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Role of microglia in ischemic focal stroke and recovery: focus on Toll-like receptors

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ABSTRACT

Stroke is the leading cause of disability in adults. Drug treatments that target stroke-induced pathological mechanisms and promote recovery are desperately needed. In the brain, an ischemic event triggers major inflammatory responses that are mediated by the resident microglial cells. In this review, we focus on the microglia activation after ischemic brain injury as a target of immunomodulatory therapeutics. We divide the microglia-mediated events following ischemic stroke into three categories: acute, subacute, and long-term events. This division encompasses the spatial and temporal dynamics of microglia as they participate in the pathophysiological changes that contribute to the symptoms and sequela of a stroke. The importance of Toll-like receptor (TLR) signaling in the outcomes of these pathophysiological changes is highlighted. Increasing evidence shows that microglia have a complex role in stroke pathophysiology, and they mediate both detrimental and beneficial effects on stroke outcome. So far, most of the pharmacological studies in experimental models of stroke have focused on neuroprotective strategies which are impractical for clinical applications. Post-ischemic inflammation is long lasting and thus, could provide a therapeutic target for novel delayed drug treatment. However, more studies are needed to elucidate the role of microglia in the recovery process from an ischemic stroke and to evaluate the therapeutic potential of modulating post-ischemic inflammation to promote functional recovery.

1. Introduction

Stroke is the fifth leading cause of death in the United States of America (Mozaffarian et al., 2016) and a leading cause of disability in adults. Approximately 10 million patients survive stroke annually. In the USA alone, the direct and indirect annual costs in 2012 were $33 billion for the acute and chronic treatment of stroke (Mozaffarian et al., 2016). The primary risk factor for stroke is age, and as life span increases the number of people suffering from stroke is rising. Slow and incomplete recovery is associated with a reduction in quality of life, which is compounded by the lack of drug treatments to facilitate the recovery process. Acute care relies on thrombolytic treatment using tissue plasminogen activator (TPA), but it can be administered only to a small fraction of patients. Furthermore, even when TPA is given within the therapeutic window (4.5 h) it has inconsistent efficacy.

Focal ischemic stroke is caused by an arterial thrombus that blocks blood flow to the brain and leads to a loss of oxygen and glucose supply to the downstream tissue. Anaerobic conditions result in protein and lipid modification, dysfunction of ionic pumps, loss of membrane potential, Ca^{2+} dysregulation, mitochondrial dysfunction, endoplasmic reticulum (ER) stress and apoptosis (Dirnagl et al., 1999). The region that is entirely dependent on the vasculature immediately downstream of the thrombosis can start to die within minutes, and forms the core of the infarct. The area surrounding the infarct, the penumbra, has a partial loss of blood flow in addition to an extracellular milieu that is dominated by chemicals diffusing from necrotic cells within the infarct area. The initial injury also includes an excessive and uncontrolled release of neurotransmitters, such as glutamate, from stressed cells that triggers excitotoxicity in local cells. The peri-infarct region may be minimally impacted by the diminished blood flow directly (if spatially distant from the thrombus), but cells in this region must cope with indirect effects such as excitotoxicity. Restoring blood flow leads to deoxygenation and the formation of reactive oxygen species that contribute to reperfusion damage (Nour et al., 2013). These neurodegenerative processes in the ischemic brain result in disruption of neural circuits such as those that control motoric, sensory and cognitive functions.

The treatment strategies for stroke can be divided into four different categories: prophylaxis, neuroprotection, thrombolysis and promotion of recovery. Prophylactic approaches include healthy lifestyle and treatment of high blood pressure and cholesterol levels. Many of the failed clinical trials for stroke were focused on neuroprotection. Most experimental stroke therapies are designed to intervene within a narrow

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time-window after the ischemic event in order to prevent the stressed cells in the peri-infarct area from dying, and it has been shown that neuroprotective efficacy is highly dependent on the time of administration (Belayev et al., 2001). The thrombolysis approach given within 4.5 h is the only one that has shown efficacy, although it is also limited, and can lead to reperfusion injury. The inability to translate the preclinical experiments to the clinic has resulted in to proposals that next generation therapeutic strategies should focus on outcomes other than lesion size (Dirnagl, 2012). Instead, the goal should be to target the pathophysiological mechanisms that dominate during the days and weeks following the initial ischemic event (Dirnagl, 2012). For example, new therapies could be developed that target microglia-mediated inflammation following a stroke, which would extend the therapeutic window from hours to days.

Microglia are the resident macrophages of the central nervous system and regulate tissue homeostasis throughout life. The proportion of glial cells in the mouse brain that are microglia is estimated at 5–12% (Lawson et al., 1990). Microglia are a distinct subtype of mononuclear macrophages, which is a class that also includes peripheral tissue macrophages, dendritic cells and monocyte-derived cells (Butovsky et al., 2012; Gautier et al., 2012; Prinz and Priller, 2014). The complex inflammatory response in the brain following a stroke includes activation of microglia and infiltration of peripheral immune cells into the brain parenchyma. In a stroke, an immune response is triggered by proteins released rapidly from dead and damaged cells. Toll-like receptors (TLRs) are a key class of receptors that regulate microglia activation. TLRs are pattern recognition receptors, imperative to the immune system for the recognition of pathogen-associated molecular patterns (PAMPs) and endogenous-derived danger-associated molecular patterns (DAMPs). They are expressed by a variety of cells, spanning the periphery and immune-privileged brain. There are a variety of TLRs (nomenclature ranging 1–11 in humans), which differ in PAMP/DAMP recognition and location (Akira and Takeda, 2004). For example, TLR4 and TLR2 are localized to the cell surface and become activated upon the recognition of lipopolysaccharide (LPS). Other TLRs, such as TLR9, are localized intracellularly within endosomal membranes and primarily recognize nucleic acids. Some TLRs, TLR4 and TLR2 in particular, can recognize DAMPs from ischemic stroke events such as heat shock proteins, fibrinogen, RNA and methylated DNA (Fadakar et al., 2014). While the expression of all TLR orthologues has been reported in mouse microglia, TLR4 and TLR2 are the most predominantly expressed TLRs in the human brain (Nishimura and Naito, 2005; Olson and Miller, 2004).

2. Microglia in ischemic stroke

In ischemic injury, microglial cells undergo changes in morphology and gene expression that are known collectively as microglial activation (Kettenmann et al., 2011). In healthy tissue, microglia have a ramified structure of highly motile processes that are actively sensing and maintaining homeostatic conditions within the area surrounding that individual microglial cell. In ischemic injury, microglial cell retracts finer processes and may exhibit chemotaxis to DAMPs released from the dead and dying cells. When activated after stroke, microglial cells progress through four morphological states that are indicative of increasing activation (Fig. 1): ramified, intermediate, amoeboid and round (Lehrmann et al., 1997; Thored et al., 2009). **Ramified** microglia are in the resting state, with small cell body and long processes and can be found in the contralateral side of the ischemic brain and in distal areas in the ipsilateral side. The **intermediate** state of activated microglia is characterized by enlarged cell body and short processes. **Amoeboid** microglial cells have very short or no processes and an amoeboid shape. Intermediate and amoeboid types of cells can be found in the peri-infarct region (Anttila and Airavaara, 2016 unpublished results; Lehrmann et al., 1997). **Round** microglia are macrophage-like and the most activated form of microglia. Round cells are found in the infarct core region (Anttila and Airavaara, 2016 unpublished results; Lehrmann et al., 1997). While following the same morphological progression, the intracellular dynamics and protein production of microglial cells can be categorized as either M1-like ('classical') or M2-like ('alternative') (Tang and Le, 2016). **M1 type is pro-inflammatory** and characterized by inducible nitric oxide synthase and nuclear factor kappa B (NF-κB) activation, and production and release of nitric oxide and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β). **M2 type is** often **anti-inflammatory** and characterized by the production of molecules that support anti-inflammation and tissue repair such as insulin-like growth

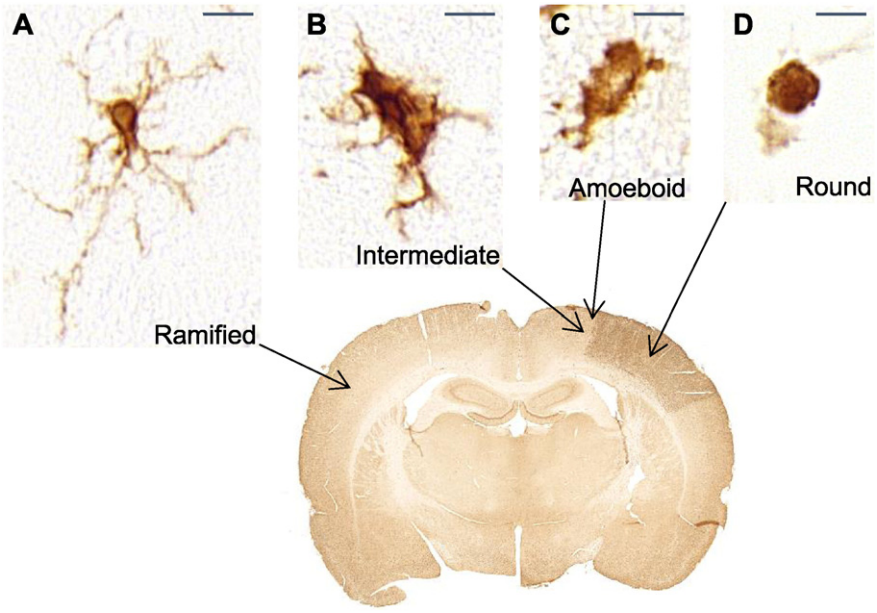

Fig. 1. Microglia can be classified into four different phenotypes based on the morphology of the cell: A) ramified, B) intermediate, C) amoeboid and D) round type. 90 min transient MCAo was induced in adult male Sprague Dawley rats as described earlier (Airavaara et al., 2009). Rat brains were perfusion-fixed 7 days post-stroke, embedded in paraffin, sectioned, and immunostained for Iba1. Ramified microglial cells are found in the contralateral side of the brain. Intermediate and amoeboid type cells are found in the peri-infarct region. Round type cells are found in the ischemic core region (dark brown). Categorization of microglia morphology is adopted from Lehrmann et al., 1997; Thored et al., 2009. Scale bar is 10 μm.