 receptors on endothelial cells through Th17-secreted cytokines, which ultimately allows further migration of Th17 and other immune cells to the site of the injury [46]. Th17 cells have a high degree of flexibility and functional adaptation. While TGF-β alone induces differentiation of these cells into Treg cells, conversely TGF-β plus IL-6 or IL-21 can lead to differentiation of Th17 cells [47]. Treg cells are known for their anti-inflammatory functions by mediating immune tolerance, secreting several immunosuppressive cytokines such as IL-10, TGF-β, and IL-35 [48]. Current data suggest that Treg cells are beneficial in the context of hemorrhagic stroke, as their depletion results in increased brain damage and neurological impairment. Corroborating this are data showing that increasing their presence reduces ICH-induced inflammatory damage, neurological impairment, and BBB breakdown [49]. Although depleting Treg cells results in increased brain damage and neurological impairment, increasing the presence of Treg cells has been observed to reduce ICH-induced inflammatory damage [48,50].

It is important to note that in the days following a stroke, including one caused by ICH, patients may develop lymphopenia and the spleen may shrink in size in both animals and humans. This condition, called stroke-induced immunodepression, is a major cause of poststroke infections. In fact, while inflammatory reactions occurring after a stroke accelerate tissue healing and destroy necrotic cells, secondary damage occurs as a result of exacerbated inflammatory reactions [21].

4. Crosstalk Between Different Immune Cells Implicated in ICH

Changes in the inflammatory response after ICH are not only the result of an individual immune cell but are also closely related to interactions between different immune cells [11]. Crosstalk between cerebral resident cells and PICs creates a delicate and complex network, and its disruption can indirectly affect the integrity of the BBB [1]. This crosstalk between immune cells is attracting the attention of researchers.

By secreting IL-1α, TNF-α, and C1q, activated microglia induce the conversion of astrocytes to the A1 type, which promotes neuron death and exacerbates inflammatory damage [51]. On the other hand, astrocytes express IL-15, CCL2, CCL5, CXCL1, and CXCL10, which allow microglial polarization towards the pro-inflammatory phenotype [52].

Activated macrophages secrete greater amounts of the inflammatory factors IL-1β and TNF-α, which promote the recruitment of Th17 cells and neutrophils, exacerbating the inflammatory response and leading to tissue damage [46]. IL-15 derived from microglia and astrocytes enhances BBB disruption by increasing the levels and activation of CD8+T (Th1) and NK cells [53]. M1-type microglia secrete IL-12 and TNF-α in the early stages of ICH, accelerating Th1 cell polarization and inhibiting Treg immunosuppressive function [54].

Subgroups of T lymphocytes showed immunoregulatory effects. The Th1 cell subpopulation promotes microglial M1 polarization through the secretion of pro-inflammatory factors such as IFN-δ [55]. In contrast, the Th2 cell subpopulation contributes to astrocytes and microglia in an anti-inflammatory way [56]. Similarly, Th2-driven IL-4 and IL-13 promote macrophage polarization toward the M2 type, which contributes to collagen deposition and tissue healing by secreting IL-10 and TGF- β [37,57]. Treg cells secrete immunosuppressive molecules, inhibit the secretion of inflammatory factors, such as TNF-α and IL-1β, by microglia, and promote microglial M2 polarization through IL-10 secretion. They eventually alleviate the inflammatory response that occurs after ICH [48,50,58].

Microglia-derived leukotriene B4 (LTB4) causes neutrophils to migrate to the hematoma site and exacerbates the effect of inflammatory damage [59]. However, IL-27 derived from microglia inhibits the production of inflammatory factors by regulating neutrophil function and reduces the formation of hematoma and edema [50].

5. Role of Microglia/Macrophages as an Immunotherapeutic Target in ICH

The central role of Mig/Møs in the maintenance of inflammation, the important dual effects of the M1 and M2 phenotypes on brain damage, their formative role in adaptive immunity, and their interaction through cross-linking with other immune cells and the clot component have made them the subject of investigation as an important potential therapeutic target [1,22,24,37]. With polarization plasticity from M1 to M2, Mig/Møs represent the crossroads of various pro- and anti-inflammatory subtypes (Figure 4) [60]. These therapeutic agents, such as minocycline, broadly speaking promote M2 polarization and inhibit aspects of acute injury response, such as MMP release and iron toxicity. Minocycline, ultimately, stabilizes the integrity of the BBB and the neuroprotective state [18].

After ICH, vessel rupture of brain parenchyma contributes to the aggregation of red blood cells (RBCs) and the formation of hematoma to oppress brain tissue structure forming primary brain injury (PBI). Erythrocyte hemolysis in hematoma results in secondary brain injury (SBI) and non-reversing neurological deficits due to the toxic hemolytic products. Inflammatory responses firmly participate in and contribute to the SBI pathophysiological processes following ICH. During this pathological process, the CNS resident microglia become activated and monocytes-derived macrophages infiltrate from the circulation at the hemorrhagic site. These microglia/macrophages act as a primary modulator for the hematoma resolution and alleviation of neuroinflammation in SBI. Specifically,

microglia polarization produces pro-inflammatory (IL-1β, IL-6, TNF-α, CXCL8, CCL2, and CCL5) or anti-inflammatory (IL-4, IL-10, IL-13, IL-1Ra, and TGFβ) mediators during the different pathological phases, participating in ICH progression [61].

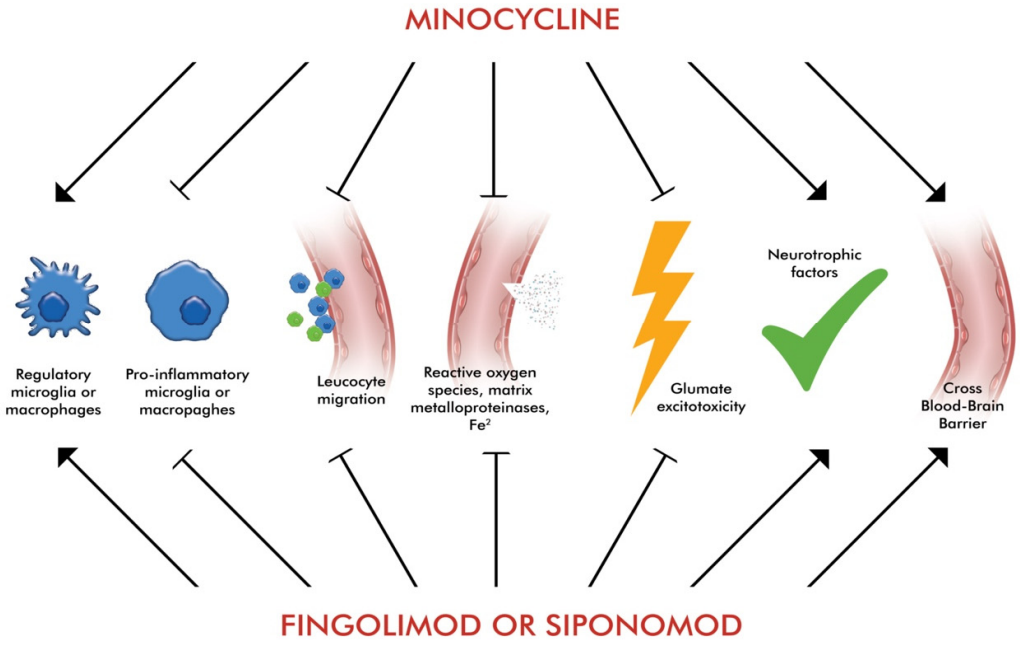

Figure 4. Schematization of exemplified microglia/macrophage polarization by intervention with selected agents (Modified from Ref. [45]).

The inhibition of M1 microglia alone is not sufficient to achieve therapeutic significance. Success in promoting M2 activation combined with inhibition of M1 microglia reveals the critical role of the M2 phenotype in the resolution of inflammation, elimination of tissue debris, and tissue healing [61].

Minocycline exerts anti-inflammatory effects by inhibiting MMPs and microglial reactivity. It also promotes microglia M2 polarization by suppressing the NOD-like receptor protein 3 (NLRP3) inflammasome. Additionally, it reduces typical markers of M1 activation (such as TNF-α and IL-1β) and increases the levels of M2 markers (such as TGF-β and IL-10) [62,63]. Furthermore, it inhibits T lymphocytic migration and reduces the secretion of chemokine and MMP (especially MMP-9).

Another modulator, fingolimod (FTY720), is a high-affinity agonist of sphingosine-1-phosphate receptor (S1PR). FTY720 can also cross the BBB and suppress inflammation by modulating S1PR-positive cells (especially Mig/Møs and astrocytes) and reducing the levels of some mediators (such as TNF-α and IL-6) [64]. Additionally, this complement significantly increases Treg cells, reduces NK cells, and limits the intravascular adhesion of leukocytes. As a result, it reduces brain damage and facilitates neurological recovery in patients [65,66].

Inhibition of pro-inflammatory cytokines is considered a useful clinical intervention. For example, IL-1Ra supplementation has been shown to significantly reduce inflammatory biomarkers and is safe and well tolerated [67].

Blocking TLR4 with agents such as eritoran, MTS510, miR-1906, and TAK-242 reduces the number of activated microglia and the production of iNOS, ROS, and pro-inflammatory factors, thus protecting the BBB against inflammatory damage [1].

The effects of many potential modulators, transcription factors, receptors, and cytokines on the shift of microglia from the M1 phenotype to the M2 phenotype are today's research topics [12,30,68–70].

Activated Mig/Møs are important source cells for chemokines, such as CCL2, CCL3, and CCL4. In addition to their role in Mig/Møs activation, these chemokines also have a direct effect on the chemotaxis of cells involved in the inflammation process and the increase in blood–brain barrier permeability [71]. Elevated serum CCL2 levels after ICH in humans have been associated with poor functional outcomes [72]. In contrast, inhibition or deficiency of CCL2 and CCL3 has been reported to result in reduced brain ischemic damage [73]. CX3CR1 deficiency, which modulates microglial activation in a mouse model, was observed to have a positive impact on functional recovery after ICH [74]. Generally, manipulating chemokine expression by Mig/Møs and chemotactic signals may constitute an approach to the treatment of ICH injury. Selected examples of therapeutic agents used in studies targeting microglia/macrophages in ICH are presented in Table 2.

Table 2. Selected examples of studies targeting microglia/macrophages in ICH.

Therapy	Major Action Mechanisms/ Administration Route	Outcomes	Reference
Animal Studies			
Minocycline	*Rat*/(intraperitoneal): - Free radical suppression - Pro-inflammatory cytokine downregulation - Anti-inflammatory cytokine upregulation - NLRP3 inflammasome suppression - MMP inhibition	- Deactivated M1 - Increased M2 polarization - BBB stabilization - Decreased inflammation - Reduced brain edema - Improved neurological functions	[69,75]
Gadolinium	*Mice*/(intraperitoneal): - Mig/Møs apoptosis	- Decreased numbers of both M1 and M2 (increase in M2b was slower than the increases in M1 and M2a, b) - Decreased neuroinflammation - Reduced brain edema - Enhanced anti-inflammatory factors - Improved neurological functions - Reduced overall mortality	[25]
MCC950	*Mice*/(intraperitoneal): - NLRP3 inflammasome suppression	- Modulated microglia phenotype - Decreased neuroinflammation - Reduced brain edema - Enhanced anti-inflammatory factors - Improved neurological functions	[76]
VK-28/Deferoxamine	*Mice*/(intraperitoneal)/*Rat*/(intramuscular): - Iron chelation (brain permeable) - Pro-inflammatory cytokine downregulation	- Increased M2 polarization - Reduced brain water content - Reduced overall mortality - Reduced neuronal death/deficits	[77,78]
siRNA	*Mice*/(intranasal): Specific silencing of CXCL16	- Increased M2 microglia - Decreased neuroinflammation - Decreased M1 microglia - Enhanced anti-inflammatory factors	[79]
Sinomenin	*Mice*/(intraperitoneal): - Anti-inflammatory responses - Immunomodulatory	- Increased M2 microglia - Decreased inflammation - Inhibited MMP-3/9 expression - Improved neurological functions	[80]

Table 2. *Cont.*

Therapy	Major Action Mechanisms/ Administration Route	Outcomes	Reference
PAP-1	*Mice*/(tail vein): Kv1.3 blockade	- Increased M2 microglia - Decreased inflammation - Enhanced anti-inflammatory response - Enhanced neurotrophic factors	[81]
TGF-β/(intravenous)	*Mice*: Immune regulation	- Increased anti-inflammatory microglia polarization - Decreased neuroinflammation - Enhanced functional recovery	[82]
Human studies			
Fingolimod (FTY720)/(oral)	- S1PR agonist - STAT3 pathway activation - Pro-inflammatory cytokine downregulation - Anti-inflammatory cytokine upregulation	- Reduced perihematomal edema - Improved neurological functions - Cell migration inhibition - Reduced vascular permeability - Decreased MMP-9 level	[83]
Edaravone/(intravenous)	- NLRP3 inflammasome suppression - Iron chelation	- Reduced perihematomal edema - Improved neurological functions - Improved daily living activity	[84]
Deferoxamine/(intravenous)	- Iron chelation (brain permeable) - Pro-inflammatory cytokine downregulation	- Increased M2 polarization - Reduced brain water content - Reduced overall mortality - Reduced neuronal death/deficits	[85]

* Observational outcome (not TGF-β therapy) in patients with earlier elevation of TGF-β in peripheral blood.

In addition to preclinical research, several clinical trials have been conducted (e.g., NCT01805895 in minocycline, NCT04088630 in fingolimod, NCT02175225 in deferoxamine, and NCT03737344 in IL-1Ra) to find the optimal treatment for ICH [ClinicalTrials.gov].

6. Strategies to Overcome Off-Target Effects and Improve On-Target Delivery

Nonspecific and/or off-target undesirable and uncontrollable systemic events can cause significant problems associated with therapeutic compound application in ICH [86]. Target-decorated nanoparticles (NPs), which enable localized and controlled delivery of these agents only to the site of injury, have recently garnered attention due to their promise for treatment and diagnostic purposes in various diseases [37]. The limited delivery of these agents to the brain is challenging for reasons such as the BBB. Various techniques to improve delivery efficiency hold great promise for overcoming this limitation [87]. To overcome the BBB challenges, the use of NP surfaces that can be conjugated with specific ligands can be considered as one of these strategies [87]. Magnetic nanoparticles (MNPs) loaded with targeting ligands and functional compound agents can be better localized to the hemorrhage site by applying an external magnetic field (EMF) [88].

7. Discussion

Cerebral hemorrhage is an important clinical condition with a high mortality rate and a poor prognosis. Currently, no effective treatment intervention is currently available [44].

Preclinical and clinical evidence shows that the inflammatory response has a crucial impact on the formation of perihematomal edema, disruption of BBB integrity, and brain cell death, which are involved in the pathophysiological process associated with ICH [44].

Diversity and plasticity are two important characteristics of the immune cells. In this context, Mig/Møs M1 phenotypes are pro-inflammatory and damage the tissue surrounding ICH. In contrast, the M2 phenotype is associated with anti-inflammatory reactions and tissue repair [89]. Therefore, M2 phenotypes play an important role in the resolution of hematoma and in the recovery phase after ICH [12,24]. For this reason, the modulation of microglia to the M2 phenotype has become an important therapeutic strategy in the treatment of ICH. Preventing excessive immune response by interfering with immune checkpoints may also be an attractive treatment method. The interaction between the programmed cell domain (PD-1/PD-L1)/cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and CD80/CD86 signaling pathways can be considered [89].

Although the basis of ICH-associated secondary injuries is inflammatory, the molecular mechanisms involved in the pathophysiology are complex and not yet clear [17]. This complex mechanism is not only dependent on an individual immune cell in the inflammatory process but also on their interactions with other immune cells and even cellular or other molecular components involved in coagulation [12,90]. The hematoma that forms shortly after an ICH event stimulates an inflammatory reaction. In the early stages, hemoglobin is released from lysed red blood cells and phagocytosed by activated Mig/Møs in the perihematomal region. Excess iron is transported out of Mig/Møs and accumulates in the neurons, leading to toxic effects on them [90].

The role of microglia/macrophage-mediated phagocytosis in clearing dead and damaged brain tissues is extremely important, especially in the subacute stage of ICH. The observation that lactoferrin treatment contributes to this phagocytosis process by reducing the amount of iron in the ICH-affected area and facilitates the hematoma resolution process suggests that it supports immune homeostasis while preventing global iron deficiency [91]. In a clinical trial, deferoxamine treatment has been observed to have good results at the 90-day follow-up [85].

Neutrophils cause brain damage owing to their phagocytic properties, degranulation of a number of cytotoxic molecules, and even secretion of NETs, which have recently become an important subject of research [92,93]. Neuronal damage is also worsened through the interaction between NETs and tissue plasminogen activator (tPA), which increases BBB disruption and cerebral hemorrhage [40]. Microglia-derived LTB4 induces neutrophil migration to the hematoma site, intensifying inflammatory damage both by the neutrophil itself and by its products, such as NETs [17,59]. In contrast, IL-27, also derived from microglia, ameliorates damage by regulating the function of neutrophils [50].

Upon stimulation after ICH, the first immune cells to be activated are microglia, which reside in the CNS [94]. Activated microglia produce a variety of pro-inflammatory cytokines, chemokines, and reactive oxygen species. In addition to acquiring phagocytic and toxic properties, these cells also gain chemotactic activity for other immune cells, especially neutrophils and macrophages, and lead to increased permeability of the BBB [12,22,95]. Furthermore, microglia polarized to the M1 phenotype intensify ICH-induced neuronal damage by amplifying early neuroinflammation through crosstalk with the immune cells [24]. The infiltration of circulating leukocytes peaks within 1-3 days after ICH and continues for weeks [38].

Secondary damage in ICH is triggered by the presence of intraparenchymal blood mainly through the initiation of the inflammatory process. Immediately after ischemic injury, microglia are activated. These cells then further activate the immune system and attract immune cells, especially neutrophils, MDM, and lymphocytes, to the injury site.

As a result, intracerebral damage further increases. There are even important interactions between the inflammatory process and components involved in coagulation. In this context, it is critical to eliminate the source of pro-inflammatory responses to restore homeostatic balance and promote healing. Microglia may be considered the most important target cells, as they play a dominant and central role in both the initiation and progression of inflammation. Furthermore, when microglia are polarized to the M2 phenotype, they play a role in preventing inflammation, resolving hematoma, and repairing damaged tissues. General anti-inflammatory medications and treatments targeting only the M2 phenotype have also been observed to not yield sufficient results. For this reason, it can be considered that approaches that modulate microglia/macrophages to the M2 phenotype would be more appropriate approaches.

The treatment options for ICH are very limited. The vast majority of patients die within a short period of time, and survivors often have significant residual disability and are at risk of significant neurological complications, including epilepsy and vascular cognitive impairment [96]. The incidence of ICH is closely related to the elderly population and the use of anticoagulant drugs [97]. Unfortunately, antiplatelet drug use is widespread among the aging population, sometimes without a clear indication. Moreover, these drugs have an impact not only on the occurrence of ICH but also on its prognosis, especially in terms of disability and mortality [98].

It is now well known that the inflammatory response plays a vital role in the development of secondary damages that affect the clinical outcome in ICH. After ICH, glial cells are activated. These lead to disruption of the blood–brain barrier, peripheral blood inflammatory cell infiltration, and production of numerous inflammatory cytokines, resulting in brain edema and neuronal injury [99]. In addition to leukocytes/macrophages, activated microglia and astrocytes are the main cellular mediators of secondary brain injury based on the local release of immune-active molecules (cytokines, chemokines, prostaglandins, proteases, ferrous iron, etc.). Inhibiting the initial upstream event that triggers the subsequent inflammatory response can significantly alleviate ICH-induced injury and reduce associated neurological deficits. For example, minocycline treatment improves clinical outcome by reducing the level of cerebral edema and neurological deficits. At the pathophysiological level, it reduces the number of microglia and macrophages around the hematoma, protects capillaries, and decreases TNF-α and MMP-12 levels [21].

Future Perspective and Conclusions

The failure of more than 250 clinical trials using more than 1000 brain-protective molecules for therapeutic purposes highlights the critical need for new approaches to developing treatments for acute stroke [21]. As the role of Mig/Møs in the pathophysiology of ICH becomes better understood, future research will become more specific, such as discovering new signaling pathways or therapeutic molecules. Despite tremendous progress, there are important unidentified aspects of the molecules involved in their polarization [99]. Studies such as transcriptomics and proteomics may offer the opportunity for more targeted intervention. Again, understanding the spatial and temporal dynamics of microglial/macrophage activation may provide more precise windows of opportunity for therapeutic interventions. This approach also enables treatment options that reduce the risk of infection due to stroke-induced immunodepression.

Additionally, the combination of microglia/macrophage regulatory agents with other therapeutics such as anti-inflammatory cytokines and iron chelators may provide synergistic effects and ultimately fewer side effects. In a two-arm clinical trial, a reduction in neurological impairment was observed in the fingolimod (FTY720) group compared with the standard treatment regimen. Moreover, a greater proportion of patients in the

combined treatment group achieved complete recovery of neurological function after 3 months. In ICH, patients show heterogeneity in terms of clinical and treatment response, highlighting the importance of personalized approaches. In this regard, biomarkers may provide the opportunity to improve patient outcomes with personalized treatment options. Localization of therapeutic agents only to the damaged area may improve clinical outcomes. This approach also plays a vital role in reducing the risk of undesirable and uncontrollable off-target systemic events.

In conclusion, modulating immunotherapies holds promise for use in intracerebral hemorrhage. However, further studies are needed to identify the most appropriate molecular targets that mediate immune cell shifts toward beneficial phenotypes and to validate them for use in clinical settings.

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