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The Role of Microglia and Astrocytes in the Neuroinflammation Process in Acute Ischaemic Stroke

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Abstract
Acute ischemic stroke triggers a complex neuroinflammatory response involving resident glial cells, particularly microglia and astrocytes. Microglia rapidly activate following ischemia and polarize into two main phenotypes: the pro-inflammatory M1 phenotype, which exacerbates neuronal damage through the release of cytokines like TNF-α, IL-1β, and reactive oxygen species (ROS), and the anti-inflammatory M2 phenotype, which promotes tissue repair via IL-10 and IL-4 secretion. Astrocytes also play a dual role: in the acute phase, they exert neuroprotective effects by regulating glutamate homeostasis, producing antioxidants (e.g., glutathione and superoxide dismutase), and supporting neurogenesis and angiogenesis; however, in the chronic phase, reactive astrocytes contribute to glial scar formation, which inhibits axonal regeneration. The dynamic interplay between microglia and astrocytes critically shapes the neuroinflammatory milieu—determining whether the response is protective or detrimental. Understanding these dual roles offers promising avenues for developing neuroprotective therapies targeting neuroinflammation after acute ischemic stroke.

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1. INTRODUCTION

Stroke is a focal dysfunction of the brain, retina, and brainstem with an acute episode lasting approximately 24 hours (Liu, 2017). Strokes, which account for 85% of cases, often result in spinal infarction and hemorrhage in the brain, retina, and brainstem. This can occur due to intracerebral and subarachnoid hemorrhage. Risk factors such as hypertension, high apolipoprotein, psychosocial factors, smoking, heart disease, alcoholism, and diabetes are strongly correlated with stroke (Feigin, 2016).

Ischemic stroke can occur due to arterial embolism in small vessels. Embolism in the brain causes a decrease in oxygen and glucose. This leads to brain *damage* and neurological deficits. Furthermore, the molecular causes of ischemic stroke have been extensively studied, and several factors have been identified. Examples of factors associated with molecular involvement include excitotoxicity, oxidative stress, and inflammation. In excitotoxicity, brain ischemia causes the release of large amounts of glutamate. This leads to overactivity of NMDA and Ca receptors $^{2+}$which is coming in excessively. This situation will end in excitotoxicity-induced *cell death*. In addition to excitotoxicity, the process-*reperfusion* induces the formation of superoxide and nitric oxide as a result of damaged neurons and astrocytes and the depletion of glutathione, the primary antioxidant for reactive oxygen protection. Inflammatory involvement can be seen after ischemia-reperfusion,

which is caused by the presence of debris and dead cells without the presence of microbes (Chamorro, 2012).

There is considerable scientific evidence demonstrating the role of the immune system in the pathophysiology of ischemic stroke. One example is astrocytes. Astrocytes are glial cells that are quite abundant in the central nervous system. These cells play a role in various pathophysiological processes of neurological diseases. After a stroke injury occurs, astrocytes are activated and play a protective role. However, the role of astrocytes remains debated as these cells can be a double-edged sword (Shen, 2021). The role of the immune system in ischemic stroke can also be seen in macrophage/microglia cells.

2. RESEARCH METHODS

The research method used in this study was a literature review, collecting and analyzing various scientific sources discussing the role of microglia and astrocytes in the neuroinflammatory process in acute ischemic stroke. The literature search was conducted through national and international journal databases, such as PubMed*, Google Scholar,* and *ScienceDirect,* using keywords*"The role of microglia in acute ischemic stroke", "The role of astrocytes in acute ischemic stroke"*, *"Neuroinflammation mechanism",* and *"Neuroinflammatory process in acute ischemic stroke".* The literature reviewed was selected based on the relevance of the topic, the credibility of the publisher, and the limited information, with a publication period between 2015 and 2021. The collected data were then analyzed and systematically arranged into several aspects, namely the pathophysiology of ischemic stroke, neuroinflammation, and the role of microglia and astrocytes in neuroinflammation after stroke.

3. RESEARCH RESULTS AND DISCUSSION

3.1 Pathophysiology of Ischemic Stroke

Platelets, emboli, and lacunar infarctions are the causes of ischemic stroke, which can lead to infarction or decreased blood flow in the arteries. Infarction in the anterior cerebral artery causes contralateral weakness and sensory loss in the lower extremities; the medial cerebral artery causes damage to the right and left hemispheres; the posterior cerebral artery causes disorders in the superficial, deep, and bilateral parts. Decreased blood flow in the vertebral basilar artery causes cranial nerve disorders, motor deficits, and sensory loss; the basilar artery causes motor deficits and cranial nerve disorders.

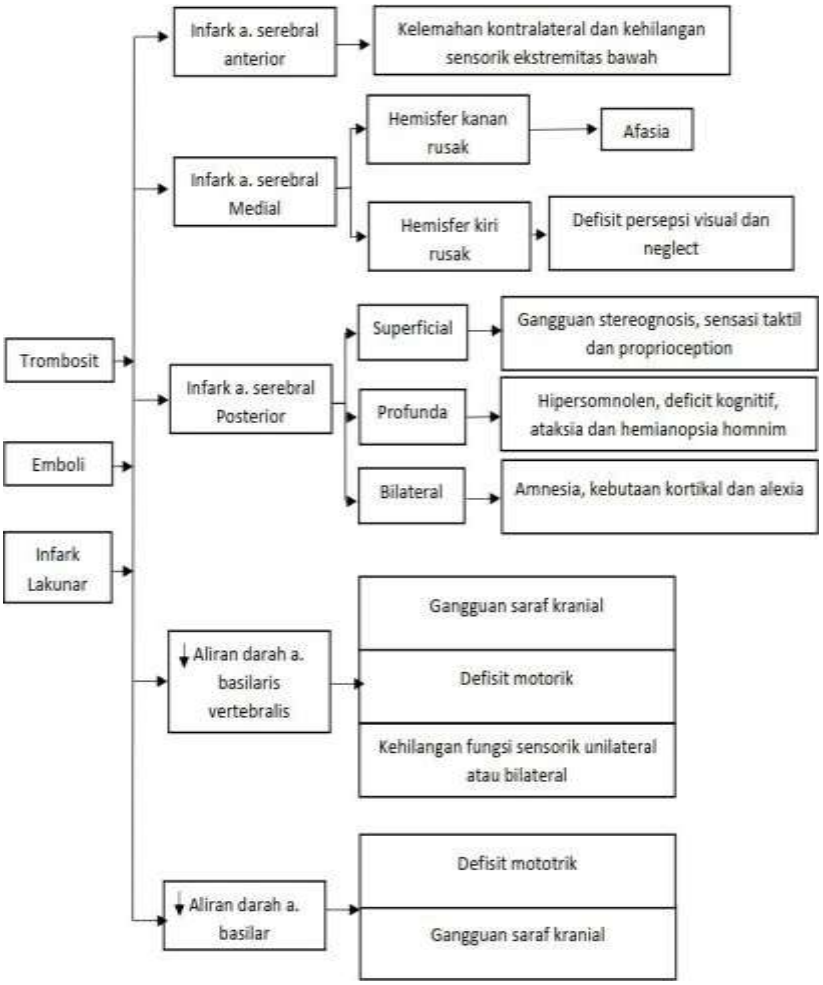

Figure 1. Pathophysiology of ischemic stroke

Ischemic stroke is caused by three factors: thrombosis, embolism, and lacunar infarction. All three cause infarction and reduced blood flow in the arteries (Randolph, 2016; Gary D. Hammer, 2018).

Arterial infarctions can occur in the anterior, medial, and posterior cerebral arteries. Arterial infarction begins with decreased blood flow, which then leads to ischemia and subsequently to arterial infarction. The anterior cerebral artery supplies blood to the medial cerebral cortex, including the frontal motor cortex, prefrontal cortex, primary motor cortex, and primary sensory cortex. The sensory and motor cortex in these areas normally function to receive sensory information and control movement of the contralateral lower extremity. An anterior cerebral artery infarction will result in reduced blood flow to the sensory and motor cortex in that area, ultimately leading to contralateral weakness and sensory loss in the extremity.

The medial cerebral artery supplies most of the lateral cerebral cortex, the basal ganglia, and the internal capsule. Medial cerebral artery infarction causes damage to the left hemisphere, resulting in aphasia, and damage to the right hemisphere, resulting in visual and perceptual deficits. *Neglect* (left-sided agnosia), can also cause contralateral hemiparesis and sensory deficits, visual field deficits, aphasia, agnosia, apraxia, and agraphia. The posterior cerebral arteries are divided into superficial and deep. The superficial posterior cerebral artery supplies the occipital lobe and the inferior portion of the temporal lobe, while the deep posterior cerebral artery supplies

the thalamus and the posterior limb of the internal capsule, as well as other deep brain structures. The occipital lobe is the location of the primary and secondary visual areas. The thalamus relays information between ascending and descending neurons, while the internal capsule contains descending fibers from the lateral and ventral corticospinal tracts.

Posterior cerebral artery infarction results in decreased blood supply in the deep areas, resulting in hypersomnolence, cognitive deficits, hypoesthesia, ataxia, and homonymous hemianopsia. In the superficial areas, it results in impaired stereognosis, tactile sensation, and proprioception. Bilateral cerebral artery infarction results in amnesia, cortical blindness, and alexia. In addition to these three arteries, decreased blood flow can also occur in the basilar artery and vertebral basilar artery. Decreased blood flow in the vertebral basilar artery results in arterial ischemia, resulting in cranial nerve (IX, X) disorders, motor deficits, and unilateral or bilateral sensory loss.

Decreased blood flow in the basilar artery results in arterial ischemia, which results in cranial nerve disorders, ipsilateral facial hypoalgesia (V), unilateral lower motor neuron facial paralysis (VII), vertigo, dysarthria (IX, X)) and motor deficits (Randolph, 2016; Asadi, 2017; Gary D. Hammer, 2018; Patti, 2021).

3.2 Neuroinflammation

3.2.1 Neuroinflammatory Mechanisms

Neuroinflammation is an inflammatory response in the brain or spinal cord. This inflammation can lead to the recruitment of immune cells, edema, tissue damage, and even cell death. Several factors can trigger nerve inflammation, including trauma, stroke, depression, tumors, and medications that can trigger neuroinflammation in the central nervous system (Kempuraj, 2016). This inflammation can be mediated by the production of cytokines, chemokines, reactive oxygen species, and other secondary messengers. These mediators can be produced by CNS resident cells such as microglia and astrocytes, endothelial cells from blood vessels in the brain, and immune cells originating from the periphery (Disabato et al., 2016).

The mechanism of neuroinflammation itself begins with a lesion on one side of the brain, such as cerebral ischemia, hemorrhage, or traumatic injury, which can induce a local neuroinflammatory reaction, in which microglia cells represent local immune cells. Neuroinflammation is a complex inflammatory process that occurs in the central nervous system as a defense mechanism against pathogens, toxins, or factors that cause neurodegeneration. This neuroinflammatory process is regulated by the activity of neurons, glia, and endothelial cells within the neurovascular system. Examples include brief, controlled inflammatory responses that are generally considered beneficial to the host organism. For example, immune signals to the brain after infection lead to subsequent reorganization of host priorities and the induction of disease behavior. Furthermore, IL-1 and IL-4 play a crucial role in maintaining learning and memory. Following traumatic central nervous system injury, IL-4-driven macrophage (M2) polarization is highly effective in promoting axonal recovery and regrowth. Immune preconditioning, or inflammation, provides a method to train the innate immune system toward a more neuroprotective phenotype (Disabato et al. 2016). Microglia cells play an active role in neuroinflammation, transmitting inflammatory signals starting from the periphery. For example, during infection, microglia become activated and function as cellular mediators of inflammation. These rapidly activated microglia can alter their transcriptional

profile and produce inflammatory cytokines and chemokines. Generally, microglial activation and increased cytokine expression are intended to protect the central nervous system and benefit the host organism (Disabato et al. 2016). Similar to microglia, astrocytes also function as inflammatory cells and can release numerous cytokines and chemokines. Astrocytes can also produce pro-inflammatory or immunoregulatory mediators appropriate to their polarization phenotype. Astrocytes can also engage in bidirectional communication with T cells, which can modulate inflammation in the central nervous system (Giovani et al. 2020).

3.2.2 Microglia and neuroinflammation

Microglia are non-specific immune system cells or innate *immunity cells* located in the central nervous system and modulating the immune response. Microglia in the brain have a specific function as early responders to tissue damage. Microglia express receptors that respond to various stimuli. Various studies have shown that microglia express different cytokine proteins for different functions. Activated microglia have two phenotypes: M1 and M2. M1 microglia are pro-inflammatory and are able to secrete cytokines such as IL-1β, TNF, IL-6, and nitric oxide, while M2 microglia are able to express anti-inflammatory mediators such as IL-10, IL-4. The results of M2 microglia expression are able to prevent the occurrence of inflammation and ongoing damage (Figure 1) (Brendeburg, 2015, and Huck, 2015).

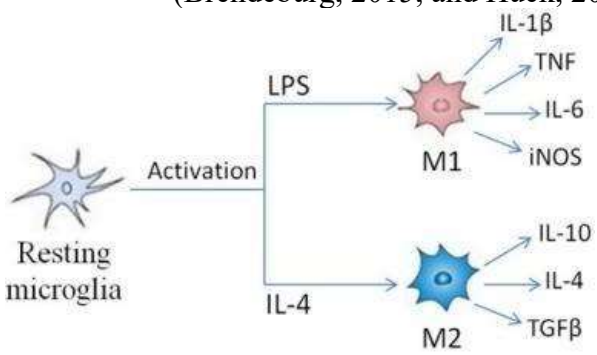

Figure 2. Two phenotypes of activated microglia, M1 and M2 (Liu R, 2017)

M1 microglia tend to cause neuronal cells to undergo apoptosis or death. Several studies have shown that the unique phenotype of M1 microglia can transform into the M2 microglia phenotype. This is evidenced by one study that linked M1 to HIV-associated *dementia*. In the HIV-associated *dementia* in this process, M1 microglia are induced, and their levels are maintained by the role of CD40L and TNFα. M1 microglia then switch to M2 through up-regulation of CD45. Under pathological conditions, the responsible stimulus likely activates microglia, causing them to change shape to initiate phagocytosis.

The role of microglia is closely related to astrocytes in releasing cytokines, which ultimately activate a cascade that modulates the neuroinflammatory response. Meanwhile, microglia are capable of producing and releasing excitotoxic metabolites that can damage surrounding tissue. Rapid-onset neuroinflammation sometimes has a good prognosis for recovery. This is different from prolonged neuroinflammation, which can damage surrounding brain tissue (Yu z, 2015; Liu r, 2017).

3.2.3 Astrocytes and Neuroinflammation

Normally, astrocytes have two main roles in the central nervous system: integrating signals and maintaining homeostasis between neurons, immunity, and

the vasculature. During neuroinflammation, astrocytes play many roles. Astrocytes limit the entry of peripheral immune cells into the CNS by limiting the blood-brain barrier and also reduce neuroinflammatory reactions (Adnyana, 2020; Linnerbauer, Wheeler, and Quintana, 2020).

Neuroinflammation caused by stroke releases inflammatory mediators. Astrocytes then respond by releasing anti-inflammatory cytokines and chemokines. Astrocytes activate the astrocyte dopamine D2 receptor (DRD2). Astrocyte dopamine receptors play a role in controlling neuroinflammation, suppressing inflammatory reactions in the lesion area by inducing the formation of αB crystalline molecules. In astrocytes lacking DRD2, there is increased production of pro-inflammatory cytokines accompanied by neuronal cell damage. A study in mice given dopamine 2 days after middle cerebral occlusion showed improved motor function accompanied by a smaller infarct area, possibly due to increased expression of neurotrophic factors by astrocytes (Adnyana, 2020).

The blood-brain barrier is formed by astrocytes, capillary endothelial cells, pericytes, and the basal lamina. The blood circulation and the central nervous system are separated by this barrier. The blood-brain barrier regulates the entry of hydrophilic substances, protects the brain from circulating pathogens, and serves as a CNS immunity. Normally, astrocytes and endothelial cells maintain the blood-brain barrier from immune cells such as leukocytes. During neuroinflammation, astrocytes and endothelial cells are unable to maintain the blood-brain barrier, resulting in a leaky blood-brain barrier and the infiltration of peripheral immune cells into the CNS. *Vascular Endothelial Growth Factor* Astrocyte-derived VEGF plays a role in a signaling cascade that increases the permeability of the blood-brain barrier. Lymphocyte-secreted pro-inflammatory cytokines and astrocyte-produced chemokines such as CCL2 and CXCL10 attract peripheral immune cells to the CNS, resulting in chronic CNS inflammation and neurodegeneration. On the other hand, astrocytes produce sonic hedgehog (Shh) to maintain the stability of the blood-brain barrier during inflammation. Immune cell infiltration is reduced. Shh induces increased secretion of angiopoietin 1, which also induces junctional A protein in endothelial cells, which can repair the permeability of the damaged blood-brain barrier. Angiopoietin 1 is necessary for blood vessel maturity (Adnyana, 2020; Linnerbauer, Wheeler, and Quintana, 2020).

3.3 The Role of Microglia in Neuroinflammation After Stroke

Neuroinflammation can occur in various brain diseases, such as ischemic stroke. An ischemic stroke, which causes brain injury, causes brain cells to undergo necrosis and apoptosis. Neuroinflammation is induced by damaged brain cells and debris. This inflammatory condition in the brain spreads to the surrounding areas. The ischemic event induced by debris and increased ROS initiates neuroinflammation and calls on surrounding microglia and astrocytes to attract leukocytes from the bloodstream. Simultaneously, microglia activation initiates their pro-inflammatory properties. This is followed by the secretion of chemokines, which also play a role in leukocyte infiltration (Liu, 2017).

Microglia are key modulators of the immune system, linked to events following ischemic stroke. When an ischemic stroke occurs, microglia change their morphology from a resting state. When perfusion begins, they are activated and assume their active form. Activated microglia are characterized by the onset of the penumbra of *motile amoeboid state* by means of phagocytosis, and microglia will

begin to engulf endothelial cells. In ischemic stroke, activation of microglia occurs in early *stages of* neuroinflammatory and lasts for several minutes. Several studies have shown that inactive microglia will lead to increased cell apoptosis after ischemic stroke (Liu, 2017).

Microglia activation during ischemic stroke directs activated microglia cells to the ischemic hemisphere. After ischemic stroke, microglia morphology changes, becoming M1 or M2 microglia. LPS and IFN-γ activate M1 microglia. This activation is associated with adverse effects after stroke. Unlike M1 microglia, M2 microglia contribute to stroke repair through anti-inflammatory effects such as IL-4. In ischemic stroke, the M2 phenotype is more numerous and dominant, although M1 microglia also progressively increase in number.*peri-infarct region*. Considering the opposing roles of microglia phenotypes in ischemic stroke, it is crucial to develop therapeutic strategies by restraining morphological transformation and promoting microglia benefits (Liu, 2017).

3.4 The Role of Astrocytes in Neuroinflammation After Stroke

Ischemic stroke is caused by occlusion of cerebral blood vessels, resulting in decreased cerebral blood flow, resulting in a complex cascade of excitotoxic and oxidative stress. This cascade activates pro-inflammatory cells such as microglia and astrocytes. Astrocytes are a type of neuroglia found in the brain. They become active within hours of cerebral ischemia. Astrocytes that undergo hypertrophy and proliferation are called reactive astrocytes. Reactive astrocytes first increase in the thalamus, hippocampus, and black substance on the first or second day after the stroke (Jayaraj et *al.*, 2019; Stuckey *et al.*, 2021).

In the chronic stage, astrocytes inhibit axon recovery and regeneration. Reactive astrocytes increase the expression of proteins such as monocyte *chemotactic protein-1,* IL-1β, *glial fibrillary acidic protein* (GFAP), *vimentin,* and *nestin,* which play a role in scar formation. Astrocyte scars first form in the thalamic nuclei seven weeks after stroke. Astrocyte scars inhibit axon regeneration, resulting in neurotoxicity, inflammation, and chronic pain (Cekanaviciute and Buckwalter, 2016; Jayaraj et *al.*, 2019; Xu *et al.*, 2020).

Astrocytes play a role in maintaining synaptic activity and neuronal excitability. Glutamate is an excitotoxic neurotransmitter for neurons. Excess glutamate is returned by astrocytes through the activity of glutamate transporter-1 (GT-1) and glutamate aspartate transporter (GLAST) in the glutamate-glycine cycle. Ischemic stroke results in decreased oxygen and glucose levels in the brain, resulting in increased glutamate concentrations. In ischemic stroke, there is a downregulation of glutamate transporter-1 (GLT-1) and glutamate aspartate transporter (GLAST), allowing glutamate to cause excitotoxic conditions, resulting in neuronal damage. The production of free radicals such as nitric oxide, superoxide, and peroxynitrite also increases. Free radicals cause neuronal death through a cascade of apoptosis and necrosis. Astrocytes maintain neurons by releasing glutathione and *superoxide dismutase*(SOD). Astrocytes also express the neuroprotective factor NrF2, which plays a role in free radical scavenging, detoxifying xenobiotics, and maintaining redox potential (Cekanaviciute and Buckwalter, 2016; Li et *al.*, 2017; Adnyana, 2020).

Astrocytes play a role in neurovascular repair after cerebral ischemia. Astrocytes produce neurotrophic factors for neurovascular tissue repair after an injury. A study in *vitro,* astrocytes act a stem *cell for* Neurogenesis. The protein β-arristin 1 (β-arr 1), produced by astrocytes in the dentate gyrus, plays a role in hippocampal neurogenesis. High-mobility group box 1 (HMGB1), released by astrocytes, also plays

a role in neurogenesis. Blood circulation after cerebral ischemia becomes unstable. Astrocytes induce angiogenesis by secreting several chemical agents such as TGF-β, GDNF, bFGF, and angiopentin-1. These growth factors stimulate the growth of new blood vessels and the proliferation of endothelial progenitor cells (EPCs) (Adnyana, 2020).

4. CONCLUSION

Stroke is defined as a sudden, acute reduction in blood supply to the brain, lasting more than 24 hours, which can result in brain tissue damage. One type of stroke is ischemic stroke, which is characterized by a neuroinflammatory process. Ischemic stroke activates microglia and astrocytes in a neuroinflammatory process followed by the release of cytokines. This neuroinflammatory process aims to repair damaged brain tissue. However, the activation of microglia and astrocytes can also have detrimental effects on brain tissue damage.

5. ACKNOWLEDGEMENT

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