Journal Club

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Intricate Interplay between Innate Immune Cells and TRPM2 in a Mouse Model of Multiple Sclerosis

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Review of Tsutsui et al.

Multiple sclerosis is a neurodegenerative disease affecting ~2.3 million people worldwide. It is an autoimmune disease of the CNS in which myelin is targeted, leading to demyelination and axon degeneration. The most commonly used animal model to study multiple sclerosis is experimental autoimmune encephalomyelitis (EAE), which can be induced in mice by subcutaneous injections of complete Freud adjuvant mixed with a myelin protein, and intraperitoneal injections of pertussis toxin. EAE recapitulates most of the symptoms observed in patients suffering from multiple sclerosis, and this model has uncovered the involvement of T and B cells in the disease (e.g., Siffrin et al., 2010). Therapeutic drugs targeting these cells are today used to treat patients, but they are only partially effective. One reason for this, suggested by additional studies, is that other cell types contribute to multiple sclerosis pathology. Among them, innate immune cells, specifically neutrophils, monocytes, dendritic cells, microglia, and monocyte-derived macrophages, have been shown to be important (Ajami et al., 2011; Yamasaki et al., 2014). Yet the underlying mechanisms remain unclear.

In a recent article published in *The Journal of Neuroscience*, Tsutsui et al. (2018) investigated the role of Transient Receptor Potential Melastatin 2 (TRPM2), a type of Ca^{2+}-permeable nonselective cation channel, in EAE in mice. TRPM2 is present in peripheral immune cells, including innate immune cells, and TRPM2-mediated Ca^{2+} influx induces production of proinflammatory cytokines by these cells. Moreover, TRPM2 expressed by microglia and monocyte-derived macrophages aggravates peripheral and spinal pronociceptive inflammatory responses in mouse models of neuropathic pain, implicating TRPM2 in neuroinflammation.

Consistent with previous work, EAE induction resulted in activation of T cells in lymphoid organs by day 10 (D10), and invasion of the spinal cord by T cells, neutrophils (marked by Gr1), and macrophages and microglia (marked by Iba1) by D21. The authors showed that intraperitoneal injection of the TRPM2 inhibitor miconazole reduced pathological outcome (reduced paralysis in tail and limbs) in EAE mice. Similar improvements were found in TRPM2-KO EAE mice. Although knocking out TRPM2 did not alter levels of T cells in the spinal cord, it reduced spinal levels of macrophages and neutrophils. In addition, levels of the proinflammatory chemoattractant cytokine CXCL2 were lower in the spinal cord of TRPM2-KO mice than in WT at D14 and D21 after EAE induction. Notably, CXCL2 in the spinal cord was mainly expressed by peripheral Iba1-expressing cells (i.e., monocyte-derived macrophages) rather than by resident microglia.

These results show that knocking out TRPM2 reduces infiltration of macrophages and neutrophils into the spinal cord of EAE mice, and that CXCL2 is produced in the spinal cord by peripheral macrophages during EAE. The authors conclude from these results that, in WT mice, TRPM2 expression in peripheral macrophages mediates CXCL2 production and release in EAE lesions of the spinal cord, resulting in neutrophil infiltration into CNS and aggravation of EAE pathology. Although this proposed mechanism is consistent with the results, it does not consider several other possible interpretations. TRPM2 is indeed expressed by multiple types of innate immune cell (Syed Mortadza et al., 2015), including monocytes (Yamamoto et al., 2008), neutrophils (Heiner et al., 2003; Najder et al., 2018), and microglia (Miyake et al., 2014). Because TRPM2 deletion in the mice by Tsutsui et al. (2018) was not specific to macrophages, the contribution of TRPM2 deletion in these other immune cells must be considered, as discussed below.

First, it is possible, although not reported to date, that monocytes and neutrophils require TRPM2 to respond to

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attractive cues, such as dendritic cells (Sumoza-Toledo et al., 2011). If this is the case, then KO of TRPM2 might affect recruitment of monocytes and neutrophils in EAE not only by decreasing the secretion of the CXCL2, which attracts them, but also by reducing the ability of the cells to respond to this chemoattractant. Thus, lower infiltration of Gr1-expressing cells, considered here neutrophils, might be the direct consequence of TRPM2 deficiency in neutrophils themselves, rather than a secondary consequence of TRPM2 deficiency in, and therefore reduced CXCL2 secretion by, macrophages. Similarly, if monocytes require TRPM2 to infiltrate the spinal cord, TRPM2 deficiency might reduce monocyte infiltration partly by limiting their capacity to respond to CXCL2. In addition, because monocytes can differentiate into monocyte-derived macrophages after entering the spinal cord Jakubzick et al. (2017), TRPM2 deficiency might indirectly prevent recruitment of macrophages by preventing recruitment of monocytes, and consequently lead to a reduction in CXCL2, without having any direct effect on CXCL2 expression or release in monocyte-derived macrophages.

Second, TRPM2 may be necessary not only for recruitment, but also for activation of various immune cells, including monocytes, neutrophils, and microglia (Knowles et al., 2013; Tripathi et al., 2018). If this is the case, TRPM2 deficiency would be expected to change which cytokines the cells produce (Qian et al., 2018). This would alter the molecular environment of the spinal cord lesion, which, in turn, might alter CXCL2 expression by macrophages independently of TRPM2 expression in these cells. Moreover, TRPM2 deficiency might prevent monocytes from differentiating into monocyte-derived macrophages, leading to reduced levels of macrophages, as Tsutsui et al. (2018) found. Because monocytes, monocyte-derived macrophages, and neutrophils are all deleterious in the spinal cord during EAE (Ajami et al., 2011; Aubé et al., 2014; Yamasaki et al., 2014), impaired activation of those cells might help to reduce neuronal damage and pathological outcome in TRPM2-deficient mice.

Finally, my team recently reported that neutrophils invade the spinal cord before monocyte-derived macrophages in EAE mice (Caravagna et al., 2018). But if it is correct, as Tsutsui et al. (2018) conclude, that decreased production of CXCL2 by macrophages in TRPM2-KO mice reduces neutrophil infiltration, neutrophils would have to enter the spinal cord after monocyte-derived macrophages. One possible explanation might be that neutrophils initially enter the spinal cord through an unidentified chemoattraction process, but their levels are maintained by macrophage-produced CXCL2. Further investigation is required to confirm this hypothesis. Using chimeric mice to study the relative times of entry of Iba1- and Gr1-expressing cells in the spinal cord would be of great relevance.

Innate immune cells are an important part of the neurodegenerative and demyelinating process during multiple sclerosis. The demonstration by Tsutsui et al. (2018) that monocyte-derived macrophages participate in chemoattraction of deleterious cells during EAE shows once again the complexity of this disease, in which monocyte-derived macrophages have previously been shown to directly initiate demyelination (Yamasaki et al., 2014). Still, whether a given pathological event is a cause or consequence of multiple sclerosis remains unclear in many cases. It has been thought that the cause of multiple sclerosis is the dysregulation of T cells. However, T cells need antigen-presenting cells to direct them against a specific antigen. Moreover, HLA Class II molecules are essential for antigen recognition by T cells, and some HLA Class II alleles are associated with an increased risk of developing multiple sclerosis (Hemmer et al., 2015). It can thus be asked whether the cause of multiple sclerosis is a dysregulation of T cells, of antigen-presenting cells which then misdirect T cells, or of molecules present on these antigen-presenting cells.

Nevertheless, Tsutsui et al. (2018) showed that targeting TRPM2, even by pharmacological means, improves outcome after EAE. Thus, this might be an effective treatment for people with multiple sclerosis. Knowledge about cytokine expression and underlying mechanisms in macrophages and other innate immune cells during EAE is necessary for a better understanding of the roles of each cell type during the disease. Hopefully, the critical need of new therapeutic targets will be filled at least partially with this new mechanism and will lead to new therapeutic strategies to treat people with multiple sclerosis.

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