Poised for action: USP18 restrains microglial activation in the white matter

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Microglia are the resident macrophage population of the central nervous system (CNS) and are required for CNS development, homeostasis, and immune defense. Dysregulated microglial activity is involved in the pathogenesis of neuro-degenerative conditions and is the dominant driver of neuro-inflammatory diseases named "microgliopathies". In this issue of The EMBO Journal, Goldmann et al reveal that white matter microglia in mice are actively maintained in a quiescent state via the ubiquitin-specific protease (Usp) 18 (Goldmann et al, 2015). Removing this molecular blocker results in aggressive type I IFN-mediated pathology, with features reminiscent of human microgliopathy. This study furthers our knowledge of the roles and regulation of microglial populations, adds insight into the process underlying neuro-inflammation, and broadens our consideration of immune regulation to include the concept of active restraint as a necessary component to avoid excessive inflammation.

See also: T Goldmann et al

Microglia are a specialized population of macrophages that form a homogeneously distributed network throughout the central nervous system (CNS) (Ginhoux et al, 2013). As part of the innate immune system, microglia constantly survey their environment for signals of external danger, such as those from invading pathogens, or the internal danger signals generated by damaged or apoptotic cells. Upon detecting such signals, microglia become activated and produce neurotropic and pro-inflammatory factors (Gertig & Hanisch, 2014). While necessary to neutralize a pathogenic or inflammatory threat to the CNS, these processes can be damaging if not restrained: excessive microglial activation is implicated in the pathogenesis of various brain diseases, and several microglia-related genes have been linked with neuropsychiatric or neurologic disorders (Prinz & Priller, 2014). Thus, at least as important as the pro-inflammatory capacities of microglia are their abilities to restrain inflammation via the secretion of dampening immuno-modulators such as IL-10 and TGF-β (Colton, 2009). Alongside this complex immunological balancing act, microglia also contribute to brain development and homeostasis through influences on neuronal proliferation and differentiation, and the formation and elimination of synaptic connections (Paolicelli et al, 2014; Squarzoni et al, 2014).

Given the diversity and impact of microglial roles within the CNS, understanding their origin, regulation, and diversity is imperative. Many studies have aimed to identify the extrinsic mechanisms leading to pathological activation of microglia (Colton, 2009), while the investigation of their equal and opposite state of quiescence has been somewhat overlooked. Goldmann et al offer a new perspective on microglial activation and show that white matter microglia is under constant molecular restraint to avoid constitutive activation and immune pathology and hints that this process might be involved in human autoimmune brain inflammation (Goldmann et al, 2015). The central finding of Goldmann et al is that the ubiquitin-specific protease (Usp) 18 (also named UBP43) is a key molecule imposing microglial quiescence in the white matter.

Usp proteins are part of a large family of deubiquitinating enzymes, many of which are involved in regulation of the immune system (Sun, 2008), but remain unstudied in the context of the CNS. The authors therefore first asked whether microglia in the murine brain expressed Usps. Microglia from both the white and gray matter contained mRNAs encoding several Usps, but Usp18 was specifically and abundantly expressed in white matter microglia. Mice lacking Usp18 exhibited a white matter microglial activation (WMMA) phenotype in the context of otherwise normal physiology, reminiscent of human microgliopathies. The authors demonstrated a significant accumulation of microglia displaying activation markers in the white matter of adult Usp18-deficient mice compared with wild-type animals, in association with increased transcription of several myelo-attracting and activating chemokines. These observations were replicated in mice bearing a Usp18 deficiency restricted to CX3CR1-expressing myeloid cells, which includes microglia; the authors thus argued against a role for other cell types in the brain (such as neurons, astrocytes, and oligodendrocytes) in driving WMMA. However, other myeloid cell populations are associated with the CNS, including perivascular and leptomeningeal macrophages, and their expression of Usp18/the protein's function in this context is unknown. While the contribution of other myeloid cell types to WMMA remains unestablished, a murine model of multiple sclerosis (MS) indicated the potential significance of the process. Spinal cord samples from these mice revealed induction of Usp18 mRNA and elevated levels of phosphorylated STAT1 proteins compared to wild-type controls. Phosphorylated STAT1

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was also detected within microglia in brain tissue samples from MS patients, though the frequency of its expression in the same cells from normal brain was not established.

To dissect the underlying mechanisms, the authors found that microglia from wild-type mice exhibited constitutive activation of type I interferon signaling pathways and upregulated Usp18 gene expression upon exposure to IFN-β. By comparison, microglia from mice lacking Usp18 constitutively expressed relatively higher levels of type I IFN-regulated genes than those from wild-type mice, accompanied by prolonged STAT1 activation. Taken together, these data suggest that type I interferon-stimulated pathways are constitutively active in white matter microglia and that Usp18 is required for their regulation in order to avoid microgliopathy. Usp18 can negatively regulate IFN-α/β signaling both by proteolytically deconjugating the interferon-stimulated gene 15 protein from its substrates (Malakhova et al., 2002) and by competing with JAK1 for binding to the type I IFN receptor subunit, IFNAR2 (Malakhova et al., 2006). The authors used novel mouse strains expressing Usp18 mutated in either functional domain to reveal that the effects seen in white matter microglia did not require Usp18’s protease activity, instead relying on a direct interaction with IFNAR2. In summary, Usp18 appears to be central to CNS homeostasis as mediated by white matter microglia (Fig 1).

This study raises several intriguing questions: what is the true nature and implication of the apparent microglial heterogeneity within the brain; where is the type I IFN coming from that is causing signaling in microglia in the steady state; and what is the physiological relevance of such tonic IFN signaling for microglia and for brain function? Within the CNS, IFN-α/β is produced during inflammation by glial cells, predominantly microglia and astrocytes, as well as by neurons (Owens et al., 2014). However, a steady-state source of IFN-α/β in the CNS has yet to be identified. As microglia arise from yolk sac primitive macrophages that colonize the brain rudiment during embryogenesis (Ginhoux et al., 2010), it seems unlikely that the observed differences between white and gray matter microglia arise as a result of lineage divergence; rather the heterogeneity of Usp18 expression might be explained by the differential expression of type I IFNs within the white matter. In which case, what is the trigger, which cell types are responsible, and what is the purpose of type I IFNs in this setting? Constitutive low-level type I IFN production is required for optimal functioning of the immune system at large, including responses to inflammatory cytokines, maintenance and mobilization of hematopoietic stem cells within the bone marrow, and phagocytosis by macrophages (Gough et al., 2012), but its role in CNS homeostasis is unexplored. Finally, when is this constitutive low-level type I IFN production triggered? Since WWMA is observed postnatally, an obvious hypothesis will be that constitutive production of type I IFN could be triggered by commensal microorganisms and could be addressed under germ-free conditions.

Overall, the study by Goldmann et al significantly advances our understanding of microglial activation and its regulation as well as of immune-mediated CNS homeostasis and serves to highlight several areas for further study, which should enhance knowledge of human microgliopathies.

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References


