HHS Public Access

Author manuscript

Pharmacol Res. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Pharmacol Res. 2019 September ; 147: 104349. doi:10.1016/j.phrs.2019.104349.

Inflammation within the Neurovascular Unit: Focus on Microglia for Stroke Injury and Recovery

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Abstract

Neuroinflammation underlies the etiology of multiple neurodegenerative diseases and stroke. Our understanding of neuroinflammation has evolved in the last few years and major players have been identified. Microglia, the brain resident macrophages, are considered sentinels at the forefront of the neuroinflammatory response to different brain insults. Interestingly, microglia perform other physiological functions in addition to their role in neuroinflammation. Therefore, an updated approach in which modulation, rather than complete elimination of microglia is necessary. In this review, the emerging roles of microglia and their interaction with different components of the neurovascular unit are discussed. In addition, recent data on sex differences in microglial physiology and in the context of stroke will be presented. Finally, the multiplicity of roles assumed by microglia in the pathophysiology of ischemic stroke, and in the presence of co-morbidities such as hypertension and diabetes are summarized.

Graphical Abstract

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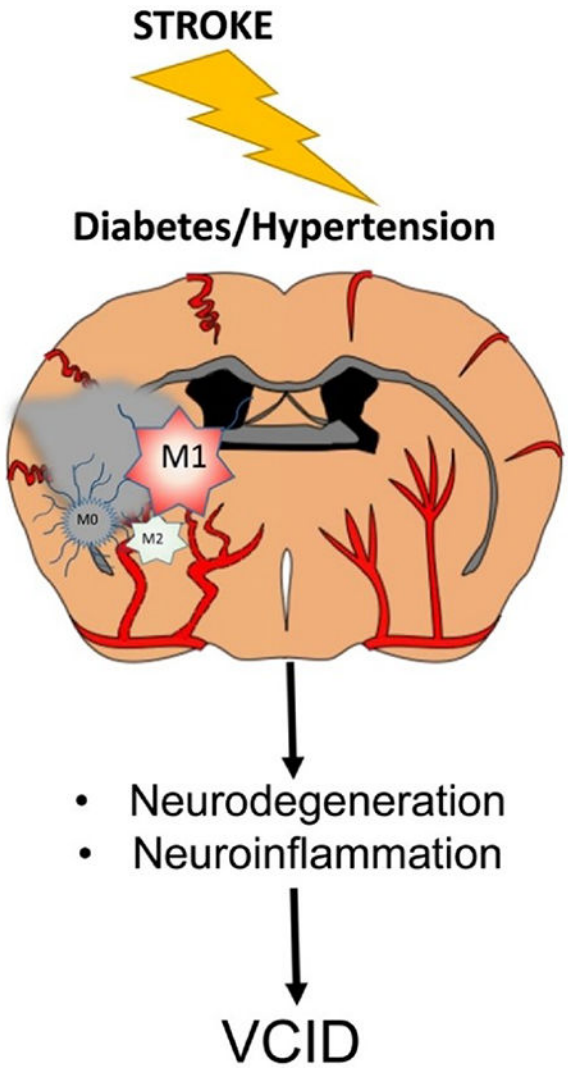
Declaration of Interests: none.



Introduction

Ischemic stroke is a leading cause of death and disability worldwide. Fortunately, recent advancements in acute stroke care including the use of tissue plasminogen activator and thrombectomy over a wider therapeutic window led to a decline in stroke mortality. Unfortunately, stroke has become a disease of survivors that suffer from functional and cognitive deficits and there is no approved treatment for stroke recovery. Thus, better understanding of the pathophysiology that restricts the endogenous reparative ability of the brain, offers potential to identify novel therapeutic targets. In this regard, emerging evidence has put neuroinflammation in the spotlight because, depending on the severity and the spatial and temporal regulation of pro and anti-inflammatory signals, neuroinflammation may play both protective and detrimental role(s) in stroke injury and recovery. There are also dynamic interactions between the innate and adaptive immune responses in both the central nervous system and the periphery, and this has been elegantly detailed in several recent review articles (1–3).

In preclinical models, post-stroke inflammation begins shortly after occlusion of a vessel and peaks in the first few days after stroke onset (4). Following an ischemic stroke, innate and adaptive cellular immune responses occur and influence stroke pathology (5). Major players of the innate immune responses after stroke are microglia, circulating monocytes, tissue macrophages, and neutrophils, while major players of the adaptive immune responses include B cells and T-cells such as CD4+ T-lymphocytes, CD8+ T-lymphocytes, γδ T cells and T regulatory cells (Tregs). In addition, dendritic cells serve as the bridge between innate and adaptive immune responses (6). Ischemic inflammation is characterized by the breakdown of the blood brain barrier (BBB), infiltration of peripheral leukocytes, activation of glial cells, and the release of damage-associated molecular patterns (DAMPs) from injured cells to trigger the activation of immune cells (7). The activated immune cells, in turn, release inflammatory cytokines and cytotoxic mediators leading to the exacerbation of the ischemic injury (7). Weeks to months after stroke onset, tissue remodeling and repair take place leading to the formation of a cavity which prevents full functional recovery (8, 9). As the first immune responders to ischemic injury, in this review we will focus mainly on the brain-resident microglial cells and refer the readers to review articles introduced above for more general neuroinflammation in stroke. We will first briefly introduce microglia morphology and physiology and then review the impact of hypertension and diabetes, major comorbidities of stroke, on microglial responses before and after a stroke in experimental models. Final discussion will focus on the relevance of the preclinical data to the failed anti-inflammatory strategies in stroke treatment and identify potential opportunities.

Microglia Biology

At embryonic day 9, yolk sac progenitors start to enter the central nervous system (CNS) before the closure of the blood brain barrier at embryonic day 13 in mice. Once these progenitors reside in the CNS, they are called microglia and self-renew for the entire lifespan of the animal (10, 11). Microglia constitute 5–10% of adult brain cells and they represent the largest population of immune cells in the brain (12). Under physiological conditions, microglia are located within brain parenchyma in contact with other brain cells such as neurons, astrocytes and oligodendrocytes (12). The microglial population is distributed in different regions in the brain ranging from 5% in the cerebral cortex and corpus callosum to 12% in the substantia nigra (13). This heterogeneity across different brain regions could stem in part from local cues produced by neural cells (14–17). In addition, microglia show heterogeneity according to age, sex, and species, and in neurological disorders such as stroke.

There is accumulating evidence that suggests that microglia could be involved in more than the response to brain injury (18). It is clear that microglia play a role in regulation of BBB (19), synaptic reorganization, postsynaptic spine formation, regulation of neurogenesis, and early wiring of neuronal circuits (12, 18, 20–23). In addition, there is evidence for a mutual interaction between microglia and astrocytes. It has been shown that microglia produce signaling molecules that regulate astrogenesis (24, 25) and maturation of astrocytes (25). In support of these data, a lag time has been described between the appearance of microglia and astrogenesis in human and mice (26–28). Astrocytes, in turn, secrete soluble factors that

increase proliferation of microglia (29, 30). A comprehensive summary of the roles of glia during brain development is reviewed by Reemst et al (25).

The BBB is a dynamic and metabolic interface that separates the CNS from the periphery and regulates the trafficking of solutes, fluid and cells, playing a crucial role in the maintenance of CNS homeostasis. The BBB is formed and stabilized by components of the neurovascular unit (NVU), which is composed of endothelial cells, astrocytes, pericytes, neurons and extracellular matrix (ECM) (19). While microglia were not traditionally considered part of the NVU, evolving NVU concept now incorporates microglia and peripheral macrophage as they certainly participate in important processes regulated by the NVU (31). Microglia regulate the BBB during development and after injury (19). Also, microglia play an important role in the stabilization and fusion of brain endothelial cells and in the sprouting, migration, and anastomosis of the cerebral and retinal vasculature during development (32, 33). There is evidence from *ex vivo* studies that microglia secrete soluble factors that can stimulate vessel sprouting without direct contact with endothelial cells (34). The loss of structural integrity and normal function of the BBB takes place in neurological disorders such as stroke (35). While it has been suggested that the opening of the BBB can be exploited therapeutically to deliver drugs that otherwise do not cross the BBB (36, 37), some studies have shown that normal diffusion of drugs into the CNS can be prevented by the accumulation of blood-derived debris and cells into enlarged perivascular spaces after BBB breakdown (38).

Microglia Phenotypes and Neuroinflammation after Stroke

Microglia develop a spectrum of functional phenotypes with different effects on neuroinflammation (Fig. 1). These phenotypes are traditionally classified as surveillance (M0), pro-inflammatory (M1) and anti-inflammatory (M2) modes and dynamically change from one mode to another (31, 39, 40).

At homeostasis, microglia are considered to be in a surveillance mode and classified as quiescent microglia (M0). These quiescent microglia are characterized by a ramified morphological phenotype with low phagocytic properties (41). However, as highlighted in a recent review, even surveillance microglia are in a very dynamic state, constantly changing their ramified processes to continuously monitor the brain parenchyma (31, 42). The mediators and markers of M1 and M2 microglia phenotypes in the context of ischemic stroke are discussed below.

The role of microglia in the acute phase of neuroinflammation after stroke

At the early stages after stroke, resident microglia and recruited macrophages assume an M2 phenotype. Although counterintuitive, this M2 phenotype is needed to remove cell debris and curb brain damage (43, 44). Following that, a proinflammatory phenotype starts to dominate in the peri-infarct area (43). This proinflammatory phenotype, known as M1 or classically activated phenotype with an ameboid morphology, is induced by stimulation with IFN-γ and LPS in addition to other factors (40, 45). The M1 phenotype is identified by a number of markers (CD16, inducible nitric oxide synthase-iNOS and tumor necrosis factor α), and secretes a number of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α,

NO, and the proteolytic enzymes matrix metalloproteinases 3 and 9 (41, 46–48). This leads to the disruption of BBB and degradation of ECM (49) resulting in the infiltration of peripheral leukocytes and plasma-derived factors such as plasma-fibronectin and fibrinogen into the brain tissue (50). All these events lead to the exacerbation of the ischemic injury (7).

The activated form of microglia can also be beneficial, however, through the phagocytosis of cellular debris and secretion of beneficial neutrotrophic factors (40). In a neonatal stroke model, depletion of microglia exacerbated vascular permeability and increased intracerebral hemorrhage (51). This indicates that modulation of microglial function rather than inhibiting microglia altogether could be a viable therapeutic approach.

In addition to resident microglia, other myeloid cells, for example, bone marrow-derived monocytes, are recruited to the CNS from the periphery after stroke. These recruited peripheral cells have similar morphology and express similar surface markers as resident microglia (6, 52). Yet, infiltrating macrophages and resident microglia show distinct gene signatures and different temporal contributions to brain injury (53). It has been shown that infiltrating monocytes can either potentiate stroke damage or differentiate into microglia-like cells to participate in post-stroke repair processes (53–55). Taken together, the role of infiltrating macrophages during ischemic stroke is controversial. One reason for the difficulty in delineating the contribution of resident microglia compared to infiltrating macrophages is the difficulty in differentiating the two cell populations (6). The recent discovery of the resident microglia pool-specific marker, transmembrane protein 119 (TMEM119), has helped in differentiating resident microglia from other myeloid cells (52). For example, after stroke in humans and using TMEM119 as a biomarker, a substantial proportion of macrophage-like cells in the lesion has been shown to be derived from the original microglia pool (56).

The role of microglia in the delayed phase of neuroinflammation after stroke

Processes such as BBB repair, neurogenesis and angiogenesis are important for functional recovery after stroke (4). Microglia can either switch to an anti-inflammatory and neuroprotective M2-like phenotype to promote a tissue repair mechanism (57) or stay in sustained M1-like phenotype aggravating injury and impeding repair.

The alternatively activated phenotype, known as the M2 anti-inflammatory phenotype, is further divided into three subtypes, M2a, M2b and M2c, according to function and stimulating factors (40, 43, 58–60). The M2a subtype is induced by stimulation with IL-4 and IL-13 and express Arginase-1, Ym1, CD206, Fizz1 and IGF-1 (59, 61). The M2b phenotype is categorized as immunomodulatory microglia. While counterintuitive, it can induced by LPS and IL-1R agonists and express CD86 and SOCS3 (40, 58, 61). The M2c subtype, referred as deactivated microglia, is induced by TGFβ, IL-10 and glucocorticoids. Phenotypic markers include SOCS3, chemokine CXCL 13 and surface receptor (SR-A1) (40). It is involved in tissue regeneration (58, 61,62). In contrast to M1 microglia, M2 microglia enhance neuronal survival *in vivo* and *in vitro* (63).

Microglia influence neurogenesis differently under different physiologic/pathologic conditions and according to different observation time points (64). In neonatal mice,

minocycline inhibits microglial function and at the same time reduces neurogenesis in the subventricular zone (SVZ) (65, 66). In aged mice, the number of microglia increases, and the removal of microglia reduces the activity of neural progenitor cells (67). After middle cerebral artery occlusion (MCAO), treatment with minocycline for 4 weeks enhances neurogenesis in the dentate gyrus (68). In another study, however, minocycline administered after MCAO reduced the number of neuroblasts and neurogenesis in the SVZ at 4 and 7 days respectively (69). These results indicate a biphasic role for microglia on neurogenesis in vivo (64, 70).

Sex Differences in Microglia Biology

Under physiological conditions, there is difference between male- and female-derived microglia at the transcriptomic and proteomic levels. This gene signature is maintained in culture and after transplant into mice of opposite sex and is not dependent on circulating hormones, because ovariectomy did not affect these observed gene signatures (71). Also, these differences have functional consequences. Microglia density and soma size are higher in the hippocampus, cortex and amygdala in 13-week-old male mice compared to age matched female mice. In contrast, the number of microglia in female Sprague-Dawley rats are higher than in males (72). This indicates that microglial density varies according to species and sex. Naive male microglia show higher antigen presenting capacity as evidenced by higher major histocompatibility complex (MHC) class I and II in the cortex. In addition, the proteomic analysis of male-derived microglia indicates higher responsiveness to immunological stimuli and higher motility capacity (73).

Sex differences in microglial functions after stroke

In the context of ischemic stroke, female microglia transplanted in male mice reduced ischemic damage indicating that there is an intrinsic sex-specific phenotype that is maintained in the absence of female hormones (71).

The ability of microglia to phagocytose endogenous structures and debris after ischemia, is mediated in part by the integrin receptor CD11b (41, 74). Females express constitutively higher levels of CD11b that remains unchanged after stroke. Whereas males express lower levels of CD11 b at baseline that significantly increases after stroke. It is suggested that the higher baseline expression of CD11b in females enhances the microglial capacity to remove the necrotic debris and ameliorate the neuronal injury after stroke (75).

Interestingly, microglia in the normal neonatal brain are active with an amoeboid morphology. In the preoptic area, there are twice as many active microglia in males as compared to females, while in the neonatal hippocampus, there are no sex differences in the number of amoeboid microglia (18, 76–78). However, in females, the hippocampus has more phagocytic microglia than that observed in male (79).

One protein that plays an important role in the regulation of inflammatory processes and immune response is S100a8, which is a TLR4-binding protein that regulates the expression of pro-inflammatory cytokines (64). Sex differences in S100a8 has been described in earlier studies. In male-derived microglia, S100a8 has been shown to be upregulated in whole brain

homogenate at homeostasis. Surprisingly, in another study, S100a8 has been shown to be upregulated at the mRNA level in female mice (80). It was suggested that differences in sample processing and microbiome could have contributed to these differences between the two studies (73).

Results from sex difference studies, however, should always be carefully interpreted. Different stages of the estrus cycle in females can affect the gene expression patterns (81). Differences in species, age, methods of isolation of different cell types and microbiome should be always be considered. In addition, it has been shown in some studies that microglia can rapidly lose epigenetic and transcriptional identity upon separation from their tissue environment (70, 82)

The impact of co-morbidities on microglia

It is recognized that one potential factor that may have contributed to the failure of successful translation of potential treatments identified in preclinical studies into clinical use is the limited use of comorbid disease models in stroke research. In this regard, hypertension and diabetes are leading contributors to the risk, severity and poor recovery of ischemic stroke. Both diseases are considered as states of chronic systemic and vascular inflammation. While the brain has been long considered an immune “privileged” organ, emerging evidence suggests that even in the absence of a brain injury, microglia sense and respond to this systemic inflammation. An intriguing example of this is the robust hippocampal microglial activation that is associated with cognitive dysfunction after a peripheral surgical intervention (83). This neuroinflammatory response is further aggravated in diabetic animals and depletion of microglia prevents the development of cognitive impairment. Thus, it is highly likely that hypertension and diabetes can directly prime the microglia through the systemic inflammatory state.

Both diseases can also indirectly influence neuroinflammation via regulation of cerebral blood flow (CBF). Diabetes and hypertension have profound effects on cerebrovascular function and structure resulting in dysregulation of autoregulatory and neurovascular coupling mechanisms (84–86) that are essential for continuous and “on demand” delivery of blood to the brain. It has been postulated that decreased CBF and a hypoxic milieu precedes development of overt symptoms of cognitive deficits in neurodegenerative diseases. As depicted in Fig 2, comorbid factors can initiate low grade systemic and vascular inflammation that causes early cerebrovascular dysfunction. Resultant decrease in CBF leads to microglial activation and neuroinflammation mediating neurodegeneration and ensuing cognitive deficits. An ischemic injury added to this pathology amplifies this vicious neuroinflammatory loop with microglia being a key player.

Diabetes and Microglia

Diabetes-mediated activation of microglia in the absence of an ischemic event has been reported in a number of studies. Drake et al. investigated microglial activation and neurovascular inflammation in the corpulent JCR:LA-cp (cp/cp) model of diabetes using an interesting positron emission tomography (PET) approach, as well as traditional

immunohistological methods (87). This model of diabetes presents with insulin resistance and obesity. As animals aged (12–15 months), there was a significant activation of microglia in the diabetic animals, as evidenced by increased uptake of the translocator protein TSPO radiotracer [18F]DPA-714. Augmented IBA-1 staining in the diabetic animals supported this finding. Vascular inflammation was evident by increased ICAM-1 and VCAM-1 staining in the cerebrovascular cross sections. The same study also reported increased microglial activation in diabetic individuals that did not have any intracranial pathology detected by MRI, collectively suggesting that diabetes can cause neuroinflammation in the absence of an ischemic event. While this particular study did not provide information with respect to different microglia phenotypes, a recent study showed that high glucose alone can increase M1/M2 ratio shifting isolated microglia to a proinflammatory phenotype. Furthermore, sham-operated diabetic animals exhibited lower M2-like microglia in the brain homogenates analyzed by flow cytometry (88).

As one would expect, an ischemic injury in the setting of diabetes further exacerbates deleterious microglial activation. In an earlier study, Hu and colleagues reported that in control animals an early beneficial M2 activation after stroke dynamically shifts to an M1 phenotype (43). A follow-up study by the same group recently expanded these findings and showed that in the db/db mouse model of type 2 diabetes, M2 microglia density is much lower and the increase in M1 microglia is much more robust. This increased activation of the deleterious microglia phenotype is associated with poor white matter integrity and worse functional outcomes in diabetic animals. Moreover, this increase in M1/M2 ratio under diabetic conditions suppresses oligodendrocyte precursor cell differentiation explaining the mechanistic basis of poor myelination while highlighting the importance of microglia phenotype in recovery. We have also shown that stroke caused by either suture or embolic occlusion of middle cerebral artery (MCAO) activates microglia to a greater extent in diabetic rats in a diet-induced model of diabetes and this effect is more pronounced in embolic stroke which causes greater hemorrhagic transformation (Fig 3) (89). While we did not use specific phenotypic markers, morphological studies showed increased cell body swelling, reduced number of microglial protrusions and summed process length in the hippocampus in both 60 min and embolic MCAO compared to control animals. Sensorimotor and cognitive deficits follow a similar pattern. In a follow-up study we reported that iron chelation by deferoxamine in the recovery period after ischemic stroke attenuates microglial activation and this is accompanied by improved functional outcomes (90). However, in these studies, microglial activation was based on morphology and microglia phenotype was not assessed by classical flow cytometry analyses.

Several other studies offer hope that detrimental microglial responses can be therapeutically modulated to improve outcomes. Exendin 4, a glucagon-like receptor 1 agonist that is clinically used for blood glucose control in diabetic patients, provides neuroprotection and improves outcomes by promoting M2 polarization even when treatment is started 4.5 h after stroke (91). We have evidence that stimulation of the Angiotensin II type 2 receptor (ATR2) by C21 improves M1/M2 ratio in favor of M2 microglia in diabetic animals, while increasing surveillance and M2 like microglia in control animals (92). Interestingly, an earlier study revealed that diabetic animals fail to launch an inflammatory response as evidenced by reduced inflammatory cytokine expression and microglial activation resulting

in impaired repair (93). The PPARγ agonist darglitazone improved outcomes by restoring the inflammatory response (94). However, it has to be acknowledged that this study employed a hypoxia-ischemia model in diabetic mice, and not a stroke model.

Hypertension and Microglia

Microglia may play a dual role in hypertension. Emerging evidence suggests that microglial activation contributes to the central regulation of blood pressure and development of hypertension as recently reviewed in two excellent review articles (31, 95). In this self-perpetuating cycle, the delicate balance of microglia phenotype can be tilted towards deleterious chronically activated M1 microglia.

Evidence for microglial activation in hypertension has been shown in different models of hypertension (96, 97). A recent study reported that chronic Angiotensin (Ang) II infusion in mice causes astrogliosis and hippocampal microglial activation and these are associated with parallel increases in TRPV4-mediated currents and Ca^{2+} events in astrocytes emphasizing the close communication between these cells (96). Central administration of a tetracycline derivative with potent anti-inflammatory activity (CMT-3) has been demonstrated to reduce microglial activation and attenuate increases in blood pressure in both spontaneously hypertensive rats (SHR) as well as in Ang II-induced hypertensive rats (97). We have shown that Ang II type 1 receptor (AT1R) blockade with candesartan or AT2R stimulation with Compound 21 prevents microglial activation and accompanying cognitive decline in aged SHRs (98). Moreover, Bhat and colleagues demonstrated that candesartan-mediated attenuation of microglial activation in SHRs is independent of its blood pressure lowering effects (99).

While limited, there is evidence for exacerbated microglial activation after stroke in the hypertensive setting. A 2001 study reported more quiescent and activated microglia in the stroke-prone spontaneously hypertensive rats (SHRSP) as compared to normotensive controls. Ischemic injury further increased activated microglia (100). Pires and colleagues showed that ischemic stroke in SHRSPs chronically treated with TNFα inhibitor etanercept caused greater infarct sizes that is characterized by reduced number of microglia and upregulation of the expression of M1 markers in the infarct core (101). Interestingly, another study reported reduced microglial activation after endothelin-1-induced stroke in SHRs (102). While microglial phenotype was not identified, microglia were reported to be dominating cell type in the ischemic hemisphere at Day 4 after permanent distal MCAO (103). A more recent report showed that estradiol-mediated neuroprotection observed in SHRs was independent of changes in microglial activation (104). Minocycline administration at reperfusion was reported to promote a protective microglial phenotype and improve recovery in the SHR model (105). While minocycline is a known microglia inhibitor, it also inhibits matrix metalloproteases (MMPs). This may be an indirect effect and may stem from inhibition of by MMPs. Our own studies also showed a robust microglial activation exacerbated by stroke in this model of hypertension. AT2R activation by C21 or AT1R blockade by candesartan, even when started at day 7 after stroke, reduced neuroinflammation and improved cognitive outcomes suggesting that modulation of the Ang II system may be a therapeutic target with high translational potential (106). Notably, all

these studies utilized the SHR or SHRSP models of hypertension. There is a need to investigate post-stroke recovery and microglial activation in other preclinical models of hypertension.

Microglia and Post-stroke Cognitive Impairment

Cognitive impairment is a common cause of disability after stroke, occurring in up to 60% of individuals and progressing to dementia in up to a third of its victims (107). Recent evidence from a large, population-based, epidemiological study in the United States revealed the chronically progressive nature of cognitive impairment, even in the absence of additional events (108). Post stroke cognitive impairment and vascular cognitive impairment, the leading diseases in the vascular contributions to cognitive impairment and dementia (VCID) spectrum of disorders, are the leading causes of Alzheimer's Disease Related Dementias (100).

In animal models of stroke, although recovery from the immediately obvious sensorimotor deficits occurs in the first week, cognitive impairment can appear later and may be progressive (106). This latter phenomenon has only recently been appreciated and can only be detected when animals are allowed to survive for weeks to months after stroke. The recognition of chronically progressive secondary neurodegeneration after stroke is not new, however, it was originally described in both animals and humans in areas remote from the infarct, including the thalamus (109, 110). More recently, this secondary neurodegeneration has been linked to neuroinflammation (111–113), in general, and microglial activation, in particular (114). This secondary neurodegeneration has been associated with development of post-stroke cognitive impairment (115) but whether interference with microglial activation is a viable therapeutic target in humans remains unknown (116).

Conclusions and Therapeutic Implications

Chronic microglial activation after stroke is a promising area of therapeutics research. In addition to the studies discussed above under diabetes and hypertension models, in preclinical models of secondary neurodegeneration, microglial modulation in favor of the M2 phenotype, with either osteopontin (117) or the CSF1R inhibitor, PLX3397 (118), has been shown to decrease tissue damage and improve functional outcomes, including cognition. In stroke patients, perispinal administration of the TNF inhibitor, etanercept, has been shown in case series to reduce functional impairment even years after stroke, by a mechanism involving reduction in microglial activation (119). Interfering with microglial function is to be undertaken carefully, however, due to an incomplete understanding of the biphasic nature of microglial response (70), the multiple involved phenotypes (39), and sex differences in microglia physiology/pathophysiology. Strategies to enhance the surveilling M0 properties as well as anti-inflammatory M2 phenotype need to be better developed. An interesting recent study by Xing and colleagues reported that while conditioned medium from endothelial cells exposed to oxygen glucose deprivation promoted an M1 like phenotype, conditioned medium from astrocytes mediated a protective/restorative M2 like microglial activation suggesting a gliovascular switch (120). Thus, given these complex interactions, we support the evaluation of microglia modulation in the context of the NVU.

Acknowledgments

This work was supported in part by a Veterans Affairs (VA) Merit Award (BX000347), VA Senior Research Career Scientist Award (IK6 BX004471) and National Institutes of Health (NIH) award (R01NS083559) to Adviye Ergul, and R01NS104573 multi-PI grant to Susan C. Fagan and Adviye Ergul.

Disclosures: Adviye Ergul is a Senior Research Career Scientist at the Ralph H. Johnson Veterans Affairs Medical Center in Charleston, SC. The contents do not represent the views of the Department of Veterans Affairs or the US Government.

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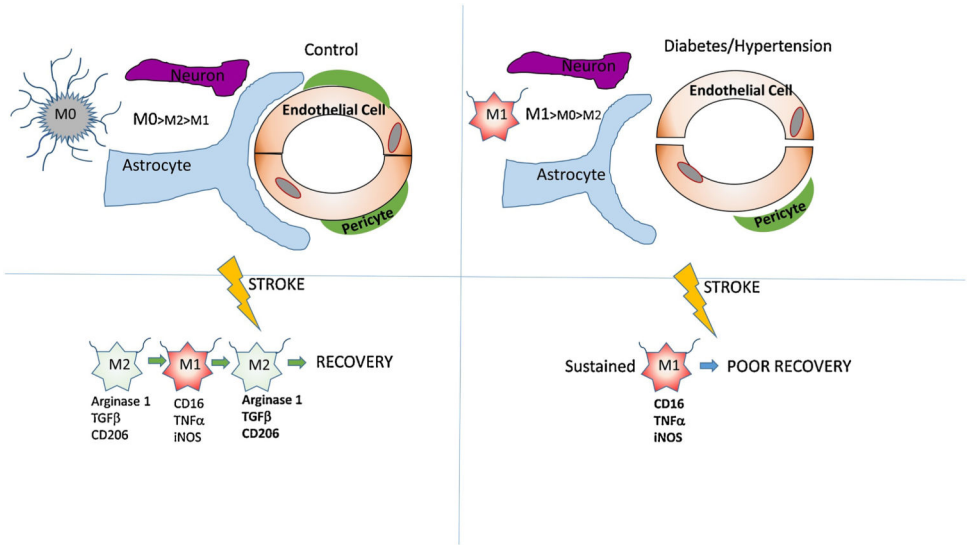

Figure 1.

A schematic model of the interaction between microglia and different components of the neurovascular unit in the absence and presence of comorbidities. In the left panel, microglia are in a resting state (M0) characterized by a higher number of process endpoints per cell that constantly probe the environment. After stroke, microglia transiently shift to the (M2) phenotype before shifting to (M1) pro-inflammatory phenotype. In the right panel, microglia are already primed for a pro-inflammatory phenotype in the presence of comorbidities and exhibit lower number of process endpoints per cell. After stroke, microglia shift directly to the (M1) phenotype and exhibit poorer recovery compared to microglia from animals with no comorbidities.

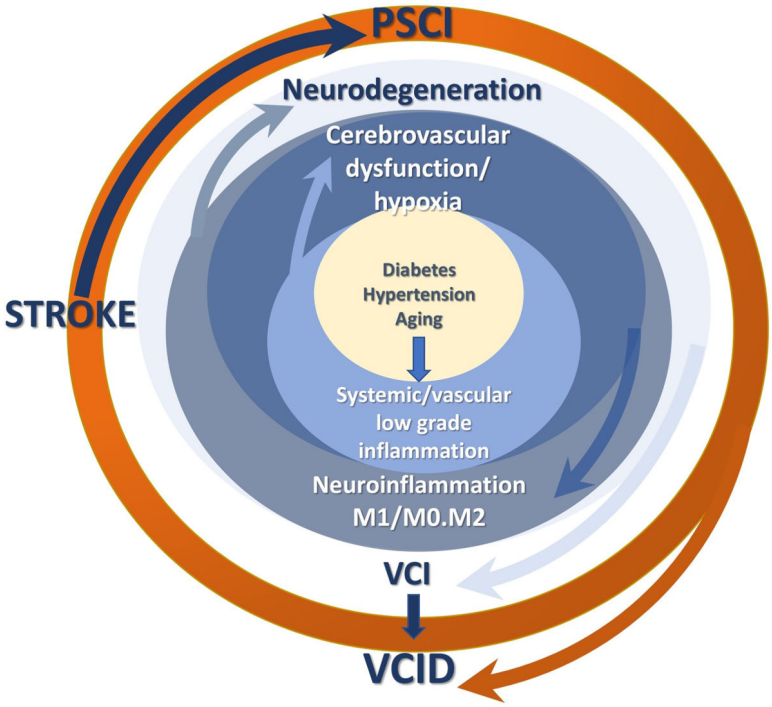

Figure 2.

A schematic model of the role of microglia phenotypes in the vicious neuroinflammatory loop initiated by comorbid conditions. Hypoxic conditions resulting from cerebrovascular dysfunction and altered CBF causes an imbalance of pro- (M1) and anti-inflammatory (M2) microglia leading to neurodegeneration and vascular cognitive impairment (VCI). An ischemic stroke overlaid on this pathology and amplifies neurodegeneration via sustained M2 activation and causes poststroke cognitive impairment (PSCI), collectively contributing vascular contributions to cognitive impairment and dementia (VCID).

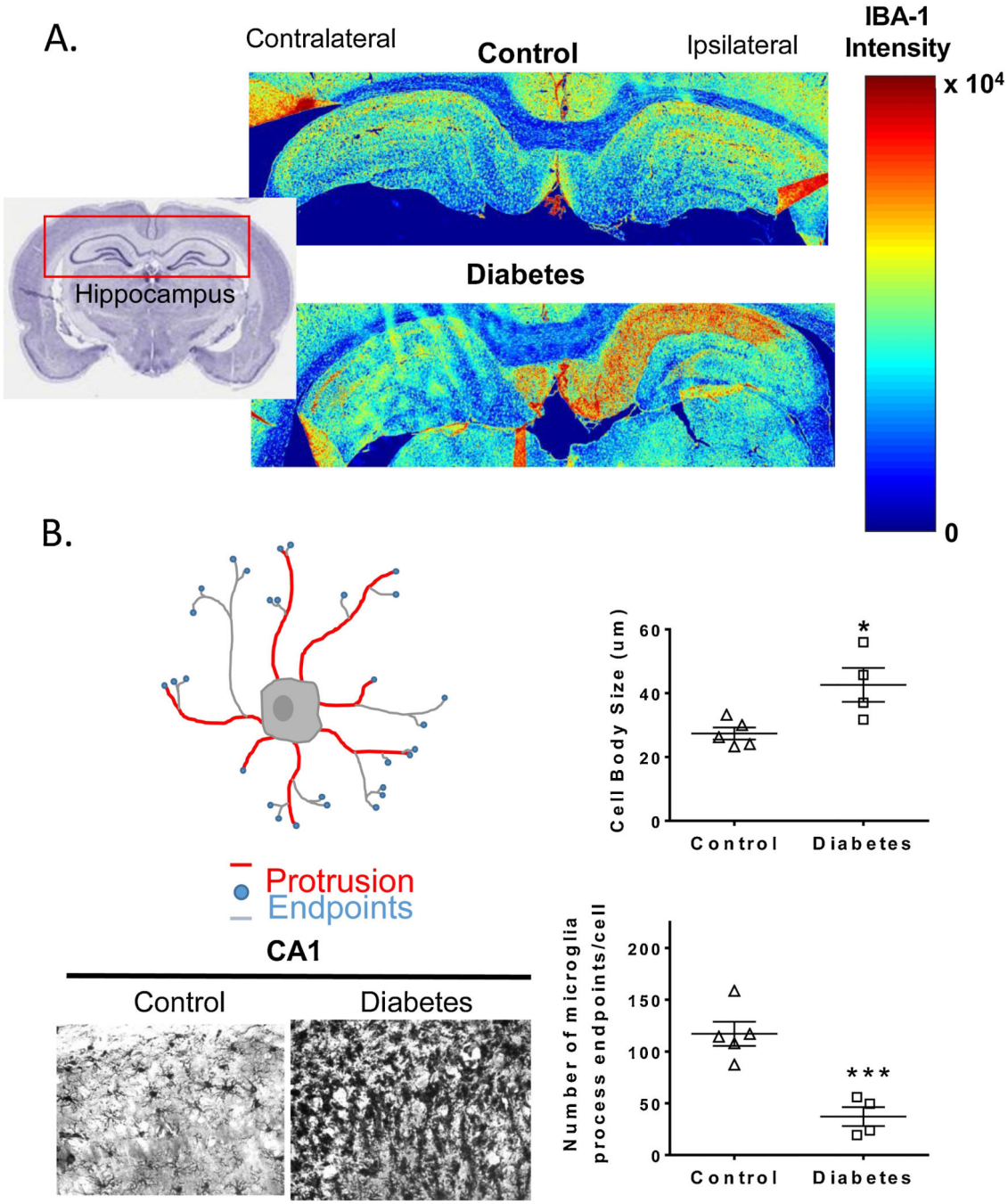

Figure 3.

The impact of stroke and diabetes on microglial morphology and density. A. Heatmaps generated by IBA-1 staining after embolic MCAO show that microglial density is significantly higher in stroked animals in the ipsilateral side in the presence of diabetes. B. Cell swelling, number of protrusions from microglia cell body (red lines), number of endpoints at the tips of microglia processes (blue circles) and process length (grey lines) calculated from 40x images show that microglia of stroked diabetic animals exhibit larger

cell body size and lower number of process endpoints per cell compared to control animals. (Adapted with permission from Ward, B. et al AJP: Heart and Circ Physiol.2018 (89)).