Journal Club

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A Role for Microglia in Retinal Development

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Review of Jobling et al.

Microglia are the resident macrophages of the CNS. Though they are best known for their traditional macrophage activities, such as the phagocytosis of cell debris and inflammatory responses after injury, microglia also play an integral role in the normal development of the brain and spinal cord. For instance, microglia engulf excessive synapses to sculpt neuronal circuits in development (Schafer et al., 2012) and phagocytose neural progenitor cells to regulate neurogenesis (Cunningham et al., 2013). However, microglia also assume roles specific to the CNS that are unique among cell members of the myeloid lineage. The loss of microglia perturbs axonal outgrowth and somatic localization of dopaminergic neurons in the embryonic brain, suggesting that they are uniquely positioned to guide neuronal circuit formation (Squarizoni et al., 2014). Additionally, some cortical neurons require microglia-secreted signals for survival (Ueno et al., 2013). Many questions remain about the role of microglia in normal development and CNS homeostasis, especially those roles that are unique to microglia versus macrophages in other tissues.

In a recent study, Jobling et al. (2018) unveiled a novel mechanism by which microglia regulate the development of the ret-

ina (Jobling et al., 2018). They began by characterizing the function of Cx3cr1—or fractalkine receptor—in retinal development. Cx3cr1 is classically associated with leukocyte adhesion (Combadiere et al., 1998) and, in the CNS, is predominantly expressed by microglia (excluding Cx3cr1⁺ populations of peripheral immune cells in the leptomeningeal, ventricular, and perivascular spaces). A Cx3cr1^{EGFP} knock-in mouse carrying an EGFP instead of the Cx3cr1 coding region was previously developed to study the function of Cx3cr1 in microglia. Surprisingly, despite the well documented role of Cx3cr1 in the migration and extravasation of leukocytes, no major deficits in migration or disease response were observed in the Cx3cr1-deficient microglia (Jung et al., 2000). The lack of obvious functional deficits in Cx3cr1-deficient microglia set the stage for the development of a range of Cx3cr1-based knock-in genetic tools to manipulate microglia, most of which also disrupt Cx3cr1 gene expression. Amazingly, it was by taking a closer look at these largely phenotypically normal mice with microglia lacking fractalkine receptors that Jobling et al. (2018) discovered a novel role for microglia in retinal circuits.

To assess the effect of partial or complete loss of Cx3cr1 on the retina, the researchers compared wild-type, Cx3cr1^{EGFP/+}, and Cx3cr1^{EGFP/EGFP} animals, which have two, one, or zero copies of the Cx3cr1 gene, respectively. Unexpectedly, they found visual deficits in Cx3cr1 knock-out mice as early as 17–30 d after birth. Electroretinograms

demonstrated dysfunctional neuronal activity soon after eye opening, with progressive cone photoreceptor loss in the peripheral retina as early as postnatal day 30. These findings demonstrated that the functioning of Cx3cr1 in microglia is essential for the proper development of retinal circuits.

The early retinal changes observed in Cx3cr1^{EGFP/EGFP} mice suggested that Cx3cr1 signaling is involved in normal retinal development. But how? Though previous studies of Cx3cr1 signaling in brain microglia showed that disrupting fractalkine signaling can lead to microglia depletion (Paolicelli et al., 2011) and changes in microglial activation (Bachstetter et al., 2011), Jobling et al. (2018) saw more microglia in the outer retina and no morphological signs of activation. Instead, the authors noticed that, in the absence of Cx3cr1, microglia extended their processes further into the periphery of the retina and made more direct contacts with developing photoreceptors. To probe whether these morphological changes in retinal microglia modified photoreceptor function, the researchers transcriptionally profiled retinas from Cx3cr1^{-/-} and Cx3cr1^{+/+} mice. This analysis unexpectedly revealed that the loss of Cx3cr1 from microglia led to changes in the expression of genes associated with the structure and function of photoreceptor cilia.

Virtually all neurons and many glia have a single primary cilium, a 1–9 μ m, tubulin-based axonemal structure anchored to the

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mother centriole and protruding from the soma (Wei et al., 2012; Dummer et al., 2016). Though the full extent of cilia-mediated activity within the brain and spinal cord is largely unknown, recent evidence has identified the primary cilium as the obligate site of signal transduction for sonic hedgehog, a diffusible signaling molecule that powerfully regulates development, and has revealed a crucial role for primary cilia in hypothalamic function (Huangfu and Anderson, 2005; Loktev and Jackson, 2013). Further, neuronal primary cilia localize specific G-protein-coupled proteins to their membrane, including receptors for neuromodulators such as somatostatin, serotonin, melanin concentrating hormone, and neuropeptide Y (Schou et al., 2015). Together, these functions point to primary cilia as a potential cellular “antenna” for intercellular signaling interactions (Berbari et al., 2009).

Importantly for human disease, genetic association studies have linked mutations in cilia genes to numerous neurological conditions such as amyotrophic lateral sclerosis (Kenna et al., 2016; van Rheenen et al., 2016).

Could deficits in photoreceptor cilia caused by the loss of microglial Cx3cr1 explain the observed visual deficits? Photoreceptor cilia, like cilia in other neurons, act as cellular antennae for signaling pathways. But they also serve as an essential bridge between the outer segment of the photoreceptor, full of light-sensitive rhodopsins necessary for light detection, and the inner segment of the photoreceptor, where mitochondria and ribosomes stockpile the machinery necessary for the cell to function. Thus, photoreceptor cilia are essential for the normal formation of inner and outer photoreceptor segments and therefore phototransduction (Rachel et al., 2012). Intriguingly, Mice lacking Cx3cr1 showed impaired elongation of photoreceptor outer segments, a potential result of ciliary defects. Additionally, immunohistochemical analyses revealed changes in the transition zone of the cilium, which is essential for sorting and trafficking proteins between the photoreceptor segments (Rachel et al., 2012). These findings suggest that microglia somehow regulate the cilium of developing photoreceptors in a way that affects photoreceptor maturation. Further, lack of Cx3cr1 correlated with photoreceptor dysfunction and eventual death, highlighting the potential role this ciliary pathway plays in the health and normal development of the retina.

Jobling et al. (2018) proposes a novel mechanism by which microglia affect the development of neural circuits. Given that microglia migrate from the yolk sac into the

brain parenchyma extremely early in development (approximately embryonic day 9.5 in mice), appearing before most mature neurons, astrocytes, or oligodendrocytes (Ginhoux et al., 2010), it is perhaps not surprising that these brain-resident cells have essential roles in sculpting neuronal development. Experiments in this study focused on the role of microglial Cx3cr1 signaling specifically in the retina because the retina is an easily accessible model of many CNS circuits, but it remains to be seen whether similar mechanisms underlie the maturation of circuits elsewhere in the brain. Cx3cr1 is also only one of many well established microglial signaling receptors, and it is of great interest to determine whether other characteristic microglial receptors play similarly important roles in the development of neuronal circuits.

This study also highlights the importance of carefully characterizing genes such as Cx3cr1 that serve as the basis for genetic lines used to probe cell function. Many experiments use the *Cx3cr1^{EGFP}* or *Cx3cr1^{Cre/CreERT}* lines to label or manipulate microglia, but these lines also disrupt normal Cx3cr1 function and the potential effect of this disruption must be considered (for review, see Wolf et al., 2013). Fortunately, the growing appreciation for microglia in brain development is matched by a new wave of tools to both label and genetically manipulate microglia in the absence of other macrophages (Bennett et al., 2016; Buttgerit et al., 2016; e.g., the marker Tmem119 and *Sall1*-based genetic lines) and to culture microglia in a state that more resembles their quiescent state *in vivo* (Bohlen et al., 2017). Pairing these new tools with our growing understanding of the importance of microglia in processes as diverse as photoreceptor development in the retina and axon guidance in the cortex promises to help elucidate the mechanistic, cell-specific interactions that dictate how microglia are involved in CNS development.

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