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Role of bone marrow-derived macrophages (BMDMs) in neurovascular interactions during stroke

Akriti Srivastava, Pranay Srivastava, Rajkumar Verma*

Department of Neuroscience, UConn Health, Farmington, CT, 06032, USA

ABSTRACT

Stroke is a leading cause of disability worldwide and hence remains a major medical concern. Besides several pathological features, such as excitotoxicity, peri-infarct depolarization, acidosis, reactive oxygen species generation, apoptosis, and necrosis, dysregulation of the immune system severely affects stroke outcomes. After stroke onset, microglia – the brain-resident macrophage immune cells – and peripheral immune cells affect stoke injury/recovery by releasing pro-inflammatory and/or anti-inflammatory cytokines depending on their microenvironment. These pro- or anti-inflammatory cytokines further affect integrity of the blood brain barrier (BBB) and modulate immune infiltration after stroke. Among peripheral immune cells, bone marrow-derived macrophages (BMDMs) play a critical role in stroke pathology which peaks between three and seven days post-stroke. BMDMs have been extensively studied for their role in exacerbation of stroke injury, however they have rarely been studied for their role in tissue repair. Nonetheless, these reparative roles are gaining attention since recent studies have shown either failure or worsening of long-term post-stroke recovery after blockade of peripheral immune infiltration. These diverse but paradoxical effects of infiltrating monocytes/macrophages encouraged us to summarize the latest findings in neuro-immune and immune-vascular interactions. This review highlights the multifaceted role of BMDMs in stroke onset and resolution, and emphasizes the significance of tapping the potential of these cells to gain better insight into disease progression and therapy.

1. Introduction

Macrophages are key players in both adaptive and innate immunity. Given their ubiquitous distribution across most organs and their role as the first line of defense against foreign attacks, it is not surprising that they are actively engaged in various pathological conditions such as type 2 diabetes, cancers, atherosclerosis, autoimmunity, periodontitis, cerebral aneurysm, and others (Hu et al., 2018). Macrophages differentiate from monocytes, the products of myeloid stem cells. One of the characteristic features of macrophages is their ability to adapt to their microenvironment, which imparts extreme structural and functional flexibility. Macrophages exert microbicidal effects over bacteria and other foreign agents and eventually engulf and destroy them (Trouplin et al., 2013). While the primary function of all macrophages remains conserved, they display significant diversity based on their ontological origin (Epelman et al., 2014). Interestingly, a layered system of co-existing macrophages consists of embryonic macrophages and those derived from progenitor cells, which are present alongside monocytes and dendritic cells. In addition, macrophages that reside in various tissues readily proliferate to establish maintenance populations for performing tissue-specific functions (Perdiguero and Geissmann, 2016). Tissue-resident macrophages exhibit high heterogeneity based upon their tissue of origin and the tissue niche required for carrying out specific functions and maintenance of tissue homeostasis. In addition to their microbicidal functions, macrophages have prominent roles in tissue development, metabolic regulation, and clearing non-functional/defective cellular components. Macrophages also orchestrate immunological events for the restoration of tissue homeostasis during pathogenic attacks (Davies et al., 2013a). For example, in the event of stroke (also known as cerebral ischemia) the initial response by CNS-resident microglia is considered critical for injury repair and involves clearance of dead cells, whereas bone marrow derived monocytes are recruited after damage to the blood-brain barrier (BBB) (Ritzel et al., 2015; Fang et al., 2018). These recruited monocytes undergo dynamic polarization, directed by the tissue niche, that either contributes to neuronal injury (Kong and Gao, 2017) or supports neuronal recovery (Kanazawa et al., 2017). Therefore, distinguishing between resident microglia and macrophages is critical to understand their functional relevance during ischemia. To this end, Dukhinova et al. (2018) described various cellular markers that are expressed in microglia but not in peripheral macrophages, which were identified using multiparameter flow cytometry (Dukhinova et al., 2018). In this review, we will describe several macrophage lineages and some of their prominent roles in various systems and diseases. We will also discuss the recently discovered role of macrophages in neurovascular interactions as well as their involvement in memory and cognition in neurovascular injury

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* Corresponding author. Department of Neuroscience, UConn Health, 263 Farmington Ave, Farmington, CT, 06032, USA.
E-mail address: raverma@uchc.edu (R. Verma).

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models such as stroke.

2. Immune system of the central nervous system

The central nervous system (CNS), which includes the brain and spinal cord, is considered the most complex as well as vulnerable organ system in the human body. Neurons, the primary cells of the CNS, impart function, and their degeneration can render the entire system impaired resulting in neurodegenerative diseases. Immune cells, both the resident microglia/macrophages and infiltrating leukocytes play significant roles in the progression of these neurodegenerative diseases. As such, the CNS is protected from external threats, immune cells, and blood components through the BBB. Immune cells are usually restricted to the meninges under normal conditions (Ransohoff and Engelhardt, 2012). Earlier studies concluded that the brain was immune-privileged due to the absence of antigen-presenting cells, the lymphatic system, and bleak expression of major histocompatibility complexes (MHC) that prevented an immune reaction against CNS-derived antigens (Louveau et al., 2015; Medawar, 1948). It was believed that CNS antigens remained sequestered within and hence, evaded the immune system (Louveau et al., 2015). However, this long-standing concept was refined with advancements in our knowledge of neuro-immune reactions (Louveau et al., 2015). Modern imaging techniques compelled researchers to rethink this dogma, and facilitated the visualization of apparent “trading” between the interstitial fluid (ISF) and cerebrospinal fluid (CSF), which was designated as the glymphatic system; this allows clearance of components from brain parenchyma to CSF. Additionally, the confirmation of lymphatic system existence in dura mater indicated drainage of ISF into lymphoid tissues through subarachnoid CSF (Engelhardt et al., 2017; Forrester et al., 2018). These findings thus altered/refined the earlier concept of CNS as an immune-privileged system.

The CNS immune system is predominated by microglia (also called as CNS resident macrophage) that originate from embryo-sac and maintain CNS homeostasis. (Ginhoux et al., 2016). Once established, they can sustain themselves with the help of resident progenitors, independent of other types of blood cells (Ransohoff and Brown, 2012). Microglia are first responders to brain insults such as stroke, however, as early as 4 h after stroke, the BBB is compromised and bone-marrow-derived macrophages (BMDMs) infiltrate and dominate the immune response, at least during the acute phase (Ritzel et al., 2015). Detailed functions of microglia and how they coordinate with infiltrated BMDMs in tailoring immune responses after ischemic insults is beyond the scope of this review and can be found elsewhere (Herz et al., 2017; Patel et al., 2013). Besides microglia, perivascular macrophages form another class of sentinels that monitor the CNS for possible external threats (Ousman and Kubes, 2012).

3. Macrophage lineages

The phagocytic system of monocytes, the precursor cells of macrophages, arises from committed hematopoietic stem cells (HSCs) present in bone marrow. Monocytes are distributed systemically through the body. Once these cells extravasate from the circulatory system and reach a target tissue they differentiate into macrophages (Murray and Wynn, 2011). Blood-resident monocytes can be distinguished from each other based on specific Cluster of Differentiation (CD) markers, such as CD14++, CD16++, and CD14 +CD16$^+$, and are categorized into classical, non-classical, and intermediate subtypes respectively (Davies et al., 2013b). Classical monocytes are the predominant monocytes in blood, while non-classical monocytes comprise merely 10% of the total monocyte population (Ziegler-Heitbrock et al., 2010). The classical monocytes subset increases in blood circulation, whereas the number of non-classical monocytes decreases (Kaito et al., 2013). Increases in classical or intermediate monocyte subsets, or vice versa, during acute and sub-acute phases of stroke, strongly correlated with the progression and severity of brain infarction (Kaito et al., 2013).

Contrary to earlier reports of macrophages being derived solely from circulating monocytes, several recent studies reported an embryonic origin of most macrophages present in adult tissues. However, some reports suggest the presence of macrophage progenitors in the yolk-sac before HSCs emerge (Perdiguero et al., 2015). Embryonic tissue is also colonized by yolk-sac macrophages that are derived from the erythro-myeloid progenitor cells in the yolk sac (Guilliams and Scott, 2017). The fetal liver can serve as one of the primary sites of hematopoiesis during embryonic development as well (Epelman et al., 2014). In summary, macrophages can be derived from three types of precursors originating in yolk-sac, fetal liver, and HSCs (van de Laar et al., 2016). Despite these findings on macrophage origin, the role of circulating monocytes in macrophage replenishment cannot be completely ruled out, and is still considered the primary source of macrophage populations that lack self-renewal properties (Röszer, 2018). The peritoneal cavity houses the majority of immune cells and is initially primed with macrophages derived from embryo; however, later on, most of them are replaced by BMDMs possessing a high proliferative capacity (Bain et al., 2016).

Monocyte subsets that exist in mice are identified by their chemokine receptors known as CCR2 (CCR2$^+$ and CCR2$^-$) and inflammatory marker lymphocyte antigen 6 complex locus C1, Ly6C (Ly6C$^+$ and Ly6C$^-$). Ly6C$^+$ and Ly6C$^-$ chemokine receptors are represented as CCR2$^+$CX3CR1$^-$ and CCR2$^-$CX3CR1$^+$, respectively (Yang et al., 2014). Ly6C$^+$CCR2$^+$CX3CR1$^-$ monocytes are designated as pro-inflammatory monocytes that migrate from bone marrow into the blood and ultimately to the site of inflammation. In contrast, Ly6C$^-$CCR2$^-$CX3CR1$^+$ monocytes, which are also called patrolling monocytes, leave the bone marrow to be recruited to the vasculature of injured/inflamed tissues and are believed to reduce inflammatory symptoms by monitoring endothelial integrity (García-Culebras et al., 2018). Ly6C$^+$CCR2$^+$CX3CR1$^-$ monocytes are recruited to the site of inflammation by following a chemokine concentration gradient, similar to classical monocytes (Fig. 1). They also reportedly replenish the disappearing pool of tissue-resident macrophages at the injury site. Patrolling, or non-classical, monocytes exhibit conflicting actions because they secrete both pro-inflammatory and anti-inflammatory molecules (Thomas et al., 2015), which could be due to the failure to separate them from intermediate monocytes on the basis of their surface markers (Chiu and Bharat, 2016).

4. Macrophages and their diverse roles

Macrophages exhibit diverse roles ranging from basic functions such as housekeeping and maintaining tissue homeostasis to more complex functions such as immunomodulation. These functions are mainly mediated by secreted growth factors that affect neighboring cells or tissues to coordinate tissue repair or maintenance. Macrophage dysfunction of any kind can lead to pathological conditions. Some of the vital functions of macrophages are listed in Table 1 (Okabe and Medzhitov, 2016). Studies conducted either through pharmacological inhibition or gene knockout of a receptor required for macrophage recruitment (CCR2) have demonstrated delayed or decreased recovery following ischemia, indicating an important contribution of these cells (Fang et al., 2018).

5. Bone marrow-derived macrophages (BMDMs): physiology and significance

BMDMs are derived from bone marrow exposed to specific growth factors, such as macrophage colony stimulating factor (M-CSF) (Weischenfeldt and Porse, 2008). BMDMs display functional heterogeneity based on the extracellular milieu and acquire two specific polarization states, namely M1 and M2. They can easily modify their metabolic functions from an inhibitory state (M1 phenotype) to a

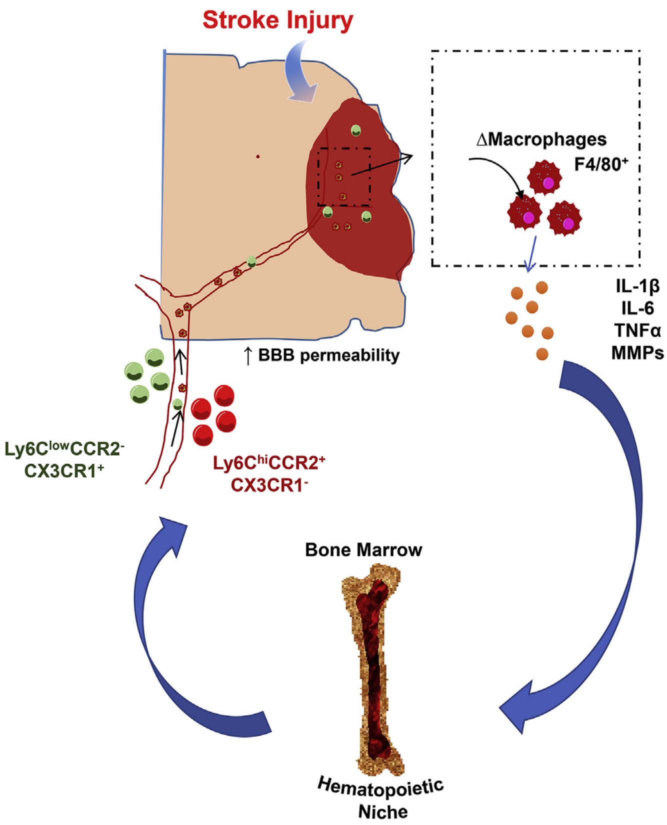

Fig. 1. Role of bone marrow in regulating resolution of inflammation. In the event of cerebral ischemia, an increased level of pro-inflammatory signals (cytokines, MMPs, etc.) attract bone marrow-derived monocytes into the circulation. Physiologically circulating monocytes expressing Ly6ClowCCR2-CX3CR1 + (patrolling monocytes) are involved in tissue homeostasis. However, compromised BBB during stroke and supraphysiological levels of pro-inflammatory cytokines signals bone marrow to deploy Ly6ChiCCR2 + CX3CR1-(Bone marrow-derived monocytes) to the site of injury. Both Ly6Chi and Ly6Clow are found in the infarct area of the brain. Recruitment of Ly6Chi monocytes via CCR2 + dictates the initial response after entering the perilesional infarct zone and can also give rise to tissue macrophages depending upon the tissue milieu. These cells are initially involved in engulfment of necrotic tissues.

repair/growth-promoting state (M2) depending upon external stimuli and show different profiles of chemokines and cytokines expression (Italiani and Boraschi, 2014). For instance, upon activation of Toll-like receptors (TLRs), nuclear factor kappa B (NFκB), or c-Jun N terminal kinase 1 (JNK1), macrophages develop into the M1 subtype that possesses pro-inflammatory properties. In contrast, in the presence of active receptors PPAR-γ and -δ or the IL-4-STAT6 pathway, macrophages develop into the M2 subtype with anti-inflammatory properties. However, there is more to macrophage polarization than just M1 and M2 phenotypes, as there could be multiple other states of macrophages depending on microenvironmental cues (Ginhoux et al., 2016). To better comprehend this activation phenomenon and its requirements, researchers use BMDMs as an *in vitro* model for macrophage polarization studies (Ying et al., 2013). BMDMs are also excellent experimental models due to their homogeneity, easy transfection, high proliferative capacity, and long lifespan; although they possess some limitations, such as instabilities in phenotypes and functions under *in vivo* conditions, which is believed to be related to their high proliferation rate (Wang et al., 2013). Since transformed macrophage cell lines (the previously preferred choice for *in vitro* immunological studies) lack clear genetic definition/detail and some natural macrophage characteristics, research focus has shifted towards primary macrophages derived from bone marrow, the BMDMs (Fejer et al., 2013). BMDMs therefore now serve as an effective *in vitro* tool to assess several morphological, physiological, and gene expression functions (Weischenfeldt and Porse, 2008).

6. Role of BMDMs in CNS injury

BMDMs are associated with several disease functions and as such offer a tremendous scope in clinical studies related to neuro-inflammatory diseases (Prinz and Priller, 2014). Just as in peripheral tissues, CNS macrophages undergo a shift in polarization state from an M2 non-inflammatory to an M1 pro-inflammatory state during brain injuries (such as stroke), spinal cord injuries, or neurodegenerative disorders (Hu et al., 2012; Kigerl et al., 2009). But often a combination of macrophages with M1 and M2 phenotypes has been observed in most neurodegenerative diseases (Prinz and Priller, 2014). In mechanical injuries to the nervous system (such as spinal cord injury (SCI), traumatic brain injury (TBI), etc.) there is a release of inflammatory cytokines and production of oxidative stress, and the initial stages are marked by the appearance of pro-inflammatory M1 macrophages followed by anti-inflammatory M2 macrophages later. Maintaining this transition optimally is thus a prime requisite of repairing after damage (Cherry et al., 2014).

A chronic inflammatory response due to continuous or progressive infiltration of BMDMs across the BBB is associated with several neurodegenerative disorders (Stephenson et al., 2018). Consequently, the focus of much current research has shifted towards unraveling the cellular and molecular events surrounding the onset of neuro-inflammatory responses in these disorders, such as stroke, TBI, and Alzheimer's disease (AD) (Moretti et al., 2015; van de Haar et al., 2016), their deleterious and favorable effects, and their potential role as unique biomarkers (Dorothée, 2018). Myeloid-derived macrophages or BMDMs are associated with progression of several neurodegenerative diseases, because they are one of the key microenvironmental components of degenerating neurons. Studies continue to define specific

Table 1
Multiple characteristic features and related functions of macrophages.

Function	Activity	Effector molecules	References
Inflammation	Induction of immune response, paracrine signaling, repair	Cytokines, antibodies, memory cells, growth factors	(Brüne et al., 2013; Kumar et al., 2018; Lee and Choi, 2018)
Tissue development	Tissue remodeling, erythropoiesis, hematopoiesis, angiogenesis, brain, and renal tubule development	Cytokines, chemokines, growth factors	(Nucera et al., 2011; Wynn et al., 2013)
Tissue repair	Macrophage plasticity, dynamic polarization, paracrine signaling	Pro-inflammatory molecules, anti-inflammatory molecules, growth factors	(Batoon et al., 2019; Brancato and Albina, 2011; Carlson et al., 2017; Chazaud, 2014; Das et al., 2015; Mescher, 2017; Novak and Koh, 2013; You et al., 2013)
Tumor progression	Tumor cell proliferation, immunosuppression, angiogenesis	Pro-tumor molecules	(Noy and Pollard, 2014; Ruffell et al., 2012; Zhang et al., 2012)
Metabolism	Metabolism orchestration, inflammation, cellular signaling in lipid metabolism, metabolic shift	Cytokines, chemokines	(Bhargava and Lee, 2012; Biswas and Mantovani, 2012; Guttenplan and Liddelow, 2018; McGettrick and O'Neill, 2013)

functions of these cells in inflammatory reactions involved in neurodegeneration (Sevenich, 2018). Although the pro-inflammatory role of BMDMs is well established in the field, several recent studies suggest that these pro-inflammatory cytokines lay the foundation for resolution of inflammation (Cuartero et al., 2013; Shichita et al., 2009; Smirkin et al., 2010). Protective effects of these monocytes can be mediated by several secreted cytokines, growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), and endothelial growth factor (EGF) (Bhat et al., 2013). Transplantation of bone marrow-derived cells (BMCs) has been proposed as a promising therapeutic regime to alleviate neuropathy associated with SCI (Koshizuka et al., 2004). BMCs regenerate neurons and myelin-synthesizing cells, and secrete some neuroprotective cytokines. However, supplementing transplanted cells with cytokines like granulocyte macrophage colony stimulating factor (GM-CSF) enhances the neuroprotective efficacy of BMCs in case of acute and sub-acute SCI (Yoon et al., 2007). The pathological conditions associated with microglial dysfunctions can potentially be treated using BMC transplantation. Furthermore, some of the earliest studies on AD reported involvement of BMC in phagocytosis of amyloid β (Aβ). These BMDMs accumulated near Aβ plaques and had better phagocytic activity than resident microglia (Larochelle et al., 2016). This evidence suggests that bone marrow-derived cells, either monocyte or macrophage, play both detrimental and neuroprotective roles that depends on microenvironments of host tissues (Newson et al., 2014).

7. BMDMs and neuroinflammatory regulation in ischemic stroke

Stroke involves three phases, acute (oxidative stress generation and excitotoxicity within the first few hours), sub-acute (neuro-inflammation that lasts for a few days), and chronic (inflammation with increased activity of microglia and astrocytes), followed by its resolution. In parallel, during the acute and sub-chronic phase, increased BBB ruptures can occur, which further allows entry of blood-derived leukocytes and exacerbates the inflammatory response. Such excessive neuro-inflammation could be fatal for the patient (Stonesifer et al., 2017). Most of the stroke literature highlights the central role of the brain-resident immune cells, microglia, in post-stroke inflammation. However, recent evidence suggests that the infiltrated myeloid cells, specifically monocytes/macrophages, become the predominant contributor of inflammation in the brain (Benakis et al., 2015; Tóth et al., 2016). Until recently, separation of activated microglia and infiltrated macrophages was not possible due to absence of specific marker proteins that could distinguish between CNS-resident and infiltrated macrophages, which led to discrepancies in the relative contribution of these cells to brain injury (Sevenich, 2018). Recently Dukhionova and collogues used a multicolor cytometry techniques to distinguish resident microglia and other heterogeneous macrophage population in the CNS which helped researcher to determine relative contribution of macrophage subtype in the brain (Dukhinova et al., 2018).

The extracellular environment regulates functional aspects of participating macrophages. Increased inflammation in a post-stroke brain results in immunosuppression to combat targeting of self-antigens, which increases vulnerability to infections that can prove fatal. Some studies suggest protective roles of macrophages, stating that the initial pro-inflammatory effects in stroke guard the cells against further damage induced by stroke and trigger reparation work to restore homeostasis (ElAli and Jean LeBlanc, 2016; Guruswamy and ElAli, 2017; Kanazawa et al., 2017). For example, infiltrating BMDMs in ischemic brains clear away debris through phagocytosis in the infarct region and promote scarring, thus facilitating tissue repair (Tanaka et al., 2003). They also release neuroprotective TGFβ1 leading to ischemic tolerance (Tanaka et al., 2003). Further studies conducted in stroke/ischemia report an increased activation of microglia with a concomitant increase in release of immune components (Ritzel et al., 2015). This is followed by widespread recruitment of BMDMs towards the ischemic site that may have translational value. Stimulation of BMDM production after ~90 minutes of stroke onset is accompanied by reductions in microglia number and impairments in cell cycle. Besides the time elapsed from the onset of stroke, polarization of macrophages is also affected by other factors like their localization at the injury site (i.e., macrophages in the main core of ischemic injury are phenotypically different from those in the outer penumbra region). BMDMs also exhibit increased phagocytic activity and induce less oxidative stress as compared to microglia (Ritzel et al., 2015). Another interesting feature of BMDMs is their ability to express neuronal markers in tissue culture, which could be extrapolated to the *in vivo* level to design a cell-mediated therapeutic approach against neuronal anomalies and CNS injuries (Brazelton et al., 2000). Therefore, all the above reports suggest that macrophages and other inflammation-evoking cells have a rather complex participation in stroke disease etiology (Gliem et al., 2016), and more studies are needed to dissect their temporal and spatial role after stroke.

8. Neurovascular unit (NVU) and its implications in stroke

Efficient brain function doesn't merely depend upon interactions between neurons but also between neurons and other cells of the entire neurovascular unit (NVU), which includes glial, endothelial, and vascular cells (McConnell et al., 2017). Glial cells surround neurons and maintain their homeotic conditions by protecting them from external insults. The vascular components of NVU are responsible for maintenance of cerebral blood flow, energy supply, cellular permeability and work together to maintain CNS homeostasis (Cai et al., 2017; McConnell et al., 2017). In addition, researchers have observed that pericytes, perivascular cells that envelop the surface of the blood vessel, occupy a major chunk of brain microvasculature to carry out various functions like angiogenesis, maintenance of BBB, etc. (Sweeney et al., 2016). Stroke perturbs the normal structure and function of the NVU, causing damage to pericytes that leads to disruption of the vascular system and BBB and elevated inflammatory reactions (Fig. 2) (LeBlanc et al., 2018). The hypoxia-induced paracrine signaling in the ischemic stroke brain degrades the vascular matrix and its native features, further compromising BBB integrity. This exacerbates neuronal damage with an increased infiltration of immune cells/molecules (Barakat and Redzic, 2016). Advancements in imaging techniques have facilitated the unearthing of the causative event in the loss of BBB integrity. This involves trafficking of trans-endothelial vesicles during the initial phase after stroke that leads to cytoskeletal changes in brain vascular endothelial cells that further predispose the BBB towards immune cell-mediated damage (Shi et al., 2016). Therefore, maintaining an intact BBB has been proposed as the primary requisite of stroke therapy (Sifat et al., 2017).

8.1. Immune responses of BMDMs in stroke resolution

BMDMs play a significant dual role in stroke etiology. BMDMs reach the site of injury 3–7 days post-stroke (i.e., during sub-acute phase). Although their morphology and some functions are similar to those of microglial cells, their phagocytic abilities are much greater. Some recent studies have also highlighted the role of these cells in maintaining the integrity of brain microvasculature (ElAli and Jean LeBlanc, 2016; Gliem et al., 2012). During the acute stroke phase, there is a rapid release of pro-inflammatory molecules accompanied by ROS generation and MMP-9 activation in the ischemic brain region that further mediates migration of leukocytes to target areas (Amantea et al., 2009; Kriz, 2006). During the sub-acute phase, there is an excessive release of cytokines and ROS generation by infiltrating leukocytes that leads to breach of the BBB, neuronal death, and even hemorrhage (Jin et al., 2010). However, during the latter reparative stages, some pro-inflammatory molecules like MMP-9 also promote remodeling of neurovasculature and brain regeneration (Jin et al., 2010). It has been shown that acute depletion of monocytes during the initial stages of stroke

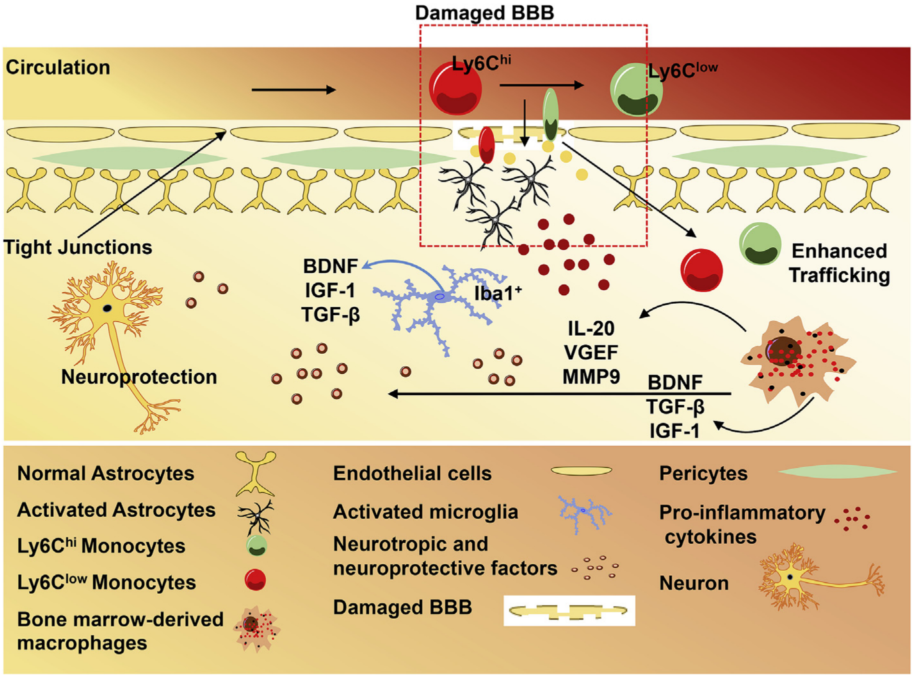

Fig. 2. Interaction of BMDMs and neurovascular (NVU) unit in stroke recovery. NVU comprises an efficient network and intricate balance of neurons and glia, including astrocytes and microglia, along with vascular endothelial cells, pericytes. Blood cells, mainly polymorphonuclear (PMN) cells, like monocytes and lymphocytes further interact with NVU. Following the acute phase of ischemic stroke (after ~7 days) there is enhanced trafficking and recruitment of monocytes and macrophages expressing Ly6C^{low} through CX3CR1. These cells carry out phagocytosis of tissue debris and are involved in production of extracellular matrix and release of various anti-inflammatory and tissue reparative substances (e.g., IL-10, TGF-β, etc.). Pro- and anti-inflammatory activity of both Ly6C^{hi} and Ly6C^{low} monocytes are regulated by the tissue microenvironment, nature of stimulus, and molecular profile of the cells. Activated microglia (may also express Iba-1$^+$ depending on their localization) display distinct shapes and expression patterns at the onset of cerebral ischemia during maturation into macrophages. They are also involved in secretion of neurotrophic factors.

hampers recovery during the latter phase (Wattananit et al., 2016). This suggests that the recruitment of monocytes to the ischemic site is a vital step that ensures proper recovery of ischemic brain later (Wattananit et al., 2016). Post-stroke recovery involves several mechanisms including resolution of inflammation, angiogenesis, and neurogenesis. Among these, enhancement of neurogenesis is one of the most exploited areas of stroke therapy and macrophages play a critical role by secreting several trophic factors essential for neurogenesis, such as brain-derived neurotrophic factor (BDNF) (Batchelor et al., 1999). The activation of M2-type macrophages speeds up vascularization of lesions in stroke-affected brains thereby promoting recovery (Kanazawa et al., 2017). Some recent studies also highlighted the significance of hematogenous macrophages in stabilizing neovessels, fibrotic scarring, as well as astrocyte-driven repair processes in some areas and also in prevention of secondary intracerebral hemorrhage (Gliem et al., 2012; Rahman et al., 2014). Functional inhibition of beneficial macrophages may also result in secondary bleeding and unresolved edema in ischemic brain (Gliem et al., 2016), which is further supported by a recent finding that macrophages are involved in stabilizing the perturbed neurovasculature in stroke models (Liu et al., 2016). Macrophages appear to accomplish this by adhering to the endothelial ends of a lesion with extending filopodia and pulling both endothelial ends together to suture ruptured vessels (Liu et al., 2016). Secretion of neurotrophic factors like Insulin-like Growth Factor-1 (IGF-1) and Fibroblast Growth Factor-2 (FGF-2) by macrophages and microglia also save dying neurons and glial cells, promote neuro-regeneration, restore synaptogenesis, and stimulate remyelination (Shichita et al., 2014).

8.2. Remodeling of neurovasculature after ischemic stroke and its subsequent restoration

Being an essential functional and structural entity for maintaining homeostasis of CNS, dysfunctional NVU is a manifestation of pathological conditions like stroke (Shabir et al., 2018). The onset of stroke negatively impacts NVU elements, besides the neurons themselves. Therefore, the trend has now somewhat drifted towards rescuing not just the neurons but also the vascular compartments (Buscemi et al., 2019). The affected brain tissue deprived of oxygen and nutrients after stroke can be rescued via angiogenesis. This phenomenon is predominantly governed by the neuro-immune interactions between microglia and peripheral immune cells like macrophages and astrocytes. All these cell types secrete angiogenesis factors, including VEGF, MMP, IL-6, and others. (Wang et al., 2018). A well-orchestrated action of NVU after ischemic insult restores the blood flow to the infarct region gradually, thus improving the damaged tissue and neurons. Any kind of relapse in NVU can lead to vascular dementia later on, which re-establishes its vital involvement in stroke pathophysiology (Posada-Duque et al., 2014). An extended network of vascular structures enhances the chances of tissue survival post-stroke by improving the necessary energy supply as well as initiating other processes that aid in restoration of homeostasis of affected tissue areas, such as neurogenesis, neuronal sprouting, glial proliferation, etc. (Yanev and Dijkhuizen, 2012). Post-mortem neuropathological assessment of stroke patients suggests that enhanced vascular density is correlated with improved chances of survival post-stroke. However, the exact spatio-temporal modifications in NVU at functional and morphological levels elicited by stroke onset remain a bit elusive but are nonetheless critical for developing effective remedies especially against the sub-acute phase of stroke (Lake et al., 2017).

The interplay between the restoration and dysfunctional signaling events involving the NVU at penumbra in ischemia largely governs long-term rehabilitation. It primarily involves tipping the balance towards repair signals while containing the damage associated, thus maintaining the structural and functional unit of NVU eventually leading to neurorestoration. Hence, it becomes imperative to focus equally on NVU signaling and its regulation for developing effective therapeutic interventions against stroke in the future (ElAli, 2016).

9. Conclusion

The CNS is an immune-privileged organ that has its own immune cells, resident microglia. It remains separated from other circulating immune cells by an intact BBB. It is only when this barrier is compromised that circulating immune cells including monocytes/macrophages infiltrate brain to participate in ongoing injury/repair mechanisms. Previously, BMDMs were considered pathogenic, especially in the case of a neurodegenerative disorder, but subsequent research revealed their additional protective role. High plasticity of BMDMs enables them to adopt a pro-inflammatory or an anti-inflammatory state in response to microenvironmental cues, which allows them to be classified into various subtypes based on their location, origin, and surface markers. BMDMs have significant involvement in the etiology of various neurodegenerative disorders, like AD, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), SCI, TBI, and stroke. Besides releasing inflammatory cytokines in their M1 state, macrophages also release angiogenic growth factors upon acquiring the M2 state. Several studies validated the neuro-inflammatory role of BMDMs in stroke models by showing a dramatic increase in the BMDM population in stroke-affected brains (Chu et al., 2015; Fang et al., 2018; Wattananit et al., 2016). BMDMs are a major participant in generating a pro-inflammatory state in the initial phases of stroke. As disease progresses, BMDMs extravasating to the site of injury gradually transform themselves to the M2 phenotype, releasing neurogenic growth factors that aid in neuronal repair. Years of research and accumulating data on BMDMs have not only unraveled their immense potential as a causative factor of neuroinflammation and neurovasculature alterations in stroke but also their benevolent contribution in neural repair. This is why they have been called a ‘double-edged sword’(Fang et al., 2018), where under specific microenvironments BMDMs can direct cellular fate towards either an inevitable end or the road to recovery. Therefore, controlling and modulating their phenotypic character could efficiently tap their neurorestorative and neurovasculature stabilizing properties in ischemia and hence break their conventional negative image. Understanding BMDM physiology under stroke conditions and identifying their contributions to disease etiology – especially their pro-inflammatory role – can aid the design of appropriate preventive measures against disease progression. Similarly, providing an environment that can enhance the anti-inflammatory properties of BMDMs in their M2 state can be one of the ways of using them in stroke resolution studies in the future. BMDMs, therefore, may someday serve as an indispensable tool for a thorough analysis of stroke and its various stages, its progression, its impact on brain damage, and eventually its possible resolution.

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Appendix A. Supplementary data

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