HHS Public Access

Author manuscript

J Neuroimmune Pharmacol. Author manuscript; available in PMC 2016 December 01.

Published in final edited form as:

J Neuroimmune Pharmacol. 2015 December ; 10(4): 576–586. doi:10.1007/s11481-015-9613-1.

Modulating the Immune Response towards a Neuroregenerative Peri-injury Milieu after Cerebral Hemorrhage

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Abstract

Cerebral hemorrhages account for 15–20% of stroke sub-types and have very poor prognoses. The mortality rate for cerebral hemorrhage patients is between 40–50%, of which at least half of the deaths occur within the first two days, and 75% of survivors are incapable of living independently after one year. Current emergency interventions involve lowering blood pressure and reducing intracranial pressure by controlled ventilations or, in the worst case scenarios, surgical intervention. Some hemostatic and coagulatherapeutic interventions are being investigated, although a few that were promising in experimental studies have failed in clinical trials. No significant immunomodulatory intervention, however, exists for clinical management of cerebral hemorrhage. The inflammatory response following cerebral hemorrhage is particularly harmful in the acute stage because blood-brain barrier disruption is amplified and surrounding tissue is destroyed by secreted proteases and reactive oxygen species from infiltrated leukocytes. In this review, we discuss both the destructive and regenerative roles the immune response play following cerebral hemorrhage and focus on microglia, macrophages, and T-lymphocytes as the primary agents directing the response. Microglia, macrophages, and T-lymphocytes each have sub-types that significantly influence the over-arching immune response towards either a pro-inflammatory, destructive, or an anti-inflammatory, regenerative, state. Both pre-clinical and clinical studies of cerebral hemorrhages that selectively target these immune cells are reviewed and we suggest immunomodulatory therapies that reduce inflammation, while augmenting neural repair, will improve overall cerebral hemorrhage outcomes.

Keywords

Lymphocytes; Microglia; Macrophages; Intracerebral Hemorrhage; Intraventricular Hemorrhage; Germinal Matrix Hemorrhage; Inflammation

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Conflicts of Interest: None.

Cerebral Hemorrhage Pathophysiology

Incidence, Outcomes, and Clinical Management

Cerebral hemorrhage, the rupturing of blood vessels within the brain tissue, accounts for 10–15% of strokes (Qureshi et al., 2009; Mracsko and Veltkamp, 2014). Occurring in approximately 25 per 100,000 people per year, cerebral hemorrhage is the leading cause of morbidity and mortality in stroke patients, having a mortality rate between 30–50% with nearly 75% of survivors incapable of living independently after one year (van Asch et al., 2010). Hospital admissions for cerebral hemorrhage cases have increased by 18% over the past decade, and admissions are expected to continue rising due to an increasing elderly population (Qureshi et al., 2009).

Current clinically approved emergency interventions involve lowering blood pressure (Anderson et al., 2008; Morgenstern et al., 2010; Sakamoto et al., 2013) or surgical craniotomy (Morgenstern et al., 2010). Hematoma evacuation is also being investigated as a potential surgical intervention, but it has yet to yield positive results (Morgenstern et al., 1998; Mendelow et al., 2005; Miller et al., 2008; Wang et al., 2009; Mendelow et al., 2013). Hemostatic therapies have also been investigated in clinical trials, such as recombinant factor VII, but despite showing promise in experimental studies, none have been clinically approved (Mayer et al., 2006; Mayer et al., 2008; Diringer et al., 2010). Unfortunately, cerebral hemorrhage is the least treatable stroke subtype, with minimal advancements being made in clinical management, despite its increasing prevalence (Kreitzer and Adeoye, 2013).

Primary Brain Injury

Most investigative therapeutic approaches for cerebral hemorrhage focus on ameliorating primary brain injury, which is caused by the mechanical pressure on brain tissue due to the hematoma mass effect, and the potential hematoma expansion (Xi et al., 2006; Keep et al., 2012; Mracsko and Veltkamp, 2014). Mechanical pressure applied to glia and neurons cause calcium influx and secretion of excitotoxic neurotransmitters, resulting in consequent cytotoxic edema and necrosis (Keep et al., 2005; Xi et al., 2006). Indeed, hematoma volume and subsequent hematoma expansion, which occurs in approximately 30% of clinical cerebral hemorrhage cases, are currently the best prognostic indicators (Davis et al., 2006; Dowlatshahi et al., 2011; Brouwers and Greenberg, 2013). Brain edema, caused by both primary and secondary brain injury mechanisms, is increasingly accepted as a valuable prognostic indicator (Thiex and Tsirka, 2007; Staykov et al., 2011).

Secondary Brain Injury

Mechanisms for secondary brain injury after cerebral hemorrhage have garnered increased research interests over the past decade. Secondary brain injury results from blood components entering the brain tissue as well as injured brain cells that trigger multiple deleterious mechanisms and subsequently augment oxidative stress, inflammatory pathways, blood-brain barrier disruption, and vasogenic edema (Aronowski and Zhao, 2011; Belur et al., 2013). Coagulation cascade activation increases thrombin formation, which stimulates the complement pathway as well as protease-activated receptors (PARs) (Hua et al., 2007;

Babu et al., 2012). PAR stimulation after hemorrhage, particularly PAR-1 activation in neurons, leads to increased NMDA receptor potentiation and consequent activation of excitotoxicity, apoptosis, and pro-inflammatory pathways (Babu et al., 2012). PAR-1 has also been implicated in playing an important role in thrombin-induced cerebral hemorrhaging (Cheng et al., 2014). Complement activation after cerebral hemorrhage leads to membranous pore formation, called membrane attack complexes, in neurons and red blood cells, causing cytotoxicity and cell lysis (Hua et al., 2000; Ducruet et al., 2009).

Red blood cell lysis releases hemoglobin, which is metabolized by heme oxygenase 1 to release iron and heme, into the surrounding tissue (Wu et al., 2006). Heme and iron are critical constituents in redox reactions that produce injurious free radicals and increase oxidative stress, causing significant tissue injury, DNA damage, blood-brain barrier disruption, and inflammation (Wu et al., 2006; Xi et al., 2006; Babu et al., 2012; Xiong et al., 2014). Administering Deferoxamine, an iron chelator, resulted in improved neurofunctional outcomes following experimental cerebral hemorrhage and reduced hydrocephalus development in other brain injury models (Nakamura et al., 2004; Klebe et al., 2014; Zhao et al., 2014a). Indeed, post-hemorrhagic hydrocephalus is a common consequence of intracerebral hemorrhage as well, and iron has been implicated as a causative factor (Chen et al., 2014; Gao et al., 2014a; Meng et al., 2014).

Inflammation is a key component of secondary brain injury following cerebral hemorrhage (Wang, 2010; Mracsko and Veltkamp, 2014; Zhou et al., 2014; Chen et al., 2015). An inflammatory response ensues immediately after blood enters the brain tissue via activation of resident immune cells and subsequent infiltration of peripheral leukocytes, leading to secretion of pro-inflammatory mediators, extracellular proteases, and reactive oxygen species that further damage brain tissue and disrupt the blood-brain barrier (Aronowski and Hall, 2005; Wang and Dore, 2007). Immune cells, particularly pro-inflammatory macrophages, also play an important role in cerebral aneurysm formation, a primary cause for cerebral hemorrhage (Hosaka and Hoh, 2014; Starke et al., 2014). Some evidence, however, suggests inflammation may play an important role in repair and recovery following central nervous system injury (Correale and Villa, 2004; Hohlfeld et al., 2006; McCombe and Read, 2008; Wee Yong, 2010). The potential neuroprotective branch in inflammation may be therapeutically exploited following cerebral hemorrhage to promote hematoma resolution as well as tissue repair and regeneration.

Review Scope

In this review, we will discuss microglia, macrophage, and T-helper lymphocyte immunology following cerebral hemorrhagic insult, the roles which the subtypes for each play in neurodegeneration or neuroprotection, as well as possible therapeutic approaches to potentially shift the inflammatory response towards a neuroregenerative phenotype. We will also discuss evidence from current cerebral hemorrhage research and address gaps in the literature that warrant further investigation. Explicating the immune response in its entirety following cerebrovascular insult will discern therapeutic immunomodulatory approaches that dampen the neurodegenerative inflammatory response in favor for a neuroregenerative one, promoting functional recovery and improving overall outcomes. Cerebral hemorrhage

pathophysiology is very complex and multi-modal approaches are being increasingly encouraged, and exploiting immunomodulatory mechanisms may prove beneficial when investigating such approaches since inflammation is an important component of secondary brain injury (Pandey and Xi, 2014).

Macrophage, Microglia, and T-helper Lymphocyte Characterization

Macrophage and Microglia Subtypes

Macrophages are a type of white blood cell of myeloid lineage found in almost all tissue types and play a quintessential role in innate (non-specific) immunity by searching for and engulfing potential pathogens (Murray and Wynn, 2011). Microglia are the resident macrophages of the central nervous system, and, unfortunately, they are extremely difficult to distinguish from infiltrated macrophages following central nervous system injury (Saijo and Glass, 2011). Traditionally, macrophages and microglia phagocytose microbes, cellular debris, apoptotic cells, cancer cells, and foreign substances. Stimulation of microglia/macrophage toll-like receptors, nod-like receptors, scavenger receptors, and/or cytokine receptors from inflammatory cytokines, pathogens, and blood products will potentiate the inflammatory response through further secretion of pro-inflammatory cytokines, such as TNF-α and IL-1β, resulting in leukocyte recruitment. Recent immunology research, however, discerned macrophages can also dampen the immune response, promoting tissue repair and regeneration. Microglia and macrophages have been classified into two predominant subtypes modeled similar to the Th1/Th2 paradigm: classically activated or M1 and alternatively activated or M2 (Murray and Wynn, 2011; Saijo and Glass, 2011). Further elucidating the role each subtype plays in cerebral hemorrhage pathophysiology may yield potential therapeutic avenues that coax the immune response to create a neural regenerative milieu in the peri-hematoma region.

Classically activated M1 macrophages and microglia are primarily responsible for the innate immune defense mechanisms, producing a pro-inflammatory response (Murray and Wynn, 2011; Saijo and Glass, 2011). The M1 phenotype can be induced by lipopolysaccharides, interferon-γ, TNF-α, or stimulation of nod-like receptors (NLR) or toll-like receptors (TLR), primarily TLR-4 (Martinez et al., 2009; Chen and Nunez, 2010). Very few definitive cell surface markers for the M1 subtype have been identified, although CD80 and CD86 are widely used. M1 macrophages and microglia also secrete TNF-α, IL-1β, IL-6, IL-12, and IL-23 pro-inflammatory cytokines (Martinez and Gordon, 2014; Wang et al., 2014). Alternatively, activated M2 macrophages and microglia are primarily responsible for mediating wound healing and produce an anti-inflammatory response (Murray and Wynn, 2011; Saijo and Glass, 2011). The M2 phenotype can be induced by IL-4, IL-10, TGF-β, and IL-13 stimulation (Martinez et al., 2009; Martinez and Gordon, 2014; Wang et al., 2014). Common cell surface markers attributed to the M2 subtype include CD163 and CD206, and M2 macrophages and microglia secrete IL-10 and TGF-β anti-inflammatory cytokines (Martinez et al., 2009; Martinez and Gordon, 2014; Wang et al., 2014). Evidence suggests macrophages and microglia do not exist as terminally differentiated M1 or M2 states, but rather have the ability to switch phenotypes depending upon their microenvironment (Stout and Suttles, 2004; Stout et al., 2005; Eggen et al., 2013; Giunti et al., 2014). Table 1

contains the macrophage/microglia subtypes, cytokines inducing their activation, common markers, secreted cytokines, as well as known mechanisms of action.

The M1/M2 paradigm, however, has limitations. Indeed, M2 microglia are being further classified into 3 subsets based on how differentiation is stimulated: M2a is stimulated by IL-4 and IL-10; M2b is stimulated by toll-like receptor activation; and M2c is stimulated by IL-10, glucocorticoids, and TGF-β (Cherry et al., 2014). M2a and M2c have the typical markers and responses attributed to alternatively activated M2, while M2b lacks certain markers and has a response similar to M1 (Cherry et al., 2014). Additionally, An increasing number of macrophage/microglia subsets in addition to M1/M2 are being classified in other diseases and disorders, such as atherosclerosis, multiple sclerosis, and lupus, including M4, Mox, Mhem, and M(Hb) (Orme and Mohan, 2012; Bogie et al., 2014; Chinetti-Gbaguidi et al., 2015). Furthermore, distinct macrophage/microglia responses have been characterized *in vitro* following stimulation by cytokines and extracellular pathogenic debris as well as *in vivo* following infection, yet the exact roles distinct microglia/macrophage subtypes play in most neurological diseases, disorders, and injuries have yet to be well elucidated. M1 and M2 may represent two extremes on a large spectrum of macrophage/microglia subsets where each subset plays a very critical immunomodulatory role in cerebral hemorrhage pathophysiology, and more research is needed to fill these gaps in our knowledge and help advance immunotherapeutic approaches.

T-helper Lymphocyte Subtypes

T-helper cells are $CD4^+$ white blood cells of lymphoid origin that develop within the thymus which are critical in regulating cell-mediated and adaptive immunity (Luckheeram et al., 2012). The two predominant paradigms for T-helper cell subtypes, analogous to the M1/M2 macrophage and microglia paradigm in that the two phenotypes oppose each other, are Th1/Th2 and Th17/Treg (Kleinewietfeld and Hafler, 2013; Bretscher, 2014). Macrophages and microglia are capable of switching phenotypes according to their microenvironment, and some evidence supports T-helper cells switching phenotypes too, particularly between Th17 and Treg, although more conclusive evidence is needed (Stout and Suttles, 2004; Stout et al., 2005; Xu et al., 2007; Kleinewietfeld and Hafler, 2013).

Th1 cells direct the immune system towards fighting intracellular pathogens by inducing a cell-mediated response. Th1 cell differentiation is kindled by IL-12 and interferon-γ stimulation, the latter of which Th1 cells secrete to further drive Th1 differentiation. Th2 cells direct the immune system towards fighting extracellular pathogens by inducing a humoral response. Th2 cell differentiation is sparked by IL-4 and IL-2 stimulation, and Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (Zhu and Paul, 2008, 2010; Luckheeram et al., 2012). Th1 cells are characterized by CCR5, CXCR3, and T-bet markers, while Th2 cells are identified by CCR3, CCR4, CRTH2, and GATA3 markers. Th1 and Th2 both drive their own differentiation while suppressing differentiation into the other through their cytokine expressions (Zhu and Paul, 2008, 2010; Luckheeram et al., 2012).

The other T-helper phenotype paradigm relies on the promotion or suppression of inflammation. Th17 cells which potentiate inflammation and have been implicated as potential sources for many autoimmune diseases differentiate through IL-6, IL-21, IL-23,

and TGF-β stimulation. T17 cells, which express CCR6 and RORγt markers, secrete IL-17, IL-21, and IL-22 (Stockinger and Veldhoen, 2007; Dong, 2008; Hirota et al., 2010; Peck and Mellins, 2010; Dong, 2011). Treg cells which dampen inflammation and mediate immune tolerance to self-antigens, differentiate through IL-2 and TGF-β stimulation (Chen et al., 2003; Mantel and Schmidt-Weber, 2011; Yoshimura and Muto, 2011). Treg cells, characterized by CD25 and FOXP3 markers, secrete IL-10 and TGF-β. Evidence suggests plasticity between Th17 and Treg cell differentiation, which is highly dependent upon their surrounding milieu (Zhou et al., 2009; Kleinewietfeld and Hafler, 2013). Interestingly, differentiation of both Th17 and Treg cells can be driven by TGF-β (Li et al., 2007). TGF-β at low concentrations and in conjunction with IL-6 or IL-21 will drive Th17 differentiation, but TGF-β at high concentrations and in conjunction with IL-10 will drive Treg differentiation (Chen et al., 2003; Mantel and Schmidt-Weber, 2011; Yoshimura and Muto, 2011). Table 1 contains the T-helper lymphocyte subtypes, cytokines inducing their activation, common markers, secreted cytokines, as well as known mechanisms of action.

Macrophage/Microglia and T-helper Lymphocyte Communication

T-helper lymphocytes, macrophages, and microglia are capable of influencing each other's differentiation as well as phenotype switching (Murray and Wynn, 2011; Saijo and Glass, 2011; Luckheeram et al., 2012). M1 macrophages secrete TNF-α, IL-6, IL-12, and IL23 (Murray and Wynn, 2011; Saijo and Glass, 2011), which recruits and induces differentiation of both Th1 and Th17 cells. In turn, Th1 cells secrete interferon-γ while Th17 cells secrete IL-17, which further augments M1 macrophage polarization and potentiates the inflammatory response (Fiorentino et al., 1991; Denning et al., 2007; Martinez et al., 2008; Savage et al., 2008; Biswas and Mantovani, 2010). M1 macrophage and Th1 / Th17 cross-talk acts as a positive feed-back loop that further drives their own differentiation and create a highly inflamed microenvironment. Analogously, M2 macrophages secrete IL-4, IL-10, and TGF-β (Martinez et al., 2008; Murray and Wynn, 2011; Saijo and Glass, 2011), which recruits and induces differentiation of both Th2 and Treg cells. In turn, Th2 cells secrete IL-4 and IL-10 while Tregs secrete IL-10 and TGF-β, which further drives M2 polarization, dampening inflammation and promoting tissue repair (Denning et al., 2007; Martinez et al., 2008; Savage et al., 2008; Biswas and Mantovani, 2010). Disproportion between macrophage/microglia and T-helper cells, in which either the M1/Th1/Th17 or M2/Th2/Treg branches are overexpressed, intensify many disorders, including allergies, asthma, cancer, autoimmune diseases, atherosclerosis, and fibrosis. Consequently, many therapeutic approaches have been developed to exploit the cross-talk between macrophages/microglia and T-helper cells, restoring homeostasis between pro-inflammatory and regenerative signaling in those aforementioned disorders (Murray and Wynn, 2011; Saijo and Glass, 2011; Luckheeram et al., 2012).

Inflammation after Cerebral Hemorrhage

Role of Macrophages and Microglia

Explicating the inflammatory component of cerebral hemorrhage pathophysiology and discerning the immune cells involved, particularly the distinct macrophage/microglia and T-helper cell subsets, could lead to novel therapeutic approaches in which the peri-hematoma

milieu is switched from a pro-inflammatory microenvironment to a neuroregenerative one. Macrophages, microglia, and T-helper cell subsets are ideal targets because of the cross-talk and capability of potentiating either the damaging branch of inflammation via M1/Th1/Th17 or the repair/regenerative branch via M2/Th2/Treg (Murray and Wynn, 2011; Saijo and Glass, 2011; Luckheeram et al., 2012). The M1/Th1/Th17 branch creates an oxidative, caustic microenvironment to destroy pathogens, but cerebral hemorrhage is a brain injury of endogenous origin, thus attenuating this branch in favor of the M2/Th2/Treg branch may be beneficial (Chen and Nunez, 2010). Macrophages and microglia are of particular interest since they are the first to be activated following hemorrhage and are quintessential drivers of the immune response (Mracsko and Veltkamp, 2014; Zhou et al., 2014). Furthermore, microglia and macrophages have great plasticity and aptly switch phenotypes between M1 and M2 in response to the pathophysiology of their microenvironment.

Activated microglia become present within 1–4 hours after cerebral hemorrhage in rodents, peak between 3–7 days, and finally return to basal levels between 3–4 weeks (Wang and Dore, 2007; Zhou et al., 2014). Following microglia activation, peripheral macrophages also infiltrate the injured tissue, although they are extremely difficult to distinguish from microglia. Microglia/macrophages also recruit neutrophils within hours of activation, which potentiate blood-brain barrier disruption and tissue damage by secreting extracellular proteases (Wang and Dore, 2007; Zhao et al., 2014b). While the activated microglia time course has been established in these experimental rodent cerebral hemorrhage models, the time course of M1 and M2 phenotype expression has yet to be well-defined. Determining the ratio of M1/M2 microglia and macrophages could be indicative of the pathophysiological milieu, as a high M1/M2 ratio implies a more oxidative, caustic inflammatory environment while a low M1/M2 ratio implies a more repair, regenerative environment. M2 microglia and macrophages have increased scavenger receptor expression levels as well as augmented phagocytic activity compared to their M1 counterparts, therefore the M2 phenotype may be important for hematoma resolution after cerebral hemorrhage (Cherry et al., 2014). Indeed, hematoma volume peaks 72 hours after experimental cerebral hemorrhage, remains relatively elevated from 3–7 days, and is finally resolved between 2–4 weeks (Zhao et al., 2009). Coincidentally, this correlates with the time course of activated microglia number, thus elucidating the time course of the M1/M2 ratio following cerebral hemorrhage may yield more information on the neuroprotective role microglia and macrophage subtypes play, as it is expected the M1/M2 ratio will rise and fall with hematoma volume, implying M2 microglia and macrophages are important for hematoma resolution.

A thoroughly investigated therapy involves enhancing hematoma resolution by peroxisome proliferator receptor gamma (PPARγ) stimulation. PPARγ stimulation enhances hematoma resolution, reduces oxidative stress, ameliorates damaging inflammation, decreases brain edema, and improves functional outcomes starting at 24 hours after cerebral hemorrhage in experimental rodent models (Zhao et al., 2007; Zhao et al., 2009). Furthermore, Pioglitazone, a PPARγ agonist, is being investigated in a clinical trial for enhancing hematoma resolution after cerebral hemorrhage. PPARγ stimulation upregulates the red blood cell scavenger receptor CD36, consequently enhancing red blood cell phagocytosis *in*

vitro and *in vivo* (Zhao et al., 2007). Furthermore, the change in the M1/M2 ratio induced by PPARγ stimulation has not been investigated. However, other studies indicate that PPARγ stimulation polarizes microglia and macrophages towards the M2 phenotype (Pisanu et al., 2014). Thus the neuroprotective effect from PPARγ treatment may include polarizing microglia and macrophages towards the M2 phenotype, reducing the M1/M2 ratio, and creating a regenerative peri-hematoma milieu that promotes tissue repair and hematoma resolution.

After cerebral vessel rupture, damage-associated molecular patterns, molecules capable of initiating and perpetuating a non-infectious inflammatory response following injury, stimulate the TLRs and NLRs of microglia, inducing microglia activation (Fang et al., 2013). TLR-4 stimulation by damage-associated molecular patterns, in addition to hemoglobin degradation products, induces M1 differentiation in macrophages and microglia. In a rodent model of cerebral hemorrhage, TLR-4 knockout mice had decreased microglia activation and macrophage infiltration at 72 hours post ictus (Fang et al., 2013). Additional studies established that TLR-4 blockade also ameliorates neurological deficits and brain edema after experimental cerebral hemorrhage (Fang et al., 2013). While TLR-4 stimulation following cerebral hemorrhage activates microglia and induces an M1-like phenotype, the effect on the M2 phenotype is unknown.

Rather than reduce the M1/M2 phenotype ratio, some studies have aimed at inhibiting microglia activation altogether. Experimental cerebral hemorrhage studies in rodents found that microglia inhibitor factor ameliorates brain injury and improves functional outcomes, which was correlated with overall reduced microglia activation (Wang and Dore, 2007; Zhou et al., 2014). One such microglia activation inhibitor is minocycline. Nervous system injury studies discerned minocycline treatment inhibits M1 microglia and macrophage polarization without affecting M2 polarization. While preclinical studies have reported that minocycline attenuates brain injury and improves functional outcomes, one study challenges minocycline's overall therapeutic potential (Wang and Dore, 2007; Zhou et al., 2014). Indeed, the authors of a study which determined that minocycline is neuroprotective also argued that long-term microglia inhibition may not be beneficial because of the role microglia play in tissue repair, suggesting that M2 microglia and macrophages may play a long-term neuroprotective role. This concept may provide an explanation for the results acquired in the negative minocycline cerebral hemorrhage study. Despite seemingly contradictory studies, minocycline is currently being evaluated in clinical trials for cerebral hemorrhage.

Evidence provided by other brain injury preclinical models suggests manipulating M1/M2 polarization by decreasing the M1 phenotype and/or by increasing the M2 phenotype has beneficial outcomes. Although microglia and macrophages are known to play a very important role in potentiating secondary brain injury after cerebral hemorrhage, since several studies also suggest they are important for functional recovery, little is known about the roles individual microglia subtypes play. Discerning the exact microglia and macrophage subtypes involved in cerebral hemorrhage pathophysiology, as well as their time course of action following injury, may yield novel, effective therapeutic approaches, since decreasing

the M1/M2 ratio by either reducing the M1 pro-inflammatory phenotype and/or increasing the M2 regenerative phenotype may improve overall outcomes.

Role of T-Helper Lymphocytes

Although cerebral hemorrhage is a brain injury of endogenous origin, and mounting an antigen specific adaptive immune response may take nearly one week to occur, increasing evidence suggests that $CD4^+$ T-helper lymphocytes play an important role in secondary brain injury. Clinical evidence shows the presence of T-helper lymphocytes within the peri-hematoma region of patients with cerebral hemorrhage (Guo et al., 2006). Pre-clinical rodent cerebral hemorrhage models indicate T-helper lymphocyte infiltration is delayed, commencing between 2–4 days post-ictus, yet some studies have reported T-helper lymphocyte infiltration occurs within one day post-ictus, peaking at 5 days post-ictus, before subsiding (Mracsko et al., 2014). Intuitively, this suggests T-helper cells, which heavily influence the surrounding milieu of injured tissue, direct the delayed immune response of cerebral hemorrhage, at least in part, by encouraging either a highly inflammatory, oxidative microenvironment or a regenerative and repair microenvironment, although preclinical evidence for the exact role of T-helper subtypes is lacking.

Fingolimod, a sphingosine-1 phosphate inhibitor, downregulates sphingosine-1 phosphate receptors in T-helper lymphocytes to reduce their egress from lymphoid tissue and subsequent infiltration into injured tissue. In a rodent model of cerebral hemorrhage, fingolimod reduced brain edema and improved neurofunctional outcomes 24 and 72 hours post-ictus, which was associated with reduced T-helper lymphocyte egress in the blood, decreased T-helper lymphocyte infiltration into the brain, and decreased IL-17 secretion (a pro-inflammatory cytokine typically secreted by Th17 cells (Rolland et al., 2011; Rolland et al., 2013). Fingolimod, however, is known to upregulate peripheral regulatory T cells with some evidence suggesting it decreases circulating Th17 cells in clinical and preclinical multiple sclerosis studies. Thus, Fingolimod's neuroprotective effects in cerebral hemorrhage may partially be explained by shifting the predominant T-helper cell response from the inflammatory Th17 subtype to the regenerative Treg phenotype, although this has yet to be confirmed. Fingolimod is currently being investigated in clinical trials for cerebral hemorrhage (Fu et al., 2014).

Another experimental rodent model of cerebral hemorrhage study investigated adoptive transfer of Treg cells, and observed that Treg cells reduced microglia activation and improved functional outcomes, however the effects on microglia and macrophage polarization were not evaluated (Yang et al., 2014). Furthermore, neural stem cell transplantation, previously shown to protect the brain from inflammatory damage, resulted in increased peripheral and infiltrated Treg cells, and was associated with increased anti-inflammatory cytokines (IL-4, IL-10, and TGF-β) and decreased pro-inflammatory cytokines (IL-6, interferon-γ) (Gao et al., 2014b). Furthermore, mammalian target of rapamycin (mTOR) inhibition following cerebral hemorrhage in rats resulted in improved neurofunctional outcomes 24 hours post-ictus which coincided with increased Treg cells, IL-10, and TGF-β, as well as reduced interferon-γ in the blood and brain (Lu et al., 2014). Unfortunately, investigative studies on the Th1/Th2 paradigm following cerebral

hemorrhage are lacking. Yet evidence suggests that inhibiting Th1 proliferation improves outcomes while inhibiting Th2 aggravates brain injury during cerebral ischemia (Theodorou et al., 2008; Gu et al., 2012).

Conclusion

In this review, we discussed the distinct macrophage and microglia, and T-helper lymphocyte subtypes, their known roles in cerebral hemorrhage pathophysiology, and current gaps in the literature. Table 2 contains a brief overview of current and past clinical trials investigating immunomodulatory therapies, which are sparse and often directly target another pathophysiological mechanism with immunomodulation as a secondary effect. Although many pharmacological approaches showed promise experimentally, none have thus far successfully translated to the clinic (Ayer et al., 2012). Although the discussion of the M1 and M2 microglia/macrophage subtypes and the Th1/Th2 and Th17/Treg subtypes is not novel, their unknown roles in cerebral hemorrhage pathophysiology warrants further investigation as possible therapeutic targets. Our goal is to advance the discussion of inflammation and secondary brain injury to involve the distinct immune cell subtypes and their probable neurodegenerative or neuroprotective roles and encourage further investigation. Indeed, modulating the immune response by shifting the peri-injury milieu from a highly oxidative, caustic environment, typically mediated by the M1/Th1/Th17 subtypes, to a regenerative and repair environment, mediated by the M2/Th2/Treg subtypes, is being increasingly encouraged in the cerebral ischemia field (Seifert and Pennypacker, 2014). Applying the same concept in polarizing towards a regenerative and repair immune response following cerebral hemorrhage may yield positive, novel therapeutic approaches, and may be more desirable than inhibiting inflammation altogether. Applying a multi-modal approach involving immunomodulatory therapies to ameliorate secondary brain injury in conjunction with conventional therapies targeting primary brain injury may improve both short and long-term outcomes and warrants further investigation.

Acknowledgments

Funding: this review was supported by NIH P01NS082184 to John H. Zhang and Jiping Tang

References

Anderson CS, et al. Intensive blood pressure reduction in acute cerebral haemorrhage trial (INTERACT): a randomised pilot trial. The Lancet Neurology. 2008; 7:391–399. [PubMed: 18396107]

Aronowski J, Hall CE. New horizons for primary intracerebral hemorrhage treatment: experience from preclinical studies. Neurological research. 2005; 27:268–279. [PubMed: 15845210]

Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. Stroke; a journal of cerebral circulation. 2011; 42:1781–1786.

Ayer A, Hwang BY, Appelboom G, Connolly ES Jr. Clinical trials for neuroprotective therapies in intracerebral hemorrhage: a new roadmap from bench to bedside. Translational stroke research. 2012; 3:409–417. [PubMed: 24323830]

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. Neurosurgical focus. 2012; 32:E8. [PubMed: 22463118]

Belur PK, Chang JJ, He S, Emanuel BA, Mack WJ. Emerging experimental therapies for intracerebral hemorrhage: targeting mechanisms of secondary brain injury. Neurosurgical focus. 2013; 34:E9. [PubMed: 23634928]

Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nature immunology. 2010; 11:889–896. [PubMed: 20856220]

Bogie JF, Stinissen P, Hendriks JJ. Macrophage subsets and microglia in multiple sclerosis. Acta neuropathologica. 2014; 128:191–213. [PubMed: 24952885]

Bretscher PA. On the mechanism determining the TH1/TH2 phenotype of an immune response, and its pertinence to strategies for the prevention, and treatment, of certain infectious diseases. Scandinavian journal of immunology. 2014; 79:361–376. [PubMed: 24684592]

Brouwers HB, Greenberg SM. Hematoma expansion following acute intracerebral hemorrhage. Cerebrovascular diseases. 2013; 35:195–201. [PubMed: 23466430]

Butcher KS, Jeerakathil T, Hill M, Demchuk AM, Dowlatshahi D, Coutts SB, Gould B, McCourt R, Asdaghi N, Findlay JM, Emery D, Shuaib A. Investigators IA. The Intracerebral Hemorrhage Acutely Decreasing Arterial Pressure Trial. Stroke; a journal of cerebral circulation. 2013; 44:620–626.

Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. Nature reviews Immunology. 2010; 10:826–837.

Chen Q, Zhang J, Guo J, Tang J, Tao Y, Li L, Feng H, Chen Z. Chronic Hydrocephalus and Perihematomal Tissue Injury Developed in a Rat Model of Intracerebral Hemorrhage with Ventricular Extension. Translational stroke research. 2014

Chen S, Yang Q, Chen G, Zhang JH. An update on inflammation in the acute phase of intracerebral hemorrhage. Translational stroke research. 2015; 6:4–8. [PubMed: 25533878]

Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25− naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. The Journal of experimental medicine. 2003; 198:1875–1886. [PubMed: 14676299]

Cheng Y, Xi G, Jin H, Keep RF, Feng J, Hua Y. Thrombin-induced cerebral hemorrhage: role of protease-activated receptor-1. Translational stroke research. 2014; 5:472–475. [PubMed: 24323711]

Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. Journal of neuroinflammation. 2014; 11:98. [PubMed: 24889886]

Chinetti-Gbaguidi G, Colin S, Staels B. Macrophage subsets in atherosclerosis. Nature reviews Cardiology. 2015; 12:10–17.

Correale J, Villa A. The neuroprotective role of inflammation in nervous system injuries. Journal of neurology. 2004; 251:1304–1316. [PubMed: 15592725]

Davis SM, Broderick J, Hennerici M, Brun NC, Diringer MN, Mayer SA, Begtrup K, Steiner T. Recombinant Activated Factor VIIIHTI . Hematoma growth is a determinant of mortality and poor outcome after intracerebral hemorrhage. Neurology. 2006; 66:1175–1181. [PubMed: 16636233]

Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nature immunology. 2007; 8:1086–1094. [PubMed: 17873879]

Diringer MN, Skolnick BE, Mayer SA, Steiner T, Davis SM, Brun NC, Broderick JP. Thromboembolic events with recombinant activated factor VII in spontaneous intracerebral hemorrhage: results from the Factor Seven for Acute Hemorrhagic Stroke (FAST) trial. Stroke; a journal of cerebral circulation. 2010; 41:48–53.

Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nature reviews Immunology. 2008; 8:337–348.

Dong C. Genetic controls of Th17 cell differentiation and plasticity. Experimental & molecular medicine. 2011; 43:1–6. [PubMed: 21270506]

Dowlatshahi D, Demchuk AM, Flaherty ML, Ali M, Lyden PL, Smith EE, Collaboration V. Defining hematoma expansion in intracerebral hemorrhage: relationship with patient outcomes. Neurology. 2011; 76:1238–1244. [PubMed: 21346218]

Ducruet AF, Zacharia BE, Hickman ZL, Grobelny BT, Yeh ML, Sosunov SA, Connolly ES Jr. The complement cascade as a therapeutic target in intracerebral hemorrhage. Experimental neurology. 2009; 219:398–403. [PubMed: 19632224]

Eggen BJ, Raj D, Hanisch UK, Boddeke HW. Microglial phenotype and adaptation. Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology. 2013; 8:807–823. [PubMed: 23881706]

Fang H, Wang PF, Zhou Y, Wang YC, Yang QW. Toll-like receptor 4 signaling in intracerebral hemorrhage-induced inflammation and injury. Journal of neuroinflammation. 2013; 10:27. [PubMed: 23414417]

Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. Journal of immunology. 1991; 146:3444–3451.

Fu Y, Hao J, Zhang N, Ren L, Sun N, Li YJ, Yan Y, Huang D, Yu C, Shi FD. Fingolimod for the treatment of intracerebral hemorrhage: a 2-arm proof-of-concept study. JAMA neurology. 2014; 71:1092–1101. [PubMed: 25003359]

Gao C, Du H, Hua Y, Keep RF, Strahle J, Xi G. Role of red blood cell lysis and iron in hydrocephalus after intraventricular hemorrhage. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2014a; 34:1070–1075.

Gao L, Lu Q, Huang LJ, Ruan LH, Yang JJ, Huang WL, ZhuGe WS, Zhang YL, Fu B, Jin KL, ZhuGe QC. Transplanted neural stem cells modulate regulatory T, gammadelta T cells and corresponding cytokines after intracerebral hemorrhage in rats. International journal of molecular sciences. 2014b; 15:4431–4441. [PubMed: 24633197]

Giunti D, Parodi B, Cordano C, Uccelli A, Kerlero de Rosbo N. Can we switch microglia's phenotype to foster neuroprotection? Focus on multiple sclerosis. Immunology. 2014; 141:328–339. [PubMed: 24116890]

Gonzales NR, et al. Design of a prospective, dose-escalation study evaluating the Safety of Pioglitazone for Hematoma Resolution in Intracerebral Hemorrhage (SHRINC). International journal of stroke : official journal of the International Stroke Society. 2013; 8:388–396. [PubMed: 22340518]

Gu L, Xiong X, Zhang H, Xu B, Steinberg GK, Zhao H. Distinctive effects of T cell subsets in neuronal injury induced by cocultured splenocytes in vitro and by in vivo stroke in mice. Stroke; a journal of cerebral circulation. 2012; 43:1941–1946.

Guo FQ, Li XJ, Chen LY, Yang H, Dai HY, Wei YS, Huang YL, Yang YS, Sun HB, Xu YC, Yang ZL. Study of relationship between inflammatory response and apoptosis in perihematoma region in patients with intracerebral hemorrhage. Zhongguo wei zhong bing ji jiu yi xue = Chinese critical care medicine = Zhongguo weizhongbing jijiuyixue. 2006; 18:290–293. [PubMed: 16700995]

Hirota K, Martin B, Veldhoen M. Development, regulation and functional capacities of Th17 cells. Seminars in immunopathology. 2010; 32:3–16. [PubMed: 20107806]

Hohlfeld R, Kerschensteiner M, Stadelmann C, Lassmann H, Wekerle H. The neuroprotective effect of inflammation: implications for the therapy of multiple sclerosis. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 2006; 27(Suppl 1):S1–7.

Hosaka K, Hoh BL. Inflammation and cerebral aneurysms. Translational stroke research. 2014; 5:190–198. [PubMed: 24323732]

Hua Y, Xi G, Keep RF, Hoff JT. Complement activation in the brain after experimental intracerebral hemorrhage. Journal of neurosurgery. 2000; 92:1016–1022. [PubMed: 10839264]

Hua Y, Keep RF, Hoff JT, Xi G. Brain injury after intracerebral hemorrhage: the role of thrombin and iron. Stroke; a journal of cerebral circulation. 2007; 38:759–762.

Keep RF, Hua Y, Xi G. Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. The Lancet Neurology. 2012; 11:720–731. [PubMed: 22698888]

Keep RF, Xi G, Hua Y, Hoff JT. The deleterious or beneficial effects of different agents in intracerebral hemorrhage: think big, think small, or is hematoma size important? Stroke; a journal of cerebral circulation. 2005; 36:1594–1596.

Klebe D, Krafft PR, Hoffmann C, Lekic T, Flores JJ, Rolland W, Zhang JH. Acute and delayed deferoxamine treatment attenuates long-term sequelae after germinal matrix hemorrhage in neonatal rats. Stroke; a journal of cerebral circulation. 2014; 45:2475–2479.

Kleinewietfeld M, Hafler DA. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Seminars in immunology. 2013; 25:305–312. [PubMed: 24211039]

Koga M, Arihiro S, Hasegawa Y, Shiokawa Y, Okada Y, Kimura K, Furui E, Nakagawara J, Yamagami H, Kario K, Okuda S, Tokunaga K, Takizawa H, Takasugi J, Sato S, Nagatsuka K, Minematsu K, Toyoda K. Stroke Acute Management with Urgent Risk-factor A, Improvement Study I. Intravenous nicardipine dosing for blood pressure lowering in acute intracerebral hemorrhage: the Stroke Acute Management with Urgent Risk-factor Assessment and Improvement-Intracerebral Hemorrhage study. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2014; 23:2780–2787. [PubMed: 25314943]

Kohler E, Prentice DA, Bates TR, Hankey GJ, Claxton A, van Heerden J, Blacker D. Intravenous minocycline in acute stroke: a randomized, controlled pilot study and meta-analysis. Stroke; a journal of cerebral circulation. 2013; 44:2493–2499.

Kreitzer N, Adeoye O. An update on surgical and medical management strategies for intracerebral hemorrhage. Seminars in neurology. 2013; 33:462–467. [PubMed: 24504609]

Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity. 2007; 26:579–591. [PubMed: 17481928]

Lu Q, Gao L, Huang L, Ruan L, Yang J, Huang W, Li Z, Zhang Y, Jin K, Zhuge Q. Inhibition of mammalian target of rapamycin improves neurobehavioral deficit and modulates immune response after intracerebral hemorrhage in rat. Journal of neuroinflammation. 2014; 11:44. [PubMed: 24602288]

Luckheeram RV, Zhou R, Verma AD, Xia B. CD4(+)T cells: differentiation and functions. Clinical & developmental immunology. 2012; 2012:925135. [PubMed: 22474485]

Mantel PY, Schmidt-Weber CB. Transforming growth factor-beta: recent advances on its role in immune tolerance. Methods in molecular biology. 2011; 677:303–338. [PubMed: 20941619]

Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime reports. 2014; 6:13. [PubMed: 24669294]

Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annual review of immunology. 2009; 27:451–483.

Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Frontiers in bioscience : a journal and virtual library. 2008; 13:453–461. [PubMed: 17981560]

Mayer SA, Brun NC, Broderick J, Davis SM, Diringer MN, Skolnick BE, Steiner T. United States NovoSeven ICHTI . Recombinant activated factor VII for acute intracerebral hemorrhage: US phase IIA trial. Neurocritical care. 2006; 4:206–214. [PubMed: 16757825]

Mayer SA, Brun NC, Begtrup K, Broderick J, Davis S, Diringer MN, Skolnick BE, Steiner T. Investigators FT. Efficacy and safety of recombinant activated factor VII for acute intracerebral hemorrhage. The New England journal of medicine. 2008; 358:2127–2137. [PubMed: 18480205]

McCombe PA, Read SJ. Immune and inflammatory responses to stroke: good or bad? International journal of stroke : official journal of the International Stroke Society. 2008; 3:254–265. [PubMed: 18811742]

Mendelow AD, Gregson BA, Rowan EN, Murray GD, Gholkar A, Mitchell PM. Investigators SI. 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]

Mendelow AD, Gregson BA, Fernandes HM, Murray GD, Teasdale GM, Hope DT, Karimi A, Shaw MD, Barer DH. investigators S. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial intracerebral haematomas in the International Surgical Trial in Intracerebral Haemorrhage (STICH): a randomised trial. Lancet. 2005; 365:387–397. [PubMed: 15680453]

Meng H, Li F, Hu R, Yuan Y, Gong G, Hu S, Feng H. Deferoxamine alleviates chronic hydrocephalus after intraventricular hemorrhage through iron chelation and Wnt1/Wnt3a inhibition. Brain research. 2014

Miller CM, Vespa P, Saver JL, Kidwell CS, Carmichael ST, Alger J, Frazee J, Starkman S, Liebeskind D, Nenov V, Elashoff R, Martin N. Image-guided endoscopic evacuation of spontaneous intracerebral hemorrhage. Surgical neurology. 2008; 69:441–446. discussion 446. [PubMed: 18424298]

Morgenstern LB, Frankowski RF, Shedden P, Pasteur W, Grotta JC. Surgical treatment for intracerebral hemorrhage (STICH): a single-center, randomized clinical trial. Neurology. 1998; 51:1359–1363. [PubMed: 9818860]

Morgenstern LB, Hemphill JC 3rd, Anderson C, Becker K, Broderick JP, Connolly ES Jr, Greenberg SM, Huang JN, MacDonald RL, Messe SR, Mitchell PH, Selim M, Tamargo RJ. American Heart Association Stroke C, Council on Cardiovascular N. Guidelines for the management of spontaneous intracerebral hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke; a journal of cerebral circulation. 2010; 41:2108–2129.

Mracsko E, Veltkamp R. Neuroinflammation after intracerebral hemorrhage. Frontiers in cellular neuroscience. 2014; 8:388. [PubMed: 25477782]

Mracsko E, Javidi E, Na SY, Kahn A, Liesz A, Veltkamp R. Leukocyte invasion of the brain after experimental intracerebral hemorrhage in mice. Stroke; a journal of cerebral circulation. 2014; 45:2107–2114.

Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nature reviews Immunology. 2011; 11:723–737.

Nakamura T, Keep RF, Hua Y, Schallert T, Hoff JT, Xi G. Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. Journal of neurosurgery. 2004; 100:672–678. [PubMed: 15070122]

Orme J, Mohan C. Macrophage subpopulations in systemic lupus erythematosus. Discovery medicine. 2012; 13:151–158. [PubMed: 22369974]

Pandey AS, Xi G. Intracerebral hemorrhage: a multimodality approach to improving outcome. Translational stroke research. 2014; 5:313–315. [PubMed: 24764218]

Peck A, Mellins ED. Plasticity of T-cell phenotype and function: the T helper type 17 example. Immunology. 2010; 129:147–153. [PubMed: 19922424]

Pisanu A, Lecca D, Mulas G, Wardas J, Simbula G, Spiga S, Carta AR. Dynamic changes in pro- and anti-inflammatory cytokines in microglia after PPAR-gamma agonist neuroprotective treatment in the MPTPp mouse model of progressive Parkinson's disease. Neurobiology of disease. 2014; 71:280–291. [PubMed: 25134730]

Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. Lancet. 2009; 373:1632–1644. [PubMed: 19427958]

Rincon F, Friedman DP, Bell R, Mayer SA, Bray PF. Targeted temperature management after intracerebral hemorrhage (TTM-ICH): methodology of a prospective randomized clinical trial. International journal of stroke : official journal of the International Stroke Society. 2014; 9:646–651. [PubMed: 24450819]

Rolland WB, Lekic T, Krafft PR, Hasegawa Y, Altay O, Hartman R, Ostrowski R, Manaenko A, Tang J, Zhang JH. Fingolimod reduces cerebral lymphocyte infiltration in experimental models of rodent intracerebral hemorrhage. Experimental neurology. 2013; 241:45–55. [PubMed: 23261767]

Rolland WB 2nd, Manaenko A, Lekic T, Hasegawa Y, Ostrowski R, Tang J, Zhang JH. FTY720 is neuroprotective and improves functional outcomes after intracerebral hemorrhage in mice. Acta neurochirurgica Supplement. 2011; 111:213–217. [PubMed: 21725758]

Romagnani S. Th1/Th2 cells. Inflammatory bowel diseases. 1999; 5:285–294. [PubMed: 10579123]

Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. Nature reviews Immunology. 2011; 11:775–787.

Sakamoto Y, Koga M, Yamagami H, Okuda S, Okada Y, Kimura K, Shiokawa Y, Nakagawara J, Furui E, Hasegawa Y, Kario K, Arihiro S, Sato S, Kobayashi J, Tanaka E, Nagatsuka K, Minematsu K, Toyoda K. Investigators SS. Systolic blood pressure after intravenous

antihypertensive treatment and clinical outcomes in hyperacute intracerebral hemorrhage: the stroke acute management with urgent risk-factor assessment and improvement-intracerebral hemorrhage study. Stroke; a journal of cerebral circulation. 2013; 44:1846–1851.

Savage ND, de Boer T, Walburg KV, Joosten SA, van Meijgaarden K, Geluk A, Ottenhoff TH. Human anti-inflammatory macrophages induce Foxp3+ GITR+ CD25+ regulatory T cells, which suppress via membrane-bound TGFbeta-1. Journal of immunology. 2008; 181:2220–2226.

Seifert HA, Pennypacker KR. Molecular and cellular immune responses to ischemic brain injury. Translational stroke research. 2014; 5:543–553. [PubMed: 24895236]

Starke RM, Raper DM, Ding D, Chalouhi N, Owens GK, Hasan DM, Medel R, Dumont AS. Tumor necrosis factor-alpha modulates cerebral aneurysm formation and rupture. Translational stroke research. 2014; 5:269–277. [PubMed: 24323710]

Staykov D, Wagner I, Volbers B, Hauer EM, Doerfler A, Schwab S, Bardutzky J. Natural course of perihemorrhagic edema after intracerebral hemorrhage. Stroke; a journal of cerebral circulation. 2011; 42:2625–2629.

Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Current opinion in immunology. 2007; 19:281–286. [PubMed: 17433650]

Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. Journal of leukocyte biology. 2004; 76:509–513. [PubMed: 15218057]

Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. Journal of immunology. 2005; 175:342–349.

Tapia-Perez H, Sanchez-Aguilar M, Torres-Corzo JG, Rodriguez-Leyva I, Gonzalez-Aguirre D, Gordillo-Moscoso A, Chalita-Williams C. Use of statins for the treatment of spontaneous intracerebral hemorrhage: results of a pilot study. Central European neurosurgery. 2009; 70:15–20. [PubMed: 19197830]

Theodorou GL, Marousi S, Ellul J, Mougiou A, Theodori E, Mouzaki A, Karakantza M. T helper 1 (Th1)/Th2 cytokine expression shift of peripheral blood CD4+ and CD8+ T cells in patients at the post-acute phase of stroke. Clinical and experimental immunology. 2008; 152:456–463. [PubMed: 18422734]

Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. Neurosurgical focus. 2007; 22:E6. [PubMed: 17613237]

van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. The Lancet Neurology. 2010; 9:167–176. [PubMed: 20056489]

Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nature reviews Immunology. 2008; 8:523–532.

Wang J. Preclinical and clinical research on inflammation after intracerebral hemorrhage. Progress in neurobiology. 2010; 92:463–477. [PubMed: 20713126]

Wang J, Dore S. Inflammation after intracerebral hemorrhage. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2007; 27:894–908.

Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Frontiers in immunology. 2014; 5:614. [PubMed: 25506346]

Wang WZ, Jiang B, Liu HM, Li D, Lu CZ, Zhao YD, Sander JW. Minimally invasive craniopuncture therapy vs. conservative treatment for spontaneous intracerebral hemorrhage: results from a randomized clinical trial in China. International journal of stroke : official journal of the International Stroke Society. 2009; 4:11–16. [PubMed: 19236490]

Wee Yong V. Inflammation in neurological disorders: a help or a hindrance? The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry. 2010; 16:408–420.

Wu G, Xi G, Huang F. Spontaneous intracerebral hemorrhage in humans: hematoma enlargement, clot lysis, and brain edema. Acta neurochirurgica Supplement. 2006; 96:78–80. [PubMed: 16671430]

Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. The Lancet Neurology. 2006; 5:53–63. [PubMed: 16361023]

Xiong XY, Wang J, Qian ZM, Yang QW. Iron and intracerebral hemorrhage: from mechanism to translation. Translational stroke research. 2014; 5:429–441. [PubMed: 24362931]

Xu L, Kitani A, Fuss I, Strober W. Cutting edge: regulatory T cells induce CD4+CD25-Foxp3− T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. Journal of immunology. 2007; 178:6725–6729.

Yang Z, Yu A, Liu Y, Shen H, Lin C, Lin L, Wang S, Yuan B. Regulatory T cells inhibit microglia activation and protect against inflammatory injury in intracerebral hemorrhage. International immunopharmacology. 2014; 22:522–525. [PubMed: 25000335]

Yoshimura A, Muto G. TGF-beta function in immune suppression. Current topics in microbiology and immunology. 2011; 350:127–147. [PubMed: 20680806]

Zambrano-Zaragoza JF, Romo-Martinez EJ, de Duran-Avelar MJ, Garcia-Magallanes N, Vibanco-Perez N. Th17 cells in autoimmune and infectious diseases. International journal of inflammation. 2014; 2014:651503. [PubMed: 25152827]

Zhao J, Chen Z, Xi G, Keep RF, Hua Y. Deferoxamine attenuates acute hydrocephalus after traumatic brain injury in rats. Translational stroke research. 2014a; 5:586–594. [PubMed: 24935175]

Zhao X, Grotta J, Gonzales N, Aronowski J. Hematoma resolution as a therapeutic target: the role of microglia/macrophages. Stroke; a journal of cerebral circulation. 2009; 40:S92–94.

Zhao X, Sun G, Zhang H, Ting SM, Song S, Gonzales N, Aronowski J. Polymorphonuclear neutrophil in brain parenchyma after experimental intracerebral hemorrhage. Translational stroke research. 2014b; 5:554–561. [PubMed: 24696130]

Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, Grotta JC, Aronowski J. Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. Annals of neurology. 2007; 61:352–362. [PubMed: 17457822]

Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009; 30:646–655. [PubMed: 19464987]

Zhou Y, Wang Y, Wang J, Anne Stetler R, Yang QW. Inflammation in intracerebral hemorrhage: from mechanisms to clinical translation. Progress in neurobiology. 2014; 115:25–44. [PubMed: 24291544]

Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood. 2008; 112:1557–1569. [PubMed: 18725574]

Zhu J, Paul WE. Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. Immunological reviews. 2010; 238:247–262. [PubMed: 20969597]

Table 1

Macrophage/Microglia and T-helper Lymphocyte Subtypes

Cell Types	Activating Cytokines	Markers	Secreted Cytokines	Mechanisms of action	References
M1	IFN-γ, TNF-α, LPS	CD86, CD80, MHC II↑, IL-1R I, TLR2, TLR4, iNOS	TNF-α, IL-12, and IL-23	Inhibits cell proliferation and causes tissue damage	(Martinez et al., 2009; Chen and Nunez, 2010; Cherry et al., 2014; Martinez and Gordon, 2014; Wang et al., 2014)
M2	IL-4, IL-13	CD163, MHC II, SR, CD206↑, (MR↑), TGM2↑, DecoyR, IL-1R II, Ym1, Fizz1, Arg-1	IL-10, TGF-β, and IL-1RA	Promotes cell proliferation and tissue repair	(Martinez et al., 2009; Murray and Wynn, 2011; Saijo and Glass, 2011; Cherry et al., 2014; Martinez and Gordon, 2014; Wang et al., 2014)
Th1	IL-12, IL-18	CD4, CXCR3, CCR5	IFN-γ, IL-2, and TNF-α	Activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses	(Romagnani, 1999; Zhu and Paul, 2008; Luckheeram et al., 2012)
Th2	IL-4	CRTH2, CCR3, CCR4	IL-4, IL-5, IL-10, and IL-13	Are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses	(Romagnani, 1999; Zhu and Paul, 2008; Luckheeram et al., 2012)
Th17	IL-2, IL-6, TGF-β, IL-1β, IL-23	CD4, CCR4, CCR6	IL-17A/F, IL-21, IL-22, CCR6 and ROR γ/γt	Creates inflammation and tissue injury in autoimmune diseases	(Stockinger and Veldhoen, 2007; Dong, 2008; Hirota et al., 2010; Peck and Mellins, 2010; Dong, 2011; Zambrano-Zaragoza et al., 2014)
Treg	IL-2, TGF-β	CD4, CD25, and Foxp3	TGF-β, IL-10, and IL-35	Are essential for maintaining peripheral tolerance, preventing autoimmune diseases and limiting chronic inflammatory diseases	(Chen et al., 2003; Vignali et al., 2008; Mantel and Schmidt-Weber, 2011; Yoshimura and Muto, 2011)

Table 2

Current and Past Clinical Trials Evaluating Immunomodulatory Therapies

Hemorrhage type	Agent	Trial	Phase	Status	References
Supratentorial Intracerebral Hemorrhage	Prophylactic forced normothermia	Systemic Normothermia in Intracerebral Hemorrhage (ICH) (SNICH)	0	Not Yet Recruiting	(Rincon et al., 2014)
Intracerebral Hemorrhage	Labetalol/Hydralazine/Enalapril	The Intracerebral Hemorrhage Acutely Decreasing Arterial Pressure Trial II (ICH-ADAPT II)	2	Recruiting	(Butcher et al., 2013)
Intracerebral Hemorrhage	Pioglitazone	Safety of Pioglitazone for Hematoma Resolution In Intracerebral Hemorrhage (SHRINC)	2	Ongoing	(Gonzales et al., 2013)
Intracerebral Hemorrhage	Minocycline	A Pilot Study of Minocycline in Intracerebral Hemorrhage Patients (MACH)	2	Recruiting	(Kohler et al., 2013)
Intracerebral Hemorrhage	Rosuvastatin	Effect of Rosuvastatin in Intracerebral Hemorrhage	2	Complete	(Tapia-Perez et al., 2009)
Intracerebral hemorrhage	Nicardipine	IV Double and Triple Concentrated Nicardipine for Stroke and ICH	4	Unknown	(Koga et al., 2014)