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Involvement of Toll-like Receptors in Ischemic Stroke Induced Neuronal Damage

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Abstract

Post-ischemic inflammation is an essential step in the progression of ischemic stroke. Infiltrating macrophages are known to serve as a key mediator of the innate immune response to danger-associated molecular pattern molecules (DAMPs) by their expression of Toll-like receptors (TLRs) because activation of TLRs of macrophages leads to the secretion of proinflammatory cytokines. This review focuses on the involvement of Toll-like receptors in ischemic stroke induced neuronal damage .

ABBREVIATIONS

DAMPs: Danger-Associated Molecular Pattern Molecules; **BBB:** Blood-Brain- Barrier; **TLRs:** Toll-like receptors; **HMGB1:** High mobility group box 1; **Prx:** Peroxiredoxin

INTRODUCTION

Innate immunity plays an important role in inflammation-related neuronal injury associated with ischemic stroke. Infiltrating macrophages are known to serve as a key mediator of the innate immune response to danger-associated molecular pattern molecules (DAMPs) by their expression of Toll-like receptors (TLRs). Since the brain has a very high glucose and oxygen demand, disturbances in the blood supply to the brain rapidly lead to the development of an ischemic infarct with accompanying necrosis of neurons and generation of DAMPs.

Danger-associated molecular pattern molecules (DAMPs)

In the ischemic brain, Heat shock proteins, β-amyloid (Aβ), hyaluronan, heparin sulfate, DNA or RNA immune complexes, oxidized low-density lipoproteins, and several other molecules, have been considered as possible DAMPs [1]. Among them, high mobility group box 1 (HMGB1) is a well characterized DAMP in ischemic brain injury that increases Blood-Brain- Barrier (BBB) permeability or promote its breakdown [2,3]. The HMGB1 level in the ischemic stroke group is significantly increased compared with that of control group, and has been correlated with the severity of neurologic impairment observed in stroke patients [4]. HMGB1, which is localized in cell nuclei in the normal brain, translocates into the cytosolic compartment and is released into the extracellular compartment in the presence of an ischemic condition. The administration of anti-HMGB1-neutralizing antibody protects the BBB and reduces infarct volume. Taken together, HMGB1 is an essential DAMP in ischemic brain injury. Another potential DAMPs in brain homogenate lysates are peroxiredoxin (Prx) family proteins which have been identified as strong inducers of inflammatory cytokines in infiltrating macrophages [5]. They are released into the extracellular compartment once the cells are about to die, functioning as DAMPs. Neutralization of Prx proteins rather than HMGB1 by specific antibodies has been shown to suppress inflammatory cytokine expression in the ischemic brain [5].

Toll-like receptors; TLRs

Toll-like receptors (TLRs) are essential receptors involved in the innate immune response to general pathogens such as bacteria and viruses. Using peripheral blood samples from MRI-diagnosed patients with acute ischemic stroke, a recent study identified a 9-gene profile, which indicates that TLR signaling is a mediator in the response to ischemic stroke [6]. TLRs are a family of at least 11 proteins, and recently, TLR2 and TLR4 have been reported to contribute to non-infectious immune-mediated injury, including ischemic brain injury [7]. Indeed, HMGB1, Prx proteins, and other DAMPs can stimulate TLR2 and TLR4. The activation of macrophages and T cells through TLR pathway induces strong inflammatory responses, because TLR2- or TLR4-deficiency significantly attenuates ischemic brain damage and suppresses inflammatory cytokine expression in infiltrating immune cells on day 1 after ischemia-reperfusion in mice [8]. Moreover, cultured monocytes are treated with serum from ischemic stroke patients, showing a strong inflammatory response that was blocked when TLR2 or -4 were blocked [9]. While these lines of evidence suggest that TLRs are activated in immune cells (T cells,

dendritic cells, and macrophages), recent study demonstrates that neurons also express TLR2 and -4, which are up-regulated and activated in response to ischemic conditions. Interestingly, in LPS treated rat hypoxia ischemic model, IL-1β is expressed mainly by neurons at 4 h post-aggression and, later, by neurons and microglial cells at 48 h. This IL-1β expression exacerbates further neuroinflammation and cerebral injury resulting in delayed onset of profound motor impairments [10].

TLR stimulation by DAMPs results in downstream activation of MyD88- and/or TRIF-dependent pathways leading to activation of nuclear factor kappa B (NFkB) - and/or IRF3-dependent gene transcription. This triggers the synthesis of primarily microglia-derived pro-inflammatory cytokines, such as TNF-α and IL1β [11]. The infiltration of macrophages becomes evident from 12h to 24h after ischemia-reperfusion and reaches a peak on day3 [12,13]. TNF-α is major mediator implicated in the pathology of the ischemic brain. It is expressed in ischemic brain tissue within 1 h after stroke onset, followed by upregulation of the TNF receptors. TNF-α exercises neurotoxic effects by inducing apoptotic neuronal cell death and enhancing MHC class II and ICAM-1 expression in astrocytes, leading to leukocyte infiltration and BBB breakdown. Furthermore, TNF-α is known to regulate synaptic signaling and synaptic plasticity and induce structural changes at the synaptic level [14]. TNF-α gene knockout (KO) mice or anti-TNF-α neutralizing antibody administration has been shown to reduce infarct volume, compared with that in control mice [1].

IL-1β is another important mediator, expressed in ischemic brain tissue within 30 min after stroke onset. IL-1β directly induces apoptosis of neuronal cells and enhances the expression of chemokine in immune cells. IL-1β is considered to be a neurotoxic mediator, given that the loss of IL-1β function is reported to reduce infarct size [15]. IL-1β is mostly produced from monocytes including macrophage which are activated by endogenous TLR ligands such as onosodium urate (MSU) crystals. IL-1β is produced in an inactivate form, pro-IL-1β, which is cleaved by caspase-1 to become an active 17 kDa form. Recently, the mechanism of IL-1β production and caspase-1 activation mediated by inflammasome has been the subject of particular attention. Multi-protein complexes known as inflammasomes (e. g. containing NLRP1, NLRP2, NLRP3, NLRP6, NLRP7, NLRP12, NLRC4, AIM2 and/or Pyrin), signaling through NLRP1 and NLRP3 inflammasomes produces cleaved caspase-1, which cleaves pro-IL-1β during the ischemic stroke [16]. Inflammasomes have been shown to be present in neurons, astrocytes, microglia, and macrophages in the ischemic brain [17]. A recent experimental study demonstrated that intravenous administration of a caspase-1 inhibitor and intravenous immunoglobulin significantly decreased caspase-1 activation, maturation of IL-1β and IL-18, and infarct size by suppressing NLRP1 and NLRP3 inflammasome activity following reperfusion in a transient mouse model of focal ischemic stroke [16].

In summary, recent findings have provided insight into a new inflammatory mechanism in the TLRs mediated innate immune system that may contribute to neuronal death during cerebral ischemia. Administration of an anti-TLR blocking antibody has emerged as a neuroprotective therapy for ischemic myocardial or renal injury [18;19]. To explore the therapeutic potential of this strategy, more detailed knowledge about the functions of TLRs mediated innate neuroinlflammation in the ischemic brain is needed.

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