Review article

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A Review on Complex Roles of Brain Macrophages/Microglia in Ischemic and Hemorrhagic Stroke Pathophysiology

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ABSTRACT:

Stroke is one of the leading causes of death worldwide. It occurs due to poor blood supply in some parts of the brain. There are two types of stroke: ischemic, which occurs due to lack of blood supply in the brain, which causes death of brain tissue and hemorrhagic, which occurs due to vessel rupture. Macrophage/microglia despite of being an immune cell plays different roles in different types of stroke. Microglia, the tissue macrophages of the brain, has under healthy conditions a resting phenotype that is characterized by a ramified morphology. Upon any homeostatic disturbance microglia rapidly alter their phenotype and contribute to processes including inflammation, tissue remodelling. After activation following a stroke they release factors which can exacerbate the damage or they can also aid in the repair of the damage. It is important to determine the function macrophage/microglial cells in order to control their activation, which could be beneficial in curing stroke in the future.We review the recent findings regarding the role of macrophages in stroke.This review will emphasize future directions towards the development of novel immunomodulatory therapeutic interventions.

KEY WORDS: Stroke, Microglia, Macrophage

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STROKE:

Stroke is one of the leading causes of death in the world. Cellular death due to poor blood flow in the brain is termed as stroke. There are two main types of stroke: ischemic and hemorrhagic. Ischemic stroke occurs due to lack of blood supply in the brain, which causes death of brain tissue due to poor oxygen supply. Hemorrhagic stroke occurs due to bleeding. Ischemic is the more common type of stroke. Two types of injuries are caused by ischemic stroke, Core (infarct) that undergoes immediate cell death by

necrosis, and the peri infarct penumbra that undergoes delayed programmed cell death [1].

Origin of Microglia: Hematopoietic stem cells, the progenitors of all blood cells differentiate into microglial cells. Some of these stem cells differentiate into monocytes and travel to the brain from the bone marrow, where they settle and differentiate into microglia. Microglial cells can persist much longer than other macrophages in the central nervous system (CNS). Microglial cells have the ability to proliferate in situ; however, bone marrow-derived cells can enter the CNS across the blood–brain barrier (BBB) and populate the microglial-cell compartment [2, 3, 4]. Though recent studies indicate precursors microglia originate in the embryonic yolk sac [5]. The direct precursors of microglia which travel to neural tube at Embryonic day 8 (E8) are the $CD45^{-}cKit^{+}$ cells. This population of cells eventually start expressing CX3CR1 and CD45 and then develop into microglia [6, 7]. These cells can be seen seeding the brain rudiment by Embryonic day 10 (E10) in rodents and have a full microglial morphology at E14 [8].

Microglial phenotypes, M1 & M2: Microglial cells have two phenotypes i.e M1 and M2. These two cells are indistinguishable from each other without their surface markers. The M1 phenotype is associated with cytotoxic properties and M2 phenotype has the ability to reduce the inflammatory response and help in tissue repair [9]. M1 phenotype increases the protein synthesis of pro-inflammatory mediators like TNFα, IFNγ, IL-1β, IL-6 etc. along with production of ROS and NO, also proteolytic enzymes (MMP) that act on the extracellular matrix, which leads to BBB breakdown [10]. The M2 microglia releases anti inflammatory mediators like IL-10, IL-4, IL-13, TGF-β and IGF-1. The release of TGF-β by microglia promotes anti inflammatory response and neuroprotection in the ischemic brain [11]. The microglial phenotypes can switch from M2 to M1 during disease progression [12]. Recent study suggests that microglia which is activated after middle cerebral artery occlusion (MCAO) can morph into reactive M1 phenotype by 7 days [13]. Many proteins have been identified as markers for M1 or M2. MHC II is commonly used as a marker for M1, which is upregulated during ischemic stroke [14]. Another marker CD68 (macrosialin) is often used to identify the M2, debris clearing state of microglia [15].

Activation of microglia: The activation of microglia begins rapidly in case of ischemic stroke [16]. The activation of the microglia occurs by the excitotoxic signals generated during the ischemic cascade [17, 18]. During activation microglia changes their phenotype. Different morphologies of microglia can be found, one with enlarged cell body found in the peri infarct regions, another can be characterized by amoeboid cell structure having rare ramifications also found in the peri infarct regions and lastly, morphology with round shape which is the highly activated form of microglia can be found nearby to the core [19]. Following activation, the microglia becomes polarized into two different phenotypes, the classically activated M1 phenotype and the alternatively activated M2 phenotype [20]. MDMs (Monocyte-Derived Macrophages) are morphologically similar to the resident microglia, though they possess more phagocytic capacity than the resident microglia [21]. MDMs are found to be contributing to the clearance of cellular debris [22]. Following stroke it takes about 3 to 7 days for MDMs to reach the injury site [23].

These cells have both pro inflammatory and anti inflammatory roles in ischemic stroke. $CCR2^+$ pro inflammatory monocytes differentiate into highly phagocytic macrophages in case of ischemic stroke [24]. It was also seen that $CCR2^+$ pro inflammatory monocytes differentiate into M2 phenotype which reduces the injury [25]. Unlike pro inflammatory monocytes, the role of anti-inflammatory monocytes in ischemic stroke is not well defined. Further studies are required in this area.

Factors released & their effects:

TNF-α has been found to be involved in necrosis, as well as in apoptotic pathways. TNF-α released from microglia has neurotoxic effect. TNF-α produced by resident brain microglia has a neuroprotective role in MCAO model. Inflammatory response may result in disruption of the blood-brain barrier. There are also evidences that TNF-α has neuroprotective roles after ischemic stroke [26, 27, 28]. After middle cerebral artery occlusion (MCAO) in mice IL-1β and TNF-α are produced by non-overlapping subsets of microglia and macrophages. IL-1β exerts neurotoxic effects in ischemic stroke and blocking its action has been shown to reduce ischemic brain damage [29, 30].

Angiopoietin like protein (ANGPTL) are a family of proteins which are structurally similar to angiopoietin but do not bind to the angiopoietin receptor. Secretion of ANGPTL by bone marrow derived infiltrating macrophages increases inflammation in ischemic mouse brain in a transient middle cerebral artery occlusion (MCAO) model by expressing pro inflammatory cytokines such as IL-1β, TNF-α etc. [31].

Matrix metallo proteinases (MMP) are responsible for the breakdown of the extracellular matrix and the BBB after ischemic stroke [32]. Microglia cells contribute to the release of MMPs (mainly MMP3 and MMP9). MMP3 and MMP9 knockout mice model are found to suffer less in ischemia [33, 34].

Neuroprotective role of microglia: Apart from inflammatory role, microglia also has a neuroprotective role after cerebral ischemia [35]. Microglia can also produce neurotrophins and growth factors (TGF-β1, FGF etc.), which can repair brain tissue following cerebral ischemia [36]. TNF has both neurotoxic and neuroprotective effects. Increase in cytokines (IL-6, IL-1β, TNF-α) following selective ablation of proliferating microglial cells exacerbates ischemic brain injury [37]. Regardless of their origin, activated macrophages /microglia play critical role in the clearance of infiltrating neutrophils after cerebral ischemia. Neutrophil infiltration occurs in the first 3 days after cerebral ischemia, after which they are replaced by macrophage like cells [38]. Macrophages reduce neuronal injury by triggering apoptosis of the neutrophils and preventing the release of cytotoxic substances into the surrounding tissue [39]. In endothelin-1-induced cerebral ischemia in rat, large-scale migration of neutrophils into the ischemic region occurs during the first day and peaks at 3 days after cerebral ischemia [40]. In this study it was found that macrophages engulf neutrophils and this condition increased with time [41].

Function of macrophages in Intra Cerebral Hemorrhage (ICH):

In a rodent model of ICH, the regions of the hematoma were infiltrated by large quantities of inflammatory monocytes caused early motor deficits [42]. Macrophages produce large amounts of pro inflammatory cytokines which exacerbates tissue damage. Peripheral macrophages/ monocytes migrate to the region of hematoma which leads to brain

damage. However, inhibition or reduction of their movement into the brain reduces the damage in ICH induced brain [43]. Microglia/macrophages have a crucial role in removing the cellular debris from hematoma [44].

In an experiment, laser beam was used to blast a blood vessel apart in the brain of a zebra fish, which represented a human microbleed. After 30 minutes, macrophage showed up at the site of injury. It was observed that the macrophage extended it's lamellipodia or filopodia, which adhered to the ends of endothelial ends and mechanically pulled the ends to mediate their ligation, thus helping in the repair of cerebrovascular rupture. This process was found to be dependent upon PI3K and Rac1 activity. The process took around three hours to complete, after which, the macrophage left the injury site [45].

CONCLUSION: Microglia play important role in ischemic as well as hemorrhagic stroke injury as it has both degenerative and protective functions. By secreting neurotrophic factors microglia actively participates in reducing the injury in brain tissues following ischemic stroke. Determining the proper roles of microglia/ macrophage and controlling their activation may hold the key in prevention and reduction in ischemic stroke therapeutics. Fine-tuning immunomodulatory interventions based on the heterogeneous profiles of microglia are urgently needed for ischemic stroke. An understanding of the mechanisms that cause microglial activation might help to provide novel therapeutic avenues for the treatment of stroke. However, it is clear that macrophages might be therapeutic targets to prevent or avoid stroke.

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