Activated microglia enhance astroglial neuroprotective pentose-phosphate pathway through the activation of the Keap1/Nrf2 system by nitric oxide

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ABSTRACT

Objectives: Both astroglia and microglia express toll-like receptor 4 (TLR4) that plays a pivotal role in the stroke-induced inflammation. Endogenous ligands for TLR4 produced in the ischemic brain induce inflammatory responses. Both reactive oxygen species (ROS) and nitric oxide (NO) produced by TLR4 activation play harmful roles in neurovascular unit damage. Although astroglia exhibit pro-inflammatory responses upon TLR4 stimulation by lipopolysaccharide (LPS), they may also play cytoprotective roles via the activation of the pentose-phosphate pathway (PPP), reducing oxidative stress with glutathione. We investigated the mechanisms by which astroglia reduce oxidative stress via the activation of PPP in concert with microglia.

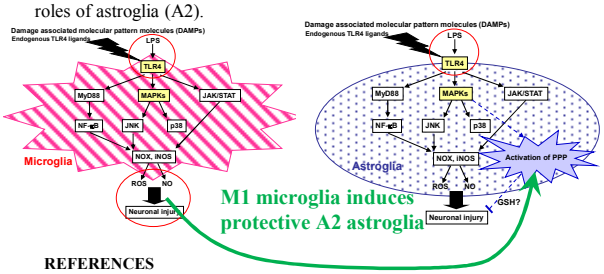
Methods: In vitro experiments were performed using cells prepared from Sprague–Dawley rats. Coexisting microglia in the astroglial culture were chemically eliminated using L-leucine methyl ester (LME). Cells were exposed to LPS (0.01 μg/mL) for 12–15 h. PPP activity was measured using [1-^{14}C]glucose and [6-^{14}C]glucose. ROS and NO production were measured using fluorescent indicators. The involvement of nuclear factor-erythroid-2-related factor 2 (Nrf2) that regulates glucose 6-phosphate dehydrogenase, the rate-limiting enzyme of PPP and glutathione synthesis was evaluated using immunohistochemistry.

Results: Cultured astroglia exposed to LPS elicited 20 % increases in PPP flux, and these actions of astroglia appeared to involve Nrf2. However, the chemical depletion of coexisting microglia eliminated both increases in PPP and astroglial nuclear translocation of Nrf2. LPS induced ROS and NO production in the astroglial culture containing microglia but not in the microglia-depleted astroglial culture. U0126, an upstream inhibitor of mitogen-activated protein kinase, eliminated LPS-induced NO production, whereas ROS production was unaffected. U0126 also eliminated LPS-induced PPP activation in astroglial–microglial culture, indicating that microglia-derived NO mediated astroglial PPP activation. SNAP, an NO donor, did indeed induce PPP activation in astroglia.

Conclusions: Astroglia in concert with microglia may play a cytoprotective role for countering oxidative stress in stroke.

MAIN POINTS

DAMPs derived from damaged tissue stimulate microglial TLR4, resulting in NO production and neuronal damage. NO released from microglia (M1), in turn, activates astroglial Keap1/Nrf2 system, inducing neuroprotective roles of astroglia (A2).



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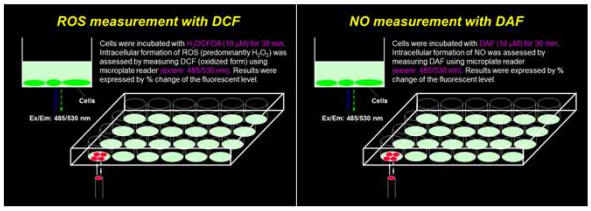
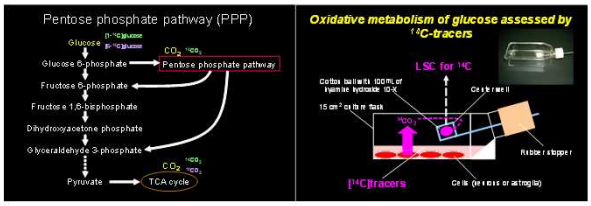
Iizumi T, Takahashi S, et al: J Neuroinflammation 13(1):99, 2016
Liddelow SA, et al: Nature 541(7638):481-487, 2017.

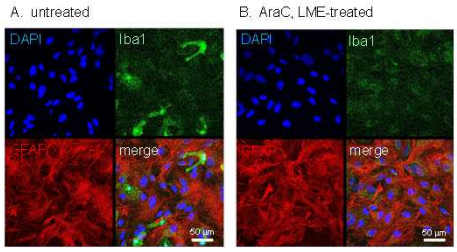
Method 1: Semi-quantitative measurement of reactive oxygen species (ROS) and nitric oxide (NO) production
(Izawa Y, Takahashi S, et al: Brain Res 1305: 64-73, 2009)



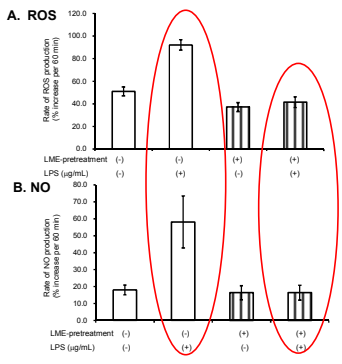
Method 2: Quantitative measurement of the PPP activity
(Hothersall et al., Arch Biochem Biophys 198:478-92,1979)



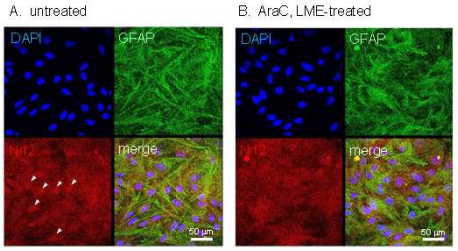
Result 1: Ara-C followed by leucine methylester (LME) treatment eliminates microglia from astroglial culture from SD rat



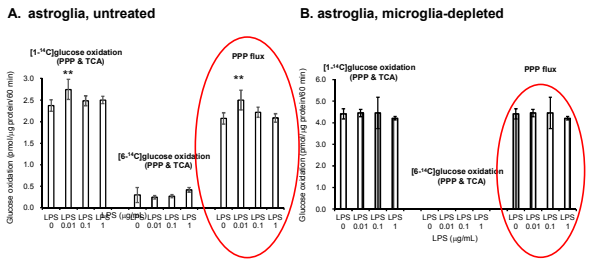
Result 2: ROS and NO are generated by co-existing microglia



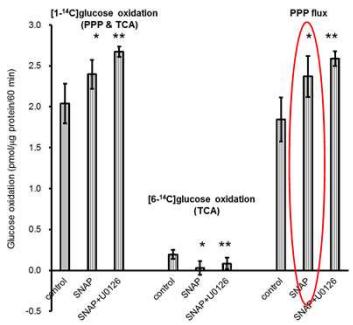
Result 3: Microglial depletion eliminates astroglial nuclear translocation of Nrf2 in response to LPS



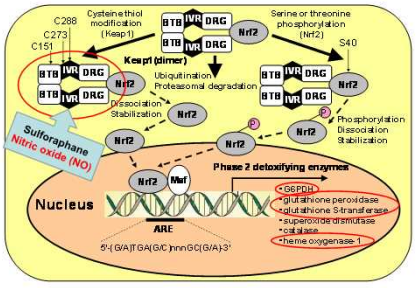
Result 4: Microglial depletion eliminates astroglial PPP activation in response to LPS



Result 5: NO donor (SNAP) activates astroglial PPP independently of U0126



A model for the Kelchlike ECH-associated protein 1 (Keap1)/nuclear factor-erythroid-2-related factor 2 (Nrf2) interaction and activation (Takahashi S, et al: Clinical Neurology 52:41-51, 2012)



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