PERSPECTIVE

Polarizing the immune system towards neuroprotection in brain ischemia

The development of ischemic brain damage is dramatically affected by the immune system, whose activation occurs immediately after the insult and may last for several days, involving a complex interplay between soluble and cellular mediators (Amantea et al., 2015). Accordingly, recent expression profiling studies have revealed that the majority of the genes modulated in the blood of stroke patients participate in the regulation of innate immune responses (Brooks et al., 2014). Moreover, in the clinical setting, serum levels of markers of acute inflammation correlate with the severity of ischemic brain damage and neurological deficit.

Both local and systemic inflammatory responses, involving soluble messengers and specialized cells activated in the brain or recruited from the periphery, exert a dualistic role on the development of ischemic cerebral injury. Brain resident microglia and blood-borne immune cells crucially contribute to the acute and chronic processes implicated in tissue injury, as well as to the regenerative and reparative mechanisms that limit the damage and provide tissue healing and recovery. In this context, an attractive approach to improve successful clinical translation of stroke therapeutics would consist in achieving a rational modulation of the immune system, by blocking its detrimental inflammatory responses while promoting its beneficial components. This perspective commentary will focus on the most recent findings regarding relevant targets and drugs for immunomodulation in stroke and their potential application in patients.

The reduction of cerebral blood flow caused by the ischemic insult prompts rapid neuronal death in the ischemic core regions and triggers the release of adenosine triphosphate (ATP) and danger associated molecular patterns (DAMPs) that stimulate purinergic and specific pattern recognition receptors (e.g., toll-like receptors), respectively, causing activation of astrocytes and microglia. Depending on the specific phenotype triggered by the environmental stimuli, microglia may be prompted to release inflammatory molecules, such as interleukin (IL)-1 and tumor necrosis factor (TNF), or to acquire an amoeboid morphology endowed with phagocytic activity that clears the damage and promotes repair. Moreover, activated microglia, damaged neurons and virtually all the other components of the neurovascular unit release toxic mediators, including cytokines, proteases and free radicals that prompt blood-brain barrier rupture and brain infiltration of circulating leukocytes. Thus, signals generated from the brain are implicated in the peripheral activation and in the cerebral recruitment of neutrophils, monocytes/macrophages, recruited from the periphery, exert a dualistic role on the development of ischemic cerebral injury. Brain resident microglia and blood-borne immune cells crucially contribute to the acute and chronic processes implicated in tissue injury, as well as to the regenerative and reparative mechanisms that limit the damage and provide tissue healing and recovery. In this context, an attractive approach to improve successful clinical translation of stroke therapeutics would consist in achieving a rational modulation of the immune system, by blocking its detrimental inflammatory responses while promoting its beneficial components. This perspective commentary will focus on the most recent findings regarding relevant targets and drugs for immunomodulation in stroke and their potential application in patients.

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stroke. Among these, minocycline has been reported to promote neurovascular remodeling during stroke recovery by facilitating alternative activation of microglia/macrophages towards a non-inflammatory protective phenotype (Yang et al., 2015). By following the concept of drug repurposing, we have recently demonstrated that acute treatment with the macrolide antibiotic azithromycin attenuates blood-brain barrier damage and cerebral ischemic damage in rodents subjected to MCAo, with a significant amelioration of neurological deficits up to 7 days after the insult (Amantea et al., 2016). Up-regulation of M2 markers has also been shown to underlie neuroprotection by Exendin-4, a glucagon-like receptor 1 agonist clinically used against type 2 diabetes in young healthy and in aged diabetic/obese mice subjected to middle cerebral artery occlusion (Darsalia et al., 2014). Interestingly, another drug widely used for the treatment of type 2 diabetes, metformin, has shown promising results in stroke animal models based on its immunomodulatory properties. Metformin is well-recognized as an activator of adenosine 5' monophosphate-activated protein kinase (AMPK). In mice subjected to MCAo, chronic metformin treatment promotes functional recovery and tissue repair via AMPK-dependent skewing of microglia/macrophages toward an M2 phenotype (Jin et al., 2014).

Thus, the preclinical findings highlighting the neuroprotective potential of M2- or N2-polarizing agents in stroke are increasing, although further studies are needed to better investigate the molecular targets that mediate immune cell shift towards beneficial phenotypes. In order to add significance to such findings, the relevance of M1-to-M2 or N1-to-N2 polarization for stroke outcome should also be validated in the clinical setting. In fact, although ischemic stroke is a leading cause of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in blood flow restoration by lysis or removal of mortality and long-term disability worldwide, the only treatments available to date consist in stimulation of inflammatory resolution and recovery especially during the late phases of the disease. BNDF: Brain-derived neurotrophic factor; CKLF-1: chemokine-like factor 1; IGF: insulin-like growth factor; IL-1: interleukin; IL-1ra: interleukin-1 receptor antagonist; INF: interferon; i-NOS: inducible nitric oxide synthase; MIP: macrophage inflammatory protein; NGF: nerve growth factor; NO: nitric oxide; PPAR: peroxisome proliferator-activated receptor; RNS: reactive nitrogen species; ROS: reactive oxygen species; RXR: retinoid X receptors; TGF-β: transforming growth factor; TLR: toll-like receptors; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor.

**Figure 1** Polarization of innate immune cells and their function in stroke. Various stimuli, including cytokines and certain receptor ligands, promote polarization of microglia/macrophages and neutrophils towards specific phenotypes. Based on their ability to release pro-inflammatory and detrimental mediators (M1 or N1 phenotypes) or immunomodulatory and pro-survival factors (M2 or N2 phenotypes), innate immune cells participate in the development of ischemic tissue damage or provide tissue healing and recovery especially during the late phases of the disease. Darsalia V, Hua S, Larsson M, Mallard C, Nathanson D, Nyström T, Sjöholm Å, Mortensen RM (2011) Myeloid-specific deletion of the mineralocorticoid receptor reduces infarct volume and alters inflammation during cerebral ischemia. Stroke 42:179-185.

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**References**


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