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INVITED REVIEW

Interferon regulatory factor 2 binding protein 2: a new player of the innate immune response for stroke recovery

Hsiao-Huei Chen[1,*], **Alexandre F. R. Stewart**[2]
1 Ottawa Hospital Research Institute, Ottawa, Canada
2 University of Ottawa Heart Institute, Ottawa, Canada

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Abstract
Ischemic brain injury triggers an inflammatory response. This response is necessary to clear damaged brain tissue but can also exacerbate brain injury. Microglia are the innate immune cells of the brain that execute this critical function. In healthy brain, microglia perform a housekeeping function, pruning unused synapses between neurons. However, microglia become activated to an inflammatory phenotype upon brain injury. Interferon regulatory factors modulate microglial activation and their production of inflammatory cytokines. This review briefly discusses recent findings pertaining to these regulatory mechanisms in the context of stroke recovery.

Key Words: *interferon regulatory factors; interferon beta; microglia; interferon regulatory factor 2 binding protein 2; stroke; inflammation; synaptic pruning; anxiety*

Correspondence to:
Hsiao-Huei Chen, Ph.D., hchen@uottawa.ca.

orcid:
0000-0003-2914-6057 (Hsiao-Huei Chen)

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M1/M2 Polarization
The ischemic brain injury of stroke triggers an acute and sustained inflammatory response of the brain-resident macrophages called microglia. Upon stroke injury, debris of dying neurons and astrocytes is recognized as damage-associated molecular patterns (DAMP) that activate Toll-like receptors (TLR) and the TLR signalling cascade. Microglia activation after stroke proceeds in an orderly transition from a proinflammatory M1 phenotype to an anti-inflammatory and restorative M2 phenotype. Bacterial lipopolysaccharides (LPS) induce M1 markers, including the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL) 1β, IL12, as well as monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1a (MIP-1a), cyclooxygenase 2 (Cox2) and inducible nitric oxide synthetase (iNOS). IL4 induces M2 markers including the anti-inflammatory cytokine IL10, arginase 1 (Arg1), mannose receptor C type 1 (Mrc1; also as CD206), Ym1 and found in inflammatory zone 1 (Fizz1) (Murray et al., 2014; Chen et al., 2015). Experimental stroke studies showed that increased pro-inflammatory M1 polarization is associated with larger infarction and worse stroke outcome, whereas anti-inflammatory M2 polarization resolves inflammation, limits stroke injury progression and promotes tissue regeneration and recovery from stroke injury (Cruz et al., 2017).

Interferon Regulatory Factor (IRF) in Microglia Polarization
Growing evidence points to the IRF family of transcription factors as playing a key role to control the microglial inflammatory phenotype, and also a central role in defending against viral infection through the activation of the interferon response. IRF1 is a transcriptional activator that is activated by ischemic brain injury and promotes M1 polarization. IRF1-null mice have a reduced infarction after ischemic brain injury (Iadecola et al., 1999). On the other hand, IRF2 binds to the same sequences as IRF1 but works as a transcriptional repressor through its interaction with the corepressor IRF2 binding protein 2 (IRF2BP2) (Childs and Goodbourn, 2003; Teng et al., 2010, 2011). Although the effects of IRF2 in the context of stroke remain to be seen, overexpression of IRF2 was shown to limit ischemia/reperfusion injury in the liver (Klune et al., 2012). Moreover, a recent study by Cruz et al. (2017) showed that microglial-specific ablation of IRF2BP2 enhanced M1 inflammatory gene expression, impaired M2 phenotypic transition of microglia and worsened ischemic brain injury after stroke (**Figure 1A**). Thus, this study indicates that IRF2 acts to counterbalance the inflammatory response caused by IRF1.

Interferon Beta (IFNβ) Protects from Stroke Injury
IFNβ expression and signalling are activated by bacterial and viral infection to limit the spread of infection. IFNβ signaling is not only important to ensure pathogen clearance but also to prevent excess inflammation (IL1β activation) and tissue destruction, a process that was reported to be dependent on IRF5 (Castiglia et al., 2016). IFNβ is also activated by ischemic brain injury (Marsh et al., 2009) and both endogenous and exogenous IFNβ reduce inflammation and infarction (Marsh et al., 2009; Inácio et al., 2015; Kuo et al., 2016). A recent report by Cruz et al. (2017) showed that activation of IFNβ signaling in the context of stroke also suppresses IL1β and limits brain in-

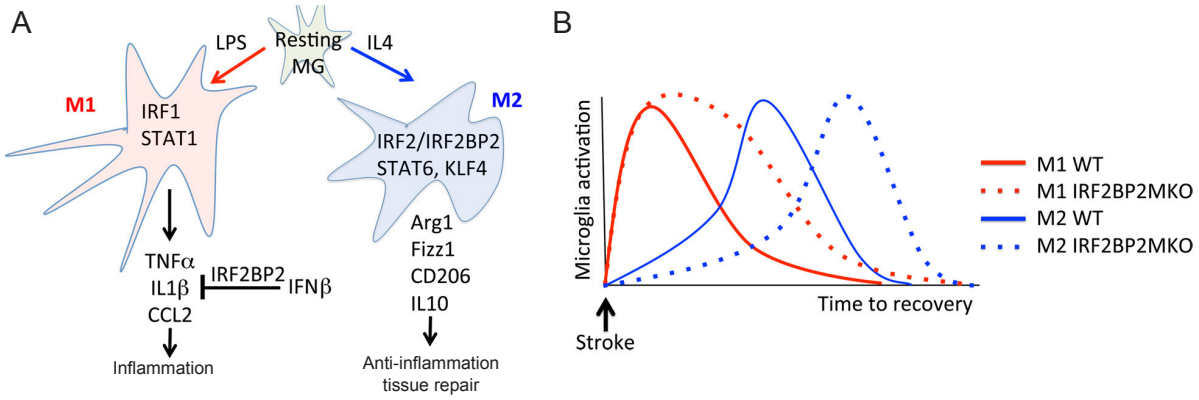

Figure 1 IRF2BP2 is required for microglia M2 polarization and anti-inflammatory effect of IFNβ.
(A) Resting MG are activated to the M1 phenotype by LPS and to the M2 phenotype by IL4. IFNβ suppresses IL1β expression and this effect requires IRF2BP2. (B) IRF2BP2-deficient MG have prolonged M1 and delayed M2 activation after stroke and this is associated with larger infarction and worsened functional deficits. IRF2BP2: Interferon regulatory factor 2 binding protein 2; IFNβ: interferon beta; MG: microglia; LPS: lipopolysaccharides; IL4: interleukin 4; IL1β: interleukin 1β; IRF1: interferon regulatory factor 1; IRF2: interferon regulatory factor 2; STAT1: signal transducer and activator of transcription 1; TNF-α: tumor necrosis factor-α; IL1: interleukin-1; CCL2: chemokine (C-C motif) ligand 2; IFN1β: interferon 1 beta; STAT6: signal transducer and activator of transcription 6; KLF4: Kruppel-like factor 4; Arg1: arginase 1; Fizz1: found in inflammatory zone 1; CD206: mannose receptor C type 1 (Mrc1); IL10: interleukin 10; WT: wild type; IRF2BP2MKO: IRF2BP2 microglia/macrophage knockout.

flammation and tissue damage. Importantly, and unexpectedly, this process requires IRF2BP2 in microglia. Since IRF2BP2 only interacts with and co-represses IRF2 (and not IRF1 or IRF5), this also implicates IRF2 in the protective effect of IFNβ signaling after stroke. Whether the IRF2 and IRF5 pathways work in parallel or converge to control IL1β remains to be determined.

IRF2BP2 Expression in Microglia/ Macrophages

The effect of IFNβ on IRF2BP2 expression in microglia is not known. In macrophages, expression of IRF2BP2 is dynamically regulated; inflammation suppresses IRF2BP2 expression whereas anti-inflammatory stimulation by IL4 elevates IRF2BP2 expression (Chen et al., 2015). As in microglia, ablation of IRF2BP2 in macrophages promotes the inflammatory M1 phenotype and interferes with IL4-induced M2 polarization and worsens atherosclerosis in susceptible mice (Chen et al., 2015).

The inflammation caused by ischemic brain injury would suppress IRF2BP2 expression and this may compromise the full beneficial effect of IFNβ to limit stroke injury. Recent studies identified several ischemia-induced microRNAs, including miR-107 (Yang et al., 2014; Bhatia et al., 2016) and miR-155 (Arruda et al., 2015) that target and suppress IRF2BP2 expression. Antagonism of miR-155 promotes stroke recovery (Caballero-Garrido et al., 2015), an effect likely mediated by increased IRF2BP2 protein levels. Future studies will be required to test whether the therapeutic effect of IFNβ for stroke recovery can be improved by antagonism of miR-107 and miR-155 in order to maintain IRF2BP2 expression.

Synaptic Pruning in Stroke Recovery

Focal ischemic lesions produce a well-documented reorganization of the sensorimotor cortex (Harrison et al., 2013), a process that is dependent on tissue repair and synaptic pruning by microglia (Paolicelli et al., 2011). Whether M2 microglia are better at synaptic pruning than M1 microglia, or target different types of synapses for pruning, are important questions that remain to be elucidated. Nonetheless, the study by Cruz et al. (2017) showed more severe functional deficits using the adhesive removal test, suggesting that in the absence of IRF2BP2, the reorganization process in the sensorimotor cortex after stroke is impaired likely due to more severe inflammation. In this paper, IRF2BP2-deficient microglia showed no difference in their phagocytic ability to engulf micro-particles after LPS stimulation compared to wild type (WT) microglia. Whether synaptic pruning *in vivo* is defective in IRF2BP2-deficient microglia remains to be seen. However, since there were fewer IRF2BP2-deficient M2 microglia in the peri-infarct area compared to littermate controls, and M2 microglia are associated with tissue repair, this could account for the delayed regression of the ischemic lesion. **Figure 1B** illustrates how IRF2BP2-deficient microglia likely have a delayed transition to the M2 phenotype that accounts for a prolonged inflammatory lesion and impaired recovery.

Inflammatory cytokines can also directly affect synaptic structure and neuronal network connectivity. TNFβ modulates synaptic transmission and synaptic scaling (Stellwagen and Malenka, 2006) and also increases the turnover of dendritic spines and axonal boutons, contributing to early synaptic abnormality in somatosensory cortex in mouse models of experimental autoimmune encephalomyelitis (Yang et al., 2013). Pathological levels of IL1β have been shown to impede synaptic long-term potentiation (Ross et al., 2003). The inflammatory cytokine IL1β is elevated not only at the area surrounding the infarction but also at the contralateral cortex 48 hours after ischemic stroke (Davies et al., 1999).

Astrocytes as Mediators of Microglial Inflammation

Although not addressed in the Cruz et al. (2017) study, astrocytes are another key player in the response to stroke injury. Like microglia, astrocytes undergo an A1 and A2 polarization in response to brain injury. A recent study by Liddelow et al. (2017) showed that activated microglia activate an inflammatory A1 phenotype of adjacent astrocytes that precipitate

neuronal death following injury. Since IRF2BP2 is expressed in astrocytes and its expression is elevated in activated astrocytes (Liu et al., 2006), the function of IRF2BP2 in astroctyes and whether it affects their A1 polarization are important questions for future studies and may be relevant to stroke therapy.

Inflammation and Anxiety

Another consequence of inflammation after stroke is the appearance of affective mood disorders (anxiety and depression) that can occur in the absence of an obvious sensory-motor deficit, long after the initial ischemic insult. Histological (Nilupul Perera et al., 2006) and positron emission tomography (PET) imaging (Gerhard et al., 2005; Gulyás et al., 2012) studies detect inflammatory brain microglia/macrophages in the area of the ischemic lesion that can persist for several months. Ischemic lesions to the left prefrontal cortex, a brain region important for mood control, can produce anxiety and depression with minimal deficits in sensory and motor function in humans and mice (Terroni et al., 2011; Vahid-Ansari et al., 2016). Inflammatory microglia are tied to anxiety-like behaviours in mice (Li et al., 2014; McGuiness et al., 2016) and mice with IRF2BP2-deficient microglia are resistant to the anxiolytic effect of enhanced postnatal care (Hari et al., 2017). It will be interesting to see whether mice with IRF2BP2-deficient microglia display more severe post-stroke affective mood disorders. Together, these reports confirm a deleterious effect of brain inflammation and that its rapid resolution after stroke is desirable for functional recovery.

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