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## Microglia/macrophage Polarization after Experimental Intracerebral Hemorrhage

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Microglia are the resident macrophages of the brain and are the first responders to immune system activation in that tissue. Under resting conditions, microglia are ramified and monitor the brain microenvironment maintaining neuron, astrocyte and oligodendrocyte coordination. In conditions such as intracerebral hemorrhage (ICH), traumatic brain injury (TBI), subarachnoid hemorrhage and cerebral ischemia, microglia become activated as part of the injury response (1–7). Upon activation, microglia transform into an amoeboid phenotype and consequently play roles in inflammatory reactions and phagocytosis of necrotic tissue. However, based on protein and mRNA expression, activated microglia have different phenotypes. Classically, these have been termed M1- and M2-polarized microglia/macrophages and they have different roles in brain injury (4). Until now, most studies on microglial polarization have focused on TBI and cerebral ischemia, and relatively little is known about the polarization of microglia/macrophages following ICH. In this commentary, we will discuss some known molecular mechanisms that mediate microglial phenotype transformation, discuss new therapeutic approaches to altering the balance of microglial activation phenotypes, and identify future directions that could aid in long-term recovery after ICH.

Under resting conditions, microglia have a ramified morphology and migrate through the brain, maintaining central nervous system and brain microenvironment homeostasis, remodeling synapses and clearing pathological neurons (8–10). Microglial cells play vital roles in brain development, adult learning and neurogenesis (11–13). Thus, microglial cells not only respond to injury, they play an active role in the healthy brain (12). Indeed, microglia interact with neurons, astrocytes, brain endothelial cells, pericytes and oligodendrocytes (14). Microglia communicate with other cells by paracrine signaling which is adjusted by cytokines and chemokines (11, 15, 16). Thus, any modulation of microglia in injury should take into account the function of those cells in normal tissue.

After ischemic and hemorrhagic brain injury, pro-inflammatory molecules are released from neurons and astrocytes and these signals result in microglia activation (4, 17, 18). M1-activated microglia/macrophages are pro-inflammatory, producing cytokines such as TNF $\alpha$  and Interleukin 1 $\beta$ , chemokines and reactive oxygen species. M1-primed microglia/

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macrophage markers include CD86, CD16, MHC II and iNOS. M2 microglia/macrophages are generally involved in phagocytosis, trophic factor release and resolution of inflammation (4), although it should be noted that there are different subtypes of M2 cells (e.g. M2a, M2b, and M2c) each of which have different markers and functions (19). It is generally suggested that M1 microglia can act to enhance injury while M2 cells may act in repair. This is probably an oversimplification and M1- and M2-primed microglia/macrophages co-orchestrate the inflammatory response to stroke, promoting remodeling and repair and, thereby, can contribute to enhanced recovery and homeostasis of the microenvironment (4).

Studies have shown that at sites of injury there is a mixture of both M1- and M2-activated microglia/macrophages. The M2-like activation seems to be transient and has a tendency to switch to M1-like activation within one week of injury (20). Initially, M1 microglia/macrophages phagocytose necrotic neurons and other debris, but once they become the dominant phenotype, their phagocytic ability is reduced, and their secretion of pro-inflammatory molecules and neurotoxic mediators is increased. This may exacerbate neuronal damage after ICH (21). Similarly, TBI studies have shown that CD206<sup>+</sup>/Iba-1<sup>+</sup> M2-activated microglia/macrophages peak at 5 days after the brain trauma. The CD16<sup>+</sup>/Iba-1<sup>+</sup> M1-activated state assumes the dominant phenotype later, accompanied by exacerbated white matter injury (22).

In ICH, there is a general lack of information about the time course of M1 and M2 microglia/macrophages activation. There is evidence that microglia are a major source of TNF $\alpha$  and interleukin 1 $\beta$  early after ICH (17) indicating the generation of M1 polarized microglia/macrophages, but the persistence of M1-activation still remains to be fully elucidated. This is important as the M1-primed response may be protective in the acute situation and it is down-regulated once the injury-induced stress attenuates. After ICH, however, this stress reaction is usually maintained for several weeks to months, and the overloaded M1 primed activation may induce neurotoxicity due to excessive secretion of pro-inflammatory cytokines and neurotoxic chemokines (23, 24).

Very little is known about the time course of microglia activation to the M2 phenotype after ICH, although it appears to play an important role in hematoma removal, healing and neuroprotection (25). In addition, as noted above, evidence indicates that there are subtypes of M2 microglia/macrophages, M2a, M2b, M2c (19). IL-4 can induce M2a-primed microglia/macrophages, which produce anti-inflammatory cytokines (IL-10) and upregulate their phenotypic markers arginase-1, CD206, Ym-1 and CD36. The role of the M2c phenotype is unclear, but it is down-regulated by IL-10, suggesting a role in tissue remodeling and matrix deposition seen after the inflammatory response decreases. In the last stage of injury, microglia/macrophages can adopt an M2b-primed phenotype, with a mixed pro- and anti-inflammatory function. M2b-like cells express both M1 and M2 markers (19). More information on M2-microglia/macrophages activation after ICH and the presence of different M2 subtypes is needed. Such studies are complicated by difficulties in discriminating infiltrating systemic macrophages and microglia, although chimera studies with green fluorescent protein labeled bone marrow can be used.

Several factors may determine the dominant microglial phenotype after injury. TBI models suggest that the severity of the ICH is one such factor (26). In a recent TBI model, repeated TBI was used to create a severe cortical lesion which led to chronic and persistent M1-primed activation that lasted for months to years (5). It should be noted that brain hemorrhage often occurs after TBI (27). Although there is a lack of recent data on microglial polarization after ICH, it is likely that hematoma size can affect M1 and M2 polarization. Additionally, studies suggest that microglial activation depends upon brain region. In white matter, the M2-primed phenotype is more likely to transform into an M1-primed phenotype than in grey matter (22). Future research should investigate the activation of microglia in different brain regions following ICH. Aging may also influence microglial polarization but recent findings have been contradictory. One study revealed an overall increase in M1-primed gene expression in aged brain, with a corresponding decrease in M2-primed gene expression (28). However, another recent study found that microglia are M2-dominated with aging and perform a more anti-inflammatory function (29). Further studies are needed assessing the effects of aging on microglial/macrophage activation after ICH.

Injured neurons release soluble molecules including TNF $\alpha$  and lipocalin 2, which can repolarize M2 to M1 microglia (30). Shifts in microglia phenotype are also controlled by intracellular signal pathways. PPAR nuclear receptors are M2-polarization regulators (31), with PPAR $\gamma$  activation promoting the M2 phenotype. Considering ICH produces large number of damaged neurons and lysed erythrocytes, PPAR $\gamma$ -stimulated M2-polarization may be suitable for removing the detrimental effects of M1-polarized microglia. Other mechanisms, such as metabotropic glutamate receptor 5 (mGluR5), which can attenuate M1 polarization, are being investigated (32). Transplantation of human bone marrow mesenchymal stem cells can strengthen M2-polarization and improve the microenvironment surrounding the injury, resulting in an anti-inflammatory response (33). Given that M2-polarized microglia/macrophages can perform protective functions and promote tissue repair and neurogenesis, a repolarization towards M2 microglia/macrophages after ICH might be an attractive method to promote recovery from neurological deficits.

Therapeutically, altering extracellular factors within the injured microenvironment and/or the intracellular network of signal pathways may balance the dynamic activation of microglia/macrophage after ICH. It is likely that efficient tissue recovery requires cooperation of both M1- and M2-like microglia/macrophages, including well-balanced transitions from one dominant phenotype to another. It is vital to remember that M1-polarized microglia/macrophages may play critical roles in early repair processes, and so such early activity should not be eliminated. In contrast, an over-long M2-dominated state after the acute pro-inflammatory period can induce fibrosis and abnormal repair (34). Since both M1 and M2 phenotypes can play detrimental and beneficial roles in the brain injury, timing the most efficient transition of M1 to M2 should be carefully and cautiously considered. It is important to evaluate the time-course of M1 and M2-polarization after ICH in order to best time switches in microglial phenotype in order to maximize effects on tissue repair.

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