Journal Club

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Stopping Inflammation in Stroke: Role of ST2/IL-33 Signaling

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Review of Yang et al.

Ischemic stroke is the second leading cause of death worldwide, according to data from the World Health Organization. It results from interruption of blood flow caused by *in situ* thrombus (blood clot) formation or embolization. Along with other mechanisms involved in the pathogenesis of stroke, postischemic inflammation accounts for brain cell death in the acute and subacute stages (Dirnagl et al., 1999). Over the past 20 years, many studies have focused on inflammatory responses triggered after ischemic stroke, with the aim of ameliorating stroke outcome. However, initial attempts to use anti-inflammatory treatments in acute ischemic stroke failed (del Zoppo, 2010). These failures may be explained by the nature of immune cells in stroke: on the one hand, they participate in the progression of postischemic injury; on the other hand, they mediate poststroke repair and regeneration.

The dual nature of the immune response to stroke is exemplified by microglia, the resident macrophages in the brain parenchyma. Microglia actively survey the surrounding environment by protracting and retracting their processes; and after acute brain injury, they undergo dramatic morphological and phenotypic changes. Specifically, when activated by injury-associated molecules, microglial cells change from having branched processes to becoming amoeboid phagocytic cells, which express novel surface antigens and produce mediators that orchestrate the inflammatory response of the brain (Perego et al., 2011). Individual microglial cells can adopt a large spectrum of activated phenotypes ranging from proinflammatory (M1 phenotype) to anti-inflammatory and neuroprotective (M2). Although microglial cells can encompass a broad spectrum of phenotypes between the M1 and M2 extremes, the M1-M2 dichotomy is generally considered as a useful framework to understand and manipulate the functional status of microglial cells.

The mechanisms driving phenotypic changes of microglia remain poorly understood and represent a potential therapeutic target to both limit brain injury and promote brain repair. Clues about this regulation might come from the study of peripheral macrophages that also adopt a range of phenotypes. One such regulatory molecule is the cytokine IL-33, a member of the interleukin-1 (IL-1) cytokine family. IL-33 exerts a neuroprotective role by promoting the shift of macrophage polarization from M1- to M2-type, reducing the levels of proinflammatory mediators and increasing the secretion of anti-inflammatory cytokines, such as IL-4 and IL-1 (Jiang et al., 2012; Pomeshchik et al., 2015). IL-33 binds to its specific receptor ST2. Although several papers have reported the neuroprotective role of IL-33 in stroke (Korhonen et al., 2015; Pomeshchik et al., 2015), the importance of IL-33/ST2 signaling cascade on microglial phenotype had never been addressed before.

Yang et al. (2017) investigated the potential beneficial effects of the activation of ST2 receptors via IL-33 in the switch of microglial cell phenotype from a proinflammatory to anti-inflammatory phenotype after ischemic stroke and asked whether activation of this pathway decreases the ischemic lesion volume. To do so, they used ST2 knock-out mice subjected to two different models of stroke. In both models, ST2-deficient mice had larger lesion volumes than wild-type mice. This deleterious effect was accompanied by neurobehavioral deficits (neuroscore and sensorimotor tests; e.g., rotarod, adhesive removal, and cylinder tests) at different times after stroke onset.

To identify the cells involved in the protective effects of ST2, the authors investigated which cells expressed this receptor and which secreted its ligand, IL-

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33. Immunohistological analyses showed that astrocytes and oligodendrocytes produce IL-33 after stroke onset. Flow cytometry and immunohistological analyses showed that ST2 receptors were expressed by astrocytes and microglial cells under physiological conditions. In addition, ST2 receptor levels were dramatically increased in these two populations after stroke. The number of ST2-positive infiltrated macrophages and neutrophils was also robustly elevated after stroke. The next step of their work was to assess the direct effects of IL-33 on microglia. *In vitro* (microglia-enriched cultures subjected to oxygen/glucose deprivation) and *in vivo* (ST2-deficient, stroke mice) data showed that the ST2/IL-33 pathway is implicated in the shift from a microglial "M1" to a "M2"-neuroprotective phenotype, by inducing the expression of M2 markers and inhibiting the expression of M1 markers (analyzed by immunohistological analyses and/or RT-PCR). Finally, the authors demonstrate, by *in vitro* and *in vivo* studies, that the neuroprotective effects of the ST2/IL-33 signaling pathway were mediated by IL-10 produced by microglial cells. *In vivo* experiments showed that the neuroprotective effects of IL-33 were abrogated in IL-10-deficient mice 3 d after stroke onset, whereas wild-type mice showed decreased lesion volumes when exposed to IL-33.

The discovery by Yang et al. (2017) links IL-33/ST2 signaling to the IL-10 pathway, for which downstream effects are known. IL-10 is one of the best studied anti-inflammatory cytokines produced by immune cells after brain injury (Iadecola and Anrather, 2011). IL-10 production impairs proinflammatory reactions by inhibiting cytokine production, downregulating MHC-II antigens, and decreasing NF-κB activity. In addition, IL-10 production may upregulate B-cell proliferation and antibody production (de Waal Malefyt et al., 1992). In stroke, many immune cells (including regulatory T and regulatory B cells) exert neuroprotective actions through IL-10 release (Liesz et al., 2009; Ren et al., 2011).

In contrast to previous data by Korhonen et al. (2015), Yang et al. (2017) did not observe any effect on the expression of IL-4 after IL-33 stroke-induced upregulation or *in vitro* IL-33 administration. IL-4 is another anti-inflammatory cytokine that has been largely reported to exert neuroprotection after stroke (Xiong et al., 2015; Liu et al., 2016). These differing findings suggest that the ST2/IL-33 signaling pathway in microglia is not at the origin of IL-4 secretion because, in the work of Yang et al. (2017), IL-33-treated microglia did not express IL-4. In agreement with this hypothesis, Zhao et al. (2015) have suggested that IL-4 released from neurons and IL-33 coming from glial cells could promote a synergistic effect and exert neuroprotective effects after stroke.

To examine the temporal profile of ST2/IL-33 signaling after stroke, Yang et al. (2017) analyzed the expression of IL-33 at 1, 3, 7, and 14 d. They detected a significant increase of IL-33 in astrocytes and oligodendrocytes exclusively 1 d after stroke, after which IL-33 expression returned to basal levels. In contrast, ST2 expression was analyzed exclusively 3 d after stroke onset. At this time, expression of ST2 was significantly elevated in microglia and astrocytes. It would have been valuable to analyze the same time points for ST2 as for IL-33, especially 1 d after stroke onset, to link the expression pattern of both molecules and to explain the discrepant expression patterns of IL-33 and ST2 3 d after stroke onset.

Astrocytes have previously been shown to participate in neuroinflammatory responses (Dong and Benveniste, 2001) and can become activated (reactive) in response to many CNS pathologies, including stroke (Pekny and Nilsson, 2005). In the study by Yang et al. (2017), astrocytes were one of the sources of IL-33 1 d after stroke onset; and, like microglia, astrocytes showed an increased expression of ST2 receptors 3 d after stroke. These results suggest that astrocytes could also contribute to the beneficial effects of IL-33 and ST2-mediated signaling after stroke. This topic deserves additional studies.

Last, the results obtained by Yang et al. (2017) are in accordance with clinical findings obtained in stroke patients (Korhonen et al., 2015). Korhonen et al. (2015) studied the plasma concentration of the soluble form of ST2 (sST2), a secreted isoform generated by alternative splicing that inhibits IL-33 signaling (Hayakawa et al., 2007). Stroke patients with high plasma levels of sST2 had greater neurological deficits 3 months after stroke, suggesting that stronger inhibition of IL-33 was associated with poorer stroke outcome (Korhonen et al., 2015).

In conclusion, the results observed by Yang et al. (2017) open new venues targeting IL-33/ST2 signaling pathways for ischemic stroke treatment. To date, there is no specific therapeutic approach available to target poststroke microglial immune responses, although some candidates have been investigated only in preclinical studies. Although some of them have been applied in other disorders, exploratory research for effective agents that regulate ischemic microglial activation is still in the early stages. Given the dual function of microglial cells after stroke, therapeutic strategies targeting microglia should be fine-tuned to selectively suppress the proinflammatory responses and/or promote anti-inflammatory effects. ST2 agonist injection might be a promising target to promote microglial beneficial responses in stroke if an effective and innocuous administration mechanism is found for stroke patients.

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