Microglia: Phagocytosing to Clean, Sculpt, and Destroy

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Introduction

Microglia are the resident macrophages and primary phagocytes of the CNS. Unlike other phagocytes, which function primarily in immunity, microglia are immune cells that are heavily involved in not only supporting brain tissue, but also shaping it. Using phagocytosis, they destroy excess functional connections between neurons (synaptic pruning) to sculpt neuronal and synaptic circuits during development and throughout adulthood. They use classical immune molecules, such as complement, to signal to neurons and glia, and they survey their microenvironment using their dynamic processes. We now appreciate that microglia are key modulators of neuronal development and plasticity, yet details about their normal homeostatic role in the healthy brain and how they contribute to disease remain elusive. Several neurodegenerative disorders involve synapse loss, and emerging evidence from several mouse models suggests that microglia mediate this loss. Although pruning is not their only role, understanding how microglia recognize and prune synapses during development is providing new insight into synapse loss and dysfunction in disease, potentially nominating new therapeutic candidates.

What Defines Microglia

Microglia were first characterized by del Rio-Hortega as a population of migratory phagocytic cells within the CNS. A long-standing mission has been to determine what defines microglia and to assess how they vary—not only across cell populations, but also across time, space, and individuals. It is becoming increasingly clear that microglia are a distinct macrophage population, differentiated and specialized from other tissue macrophages by microenvironment cues unique to the CNS (Gosselin et al., 2014; Lavin et al., 2014). Even so, the transcriptional profiles of microglia are remarkably diverse. Their profiles appear to vary by cell age, developmental stage, resident brain region, sex, and even gut microbiota (Butovsky et al., 2013; Hickman et al., 2013; Grabert et al., 2016; Matcovitch-Natan et al., 2016), suggesting that the cells' functional roles are shaped by complex regulatory networks in their local milieu. An intriguing question is whether this microglial heterogeneity shapes circuit wiring (or vice versa) in the developing brain and whether this underlies region-specific vulnerability in disease. Further studies are needed to obtain a more comprehensive profile of microglia, and particularly to assess how that profile changes in disease. This would provide insight into their remarkable plasticity in the living brain and the molecular pathways that underlie it.

Phagocytic Functions in the Brain

Microglia are the local phagocytes of brain parenchyma, where they rapidly and efficiently clear dead or dying cells and debris. They have many roles, but being our brain's innate immune cells, they react to damage signals (Ransohoff and Cardona, 2010). They migrate to injury, extend their processes to it, and produce cytokines, chemokines, and other pro-inflammatory and anti-inflammatory signals for repairing injury and maintaining homeostasis. In addition, because they enter the brain early in development (embryonic day ~9.5 in mouse) (Ginhoux et al., 2010), they are well poised to impact the developing brain. Indeed, deletion of microglia-related genes or dysregulation of inflammatory markers leads to altered brain wiring and produces behavioral deficits associated with neuropsychiatric or neurodevelopmental disorders (Frost and Schafer, 2016). Microglia are also involved in spatial patterning, engulfing cells undergoing apoptosis (programmed cell death) as the embryonic brain matures and clearing apoptotic cells during adult neurogenesis. Together, these findings suggest that microglia play an important role in shaping the brain; however, signaling mechanisms underlying the crosstalk between microglia and other cell types, including neurons and astrocytes, still remain unclear.

Microglia Shape Brain Wiring by Targeting Synapses

Microglia and immune-related proteins are critical for the refinement of neuronal connectivity in the developing brain. Manipulating microglia or microglia-related functions leads to sustained defects in synaptic connectivity, brain wiring, and plasticity-associated tasks (Hong et al., 2016b). Under healthy basal conditions, microglia connect with synapses using their highly motile processes (Fig. 1). The frequency of these connections is regulated by neuronal activity or sensory (visual) experience, suggesting that these contacts have functional implications. Indeed, microglia sculpt synaptic connectivity by engulfing the neuronal terminals that form the synapse, a process known as synaptic pruning (Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2012).

Complement as an "Eat Me" Signal for Synaptic Engulfment

How do microglia target synapses for phagocytosis? One mechanism involved in both neural development and models of neurodegeneration is the classical

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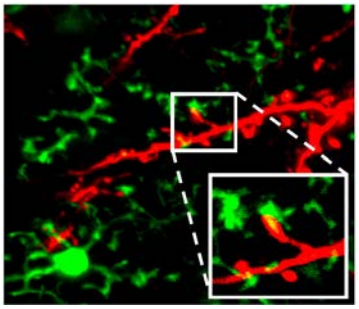

Figure 1. Microglia dynamically interact with synaptic elements in the healthy brain. Two-photon imaging in the olfactory bulb of adult mice shows processes of CX3CR1-GFP-positive microglia connecting to tdTomato-labeled neurons. Image courtesy of Jenelle Wallace at Harvard University. Reprinted with permission from Hong S and Stevens S (2016), Figure 1. Copyright 2016, Elsevier.

complement cascade (Stevens et al., 2007; Schafer et al., 2012; Hong et al., 2016a; Lui et al., 2016). In the peripheral immune system, classical complement proteins are "eat me" signals that promote rapid clearance of invading pathogens or cellular debris, which is done in part by macrophages expressing complement receptors (CRs) including CR3. In the developing visual thalamus, C1q (the initiating protein of the cascade) and C3 (a downstream protein) localize to subsets of immature synapses, likely marking them for elimination (Stevens et al., 2007). Microglia, which express CR3, phagocytose these synaptic inputs through the C3–CR3 signaling pathway (Schafer et al., 2012). Significantly, mice deficient in C1q, C3, or CR3 have sustained defects in synaptic connectivity. This complement-dependent synaptic pruning is significantly downregulated in the mature brain, suggesting that it is a highly regulated process, likely restricted to refinement stages of development.

When Synapses Are (Wrongly) Marked as Debris

Reactive microglia and neuroinflammation are hallmarks of Alzheimer's disease (AD) and other neurodegenerative disorders, including Parkinson's disease, ALS, and frontotemporal dementia (Ransohoff, 2016). Long considered to be events secondary to neurodegeneration, microglia-related pathways have been identified by emerging genetic and transcriptomic studies as central to AD risk and pathogenesis (Guerreiro et al., 2013; Jonsson et al., 2013; Lambert et al., 2013; Zhang et al., 2013; Karch and Goate, 2015; Villegas-Llerena et al., 2016; Efthymiou et al., 2017). Large-scale genome-wide association studies (GWAS) have identified more than 20 loci that are causally linked to AD. Of these, approximately half are expressed or exclusively expressed in microglia or myeloid cells, including TREM2 (triggering receptor expressed on myeloid cells 2), CD33 and members of the classical complement cascade, apolipoprotein J (ApoJ)/Clusterin, and complement receptor 1 (CR1) (Colonna and Wang, 2016; Villegas-Llerena et al., 2016). These findings (Griciuc et al., 2013) implicate microglia as critical or even causal players in AD pathogenesis; however, their biological significance remains elusive.

Region-specific synapse loss and dysfunction are early hallmarks of AD. Microglia and immune-related pathways have been implicated in AD pathogenesis through GWAS, but their role in synapse loss and cognitive impairment remains elusive (Hong et al., 2016b). Complement proteins are often upregulated in AD and localize to neuritic plaques along with microglia, but these processes have been regarded largely as secondary to plaque-related neuroinflammation. However, in multiple AD mouse models, C1q and C3 have been associated with synapses before overt plaque deposition and are localized to brain regions vulnerable to synapse loss (Hong et al., 2016a). In addition, microglia in adult mice phagocytose synaptic material in the presence of soluble oligomeric amyloid-β, a key synaptotoxic species in AD. This engulfment is dependent on CR3, and blocking the complement cascade (C1q, C3, and CR3) protects the synapses (Hong et al., 2016a). Similarly, complement activation and microglia-mediated synaptic pruning are drivers of neurodegeneration caused by progranulin deficiency in mice (Lui et al., 2016). Together, these results suggest that a key developmental pruning pathway involving complement and microglia is reactivated early in the disease process to "mark" vulnerable synapses for destruction (Fig. 2).

It is important to understand what other pathways, besides complement, are critical for pruning. For example, CX3C chemokine receptor 1 (CX3CR1, also known as the microglial fractalkine receptor) is necessary for synapse maturation, elimination, and functional connectivity (Paolicelli et al., 2011, Zhan et al., 2014); however, whether or how fractalkine signaling regulates synaptic engulfment is not yet clear. Another pathway could involve neuronal activity that modulates synaptic engulfment in the developing brain (Schafer et al., 2012). Several other immune-related molecules have recently been identified as mediators of synaptic refinement and plasticity in the

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developing and mature brain (Boulanger 2009). These include neuronal pentraxins (NP1, NP2), neuronal activity–regulated pentraxin (Narp), and components of the adaptive immune system (e.g., the major histocompatibility class I [MHCI] family of proteins and receptors) (Huh et al., 2000; Bjartmar et al., 2006; Syken et al., 2006; Lee et al., 2014).

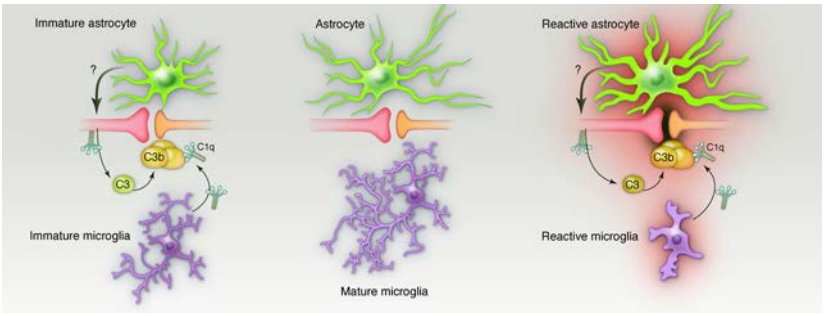

Figure 2. Complement-mediated synapse elimination during development and in neurodegenerative diseases. Left, In the developing brain, astrocytes induce the production of C1q in neurons through a molecular signal (?) that was recently identified as TGF-β (Bialas et al., 2013). Neuronal and microglia-derived C1q tags the weak or superfluous synapses for removal through the classical complement pathway, resulting in C3 cleavage and synaptic C3b deposition. Complement-tagged synapses are removed through phagocytosis by microglia. Center, In the absence of activated complement, synapses remain stable. Right, We propose that complement-mediated synapse elimination drives the development and/or progression of neurodegenerative diseases. As observed in the developing brain, reactive astrocytes release signal(s) (?) that induce C1q production in neurons. Neuronal and microglia-derived C1q is then recruited to synapses, which triggers the activation of downstream classical complement components, produced in excess by reactive astrocytes microglia, and neurons, resulting in microglia-mediated synapse elimination. Modified with permission from Stephan et al. (2012), Fig. 2. Copyright 2012, Annual Reviews.

It is intriguing to speculate that components of the complement pathway may be interacting with one of several of these immune-related molecules to mediate CNS synapse elimination in health and disease. In neurodegenerative diseases, there is aberrant neuronal activity in distinct brain networks (Seeley et al., 2009), perhaps triggering region-specific microglial phagocytosis of synapses. Knowing the positive and negative signals that regulate pruning, as well as those that guide microglia to specific synapses, will be important for nominating therapeutic candidates.

Redefining the Role of Microglia in Health and Disease

Loss of synaptic integrity has been linked to a host of developmental and neurodegenerative diseases, potentially implicating microglia-mediated pruning. Indeed, data from multiple models of neurological diseases, including AD, frontotemporal dementia, glaucoma, and West Nile virus–induced memory impairment, suggest that region-specific activation of the microglia–complement signaling pathway leads to synapse loss (Stevens et al., 2007; Hong et al., 2016a; Lui et al., 2016; Vasek et al., 2016). In addition, C4 (complement component 4) is a strong risk factor for schizophrenia (Sekar et al., 2016), indicating that this pathway could also underlie neurodevelopmental and neuropsychiatric diseases such as autism and schizophrenia. Microglia-mediated pruning may thus be involved in a variety of diseases, but its activation may differ in time, place, and magnitude. As such, it is imperative that we catalog pruning activity in the healthy brain and understand how microglia recognize and engulf specific synapses. This knowledge could not only lead to novel biomarkers for disease severity (e.g., of cognitive decline) but could also identify the time and place where intervention to protect synapses may be the most efficient. Further, learning how to modulate microglial functions may lead to drug candidates with broad therapeutic potential across multiple diseases.

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