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EDITORIAL

Editorial Focus: White matter-associated microglia (WAMs) represent an important link between aging, white matter disease and microglial activity

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Microglia constitute the brain's resident immune cells and play an active role in the response to aging, injury and neurodegeneration. Since their discovery over half a century ago, reports that microglia undergo activation in response to in vitro stimulation were soon followed by their characterization into the rigid dichotomy of M1/M2 pro- and anti-inflammatory activation states [1, 2]. In recent years, however, the emergence of transcriptomics has opened the door for the identification of multiple transcriptional states of microglia, suggesting a spectrum rather than a polarization of activation states. This includes microglial phenotypes specific to disease states, such as tumour-associated microglia (TAMs) or disease-associated microglia (DAM) implicated in Alzheimer's disease (AD) [3–5].

Within the white matter of the brain, activated microglia increase in an age-dependent manner and are associated with clinical evidence of white matter disease [6, 7]. Due to the difficulty of studying microglia in vivo, white matter microglia activity remains poorly understood, although recent transcriptomics support their pro-inflammatory nature [8].

To address this outstanding knowledge gap, in their recent work, Safaiyan et al. investigate the question: do the microglia in the aged white matter have a unique molecular signature? Here, the authors identify a novel phenotype of microglia they term "white matter-associated microglia" (WAMs) in 18–20 and 24-month-old mice, using single-cell RNA sequencing (scRNA-seq) for the former cohort and droplet-based scRNA-seq (Drop-seq) for the latter [9]. Strengthening their findings, the authors further validated the presence of WAMs in the datasets from two previously published studies. The authors utilized a wide range of murine models across different ages in subsequent experiments to elucidate WAMs as an age-dependent phenotype defined by genes implicated in HIF-1 signalling, glycolysis, lysosome activity, cholesterol metabolism, antigen processing pathways and clearance of myelin debris.

Strengths of this study include the utilization of scRNA-seq with a previously developed automated dissociation protocol to inhibit ex vivo transcription and generate data more representative of in vivo transcription [10]. Furthermore, microglia from white matter tracts (corpus callosum, optical tracts and medial lemniscus) and grey matter were analyzed separately to account for tissue-specific responses to aging. Aged white matter is often subject to ischemic conditions caused by cerebrovascular disease, and due to the high myelin content, age-related degeneration releases lipid-rich debris. In contrast, the grey matter has a more robust blood supply and accumulates debris related to proteinopathies. In line with these considerations, the authors report that the WAMs

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showed the most significant upregulation of genes related to hypoxia and phagocytose myelin debris. Unlike the WAM phenotype, "activated" microglia (which may be similar to what is conventionally called pro-inflammatory microglia) were present in both grey and white matter, although in higher numbers in the white matter (24% vs 5%). This confirms the existence of a phenotype unique to the aged white matter separate from activated microglia present in both tissue types. Future studies are needed to investigate potential interactions and influences between activated microglia and WAMs.

In clinical studies of AD, one of the earliest neuroimaging features is abnormalities of the white matter [11]. However, most recent studies of microglia in AD have been focused on DAM associated with amyloid accumulation [12], without a clear understanding of unique white matter microglia responses. To address this question, Safaiyan et al. used the transgenic APP/PS1 mouse model to investigate both the WAM and DAM phenotype. The authors report that WAM can be detected in addition to the DAM1 and DAM2 phenotypes previously described but appear at earlier age points with a distinct expression profile [3]. WAMs contain upregulated genes related to atherosclerosis, cytokine signalling and apoptosis in comparison to DAM2 which upregulate AD, Parkinson's and Huntington's disease-related genes. Since TREM2 is known to be a key regulator of the DAM phenotype, the authors used a TREM2 knockout mouse and established that in aging, the WAM phenotype was also dependent on TREM2 expression. They also investigated *Apoe* expression, which was more highly expressed in the DAM phenotype versus WAM. Using $Apoe^{-/-}$ mice the authors report that in aging the WAM phenotype is independent of APOE in comparison to the DAM phenotype. In the APP/PS1 mouse model, however, both WAM and DAM phenotype are APOE dependent. Therefore, the mechanisms regulating the WAM phenotype differ in "normal" aging versus aging in the context of genetically induced AD.

The identification of the WAM phenotype raises the important question of their functional consequences within the white matter. The authors report that WAMs degrade myelin, evidenced by positive MBP immunohistochemistry and galectin-3 in Iba1 + cells within the corpus callosum, which was exacerbated in a PMD (Pelizaeus-Merzbacher disease) mouse model that displays aggressive demyelination. The authors argue that the role of WAMs in the aged white matter is protective. In the absence of TREM2, there was an increase in microglia with irregular phenotype and inadequacy in the degradation, but not phagocytosis, of myelin debris. WAMs may be actively clearing myelin debris but not necessarily phagocytosing myelin in a deleterious manner. If this is accurate, then future work investigating the response of microglia to increasing debris levels and resulting shifts in phenotype is required.

As the scRNA-seq data of this work were generated exclusively from murine microglia, future studies should assess human microglia from the aged white matter which may have unique molecular signatures [13]. Additionally, given the compelling findings of Safaiyan et al., spatial transcriptomics should be utilized in future work to assess differences across various white matter tracts and grey matter regions in the normal, aged and diseased brain. The identification of an age-dependent increase of WAMs in aged mice devoid of additional comorbidities represents an important contribution, as aging itself is a predominant risk factor for stroke and neurodegenerative diseases. It raises important questions for future study as to how the WAM phenotype may affect the microglial response to future injury or neurodegeneration. Can WAMs shift into a more pro-inflammatory or DAM phenotype with further injury, representing a continuum of cell identities? Understanding the coexistence of multiple microglial phenotypes within the same brain across various anatomical regions and the mechanisms driving spatial and temporal shifts between these phenotypes opens the door to exciting areas of future research.

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