Planas AM.

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Brain Behav Immun. 2015 Nov;50:39-40. Epub 2015 Jul 30.

doi: 10.1016/j.bbi.2015.07.027.

Brief Commentary

Immunomodulatory Role of IL-33 Counteracts Brain Inflammation in Stroke

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Stroke induces cell necrosis and triggers inflammation as manifested by pro-inflammatory cytokine production, glial reactivity and leukocyte infiltration. The acute inflammatory reaction is thought to exacerbate brain damage. Some anti-inflammatory treatments are protective in experimental animal models of stroke. However, efficacy of these treatments has not yet been demonstrated in the clinical setting. Peripheral immune cells can gain access to the brain parenchyma and interact with resident cells to affect tissue responses and contribute to clearance of the damaged tissue. Also, during the days that follow stroke, the inflammatory response is attenuated by immunomodulatory cells such as regulatory T (Treg) (Liesz et al., 2009) or B (Ren et al., 2011) lymphocytes that produce the anti-inflammatory cytokine IL-10. Macrophages, and probably microglia as well, can be activated to acquire different phenotypes depending on the environment. *In vitro*, two prototypical phenotypes known as M1 and M2 develop after exposure to the pro-inflammatory mediators LPS/IFN-γ, and IL-4, respectively. The situation is more complex *in vivo* where the variety of phenotypes that macrophages and microglia can acquire in disease conditions are much greater than the archetypal M1/M2 phenotypes induced *in vitro* upon cytokine stimulation. Typically, cytokines released by polarized T helper type 2 (Th2) lymphocytes induce alternative M2 macrophage polarization that contributes to resolution of inflammation and tissue repair. The expression of markers of alternative polarization has been reported in microglia/macrophages after brain ischemia (Perego et al., 2011).

The cytokine interleukin-33 (IL-33) signals through the IL-1 receptor-related protein ST2 that is expressed by Th2 but not Th1 cells (Xu et al., 1998). IL-33 induces the production of Th2-associated cytokines, including IL-4, IL-5, and IL-13 (Schmitz et al., 2005), and facilitates immunomodulation by expanding Treg cells (Turnquist et al., 2011). In this issue of *Brain, Behavior and Immunity*, Korhonen et al., report the effects of systemic administration of IL-33 in a mouse model of stroke (Korhonen et al., 2015). These scientists induced focal brain ischemia by permanent occlusion of the middle cerebral artery in mice and administered IL-33 i.p. or i.v. either before or immediately after ischemia. Regardless of treatment regimen, IL-33 reduced infarct volume at 24h and ameliorated the neurological deficits during the week following stroke. These beneficial effects of IL-33 did not seem to be mediated by Treg given that they were not abrogated by treatment with a blocking antibody against CD25. However, IL-33 treatment reduced splenic TNF-α while it increased the production of IL-4 in the spleen and the peripheral ischemic brain tissue. Notably, administration

of a blocking antibody against IL-4 one hour prior to ischemia partially prevented the protective effect of IL-33, suggesting that it was, at least in part, mediated by IL-4. Given that IL-4 promotes the alternative polarization of macrophages and microglia, Korhonen et al. went on to examine the expression of the M2 marker Arginase-1 (Arg1) in brain tissue. They found increased expression of Arg-1, suggesting that IL-33 promoted the alternative activation of macrophages/microglia in ischemic tissue, thus counteracting the inflammatory response.

But which cellular target of IL-33 is responsible for the beneficial effect of IL-4 in stroke? It has been previously reported that IL-33 acts as a danger signal and increases the production of TNF-α after LPS stimulation of macrophages (Espinassous et al., 2009). The current study (Korhonen et al., 2015) found that IL-33 induced astrocyte activation as assessed by an increase in the production of IL-6 in cultured astrocytes. However, this was in contrast to *in vivo* IL-33 treatment that attenuated the astroglial reaction induced by ischemia. Furthermore, IL-33 did not prevent death of cultured neurons exposed to oxygen and glucose deprivation. Together, these findings suggested that the protective effect of IL-33 *in vivo* was not mediated by a direct action on neurons or glia. Instead, the results suggested that the beneficial effects could be mediated by T-cells since they released IL-4 after exposure to IL-33 and the conditioned medium of IL-33-treated T cells exerted anti-inflammatory effects in cultured astroglia.

The relevance of these findings to human stroke is currently unknown. Korhonen and collaborators examined the plasma concentration of soluble ST2 (sST2) in stroke patients. ST2 is a secreted isoform generated by alternative splicing that inhibits IL-33 signaling (Hayakawa et al., 2007). Patients with higher levels of sST2 had greater neurological deficits at three months, indicating that a more potent inhibition of IL-33 was associated with a bad stroke outcome. Although this study supports IL-33 treatment as potentially beneficial in ischemic stroke, there are several risks that need to be considered. Stroke induces immunosupppression in mice (Prass et al., 2005) and humans (Chamorro et al., 2007), thereby increasing the risk of post-stroke infection associated with an excessive anti-inflammatory response. Post-stroke infection is more frequent in patients with severe stroke (Chamorro et al., 2007). Korhonen et al. induced mild ischemia in mice and therefore investigation of the effects of IL-33 treatment in more aggressive stroke models is required to underscore possible threats to the immunological defense that might exaggerate the risk of post-stroke infection.

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Acknowledgment

Current research of the author is financed by the Spanish Ministry of Economy MINECO (SAF2014-56279), the European Community FP7 (grants n°278850, nº607962, and nº603043), and the Catalan Government (AGAUR, SGR-574).