Dissecting the complex landscape of neuroinflammation at single-cell resolution

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How the immune system interfaces with the central nervous system (CNS) to promote health and disease remains an enigmatic puzzle. Once thought to be an immune-privileged niche, the CNS is actively patrolled by a repertoire of myeloid cells, including tissue-resident microglia (MG) found in brain parenchyma and CNS-associated macrophages (CAMs) found in the meninges, choroid plexus, and perivascular spaces. While MGs and CAMs play an important role in maintaining homeostasis, an aberrant inflammatory response by these immune effectors is a key feature of most forms of neuropathology, from neurodegenerative disorders like Alzheimer's Disease to demyelinating diseases such as multiple sclerosis (MS)[1]. Furthermore, in MS and experimental autoimmune encephalomyelitis (EAE; a mouse model of MS[2]) many peripheral immune cells, including monocytes and dendritic cells, also invade the CNS.

It has been technically challenging to dissect the role that invading, peripheral immune cells play in disease progression versus the existing CAMs and MGs, due to a lack of adequate tools. Now, owing largely to the power of single-cell transcriptional profiling and transgenic mouse lines that distinguish the different myeloid cells found in the CNS, a new study by Jordão et al. has provided a roadmap for understanding how myeloid cells respond to acute inflammation and contribute to the progression of EAE[3]. This work has significant implications for the development of targeted interventions to treat inflammatory, demyelinating processes, like MS.

Initially, Jordão and colleagues isolated and performed single-cell RNAseq (scRNAeq) on a total of 3,461 myeloid cells from several CNS compartments at various points of EAE progression, as well as on a subset of cells from normal, healthy mice. Using t-distributed stochastic neighbor embedding (t-SNE; a form of analysis utilized in scRNAseq experiments that determines how closely related the expression profiles of individual cells are), six major cell types were identified across all brain regions and time points. Interestingly, each cell type further subdivided into two distinct clusters, one represented by cells from healthy mice (homeostatic clusters) and the other representing cells from mice with EAE (disease-associated clusters).

This clear distinction between homeostatic ("h") and disease-associated ("da") myeloid cells leads to a natural follow-up question, i.e. "Why are these cells so distinct?" In the case of MG, daMGs displayed increased expression of chemokines and genes responsible for cell proliferation. To determine whether daMGs were truly more proliferative than hMGs, the team employed a transgenic mouse line that labels individual MG, each with a different fluorescent reporter. In the parenchyma, daMGs were shown to undergo clonal expansion during the course of EAE, which nicely corroborated their gene expression profile obtained from the single-cell dataset.

The gene expression differences between hCAMs and daCAMs shed light on one of the major debates in EAE models and MS, which has centered around how lymphocytes are primed to target myelin[4,5]. Because peripheral monocytes and dendritic cells invade the CNS early in the inflammatory progression, there are many potential antigen-presenting cells (including the central myeloid cells, CAMs and MGs) within in the brain that could prime and recruit T-cells and B-cells. Compared to hCAMs, daCAMs upregulated genes responsible for MHC class II presentation of antigens to lymphocytes, suggesting that they may be functionally responsible for this priming. To test this hypothesis, the progression of EAE was assessed in a transgenic mouse, in which MHC class II presentation was abolished in all CAMs and MGs. Unexpectedly, abolishing the antigen-presenting capacity of these cell types had no effect on disease course or severity. Only when MHC class II machinery was conditionally deleted from both central and peripheral myeloid cells, did mice become resistant to EAE. Lymphocytes invading the CNS also showed significantly longer contacts with peripherally-derived monocytes than tissue-

resident macrophages suggesting that it was the peripherally invading myeloid cells that are critical for recruiting T-cells, and that the robust expression of antigen-presenting machinery in daCAMs is redundant.

In many fields, the application of scRNAseq has revolutionized our understanding of the complex and dynamic nature of cellular processes[6]. In the past, myeloid profiling of cells occurred in bulk. That approach, while providing insights into the common biology occurring collectively in a group of cells, collapsed the heterogeneity of how individual cells might be responding differently to a particular stimulus. This work of Jordão et al. confirms the vastness of this heterogeneity by demonstrating just how distinct myeloid cells in the brain can be, not just indicated by their cell type (ie. MG, CAM, monocyte-derived), but based on their location and disease-state. Using this single-cell dataset, we can now define markers that may be more relevant to classifying a distinct cellular process. For example, Jordão et al. demonstrate that while classic MG markers like *Tmem119* are more dynamic in their expression among different MG sub-clusters, other targets like *Olfml3* and *Sparc* may serve as more universal microglial-markers. Additionally, *Ms4a7* was expressed by all CAMs across all brain regions and stages of inflammation, and may be a robust marker for studying relevant biology in this population of cells.

Beyond illustrating the complexity underlying the immune response in EAE, this work also sets the stage for the development of novel, targeted disease-modifying therapies (DMTs) that can be used in inflammatory demyelinating diseases like MS[7]. Prior DMTs have utilized hammer-like approaches to target inflammation broadly, in the hopes of stemming the course of autoimmune destruction. From this work, we have gained evidence that while monocyte-derived myeloid cells are critical for antigen presentation early in disease course, they diminish in numbers during the chronic stage of inflammation. Concomitantly, MGs undergo clonal expansion over the course of disease and are the predominant myeloid cell type present in the parenchyma during chronic inflammation. Therapies aimed at countering inflammation early versus late during the demyelinating process must therefore target different cellular processes and responses. Armed with the single cell data for each of the relevant cell types, future efforts can target specific cells and minimize broad off-target effects.

Important questions still remain unanswered. For instance, there are multiple mouse models of EAE that utilize different antigens to elicit inflammation or toxic agents that damage myelin, and there is an active debate regarding which models have the most translational relevance. Application of this single cell approach to the different models could ascertain the commonality in immune responses within these disparate modalities of inciting demyelination in mice. Ultimately, application of single-cell profiling on brain biopsy samples or post-mortem tissue from MS-affected patients could allow for definitive mapping of conserved cellular immune responses in mice and humans, which would greatly accelerate the development of therapeutic interventions for inflammatory conditions that affect the central nervous system.

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