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Distinct origins, gene expression and function of microglia and monocyte-derived macrophages in CNS myelin injury and regeneration

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ABSTRACT

Central nervous system (CNS) injury incurs a rapid innate immune response, including that from macrophages derived from endogenous microglia and circulating monocytes infiltrating the lesion site. One example of such injury is the demyelination observed in the autoimmune disease multiple sclerosis (MS), where macrophages are implicated in both myelin injury and regeneration. Although initially microglia and monocyte-derived macrophages were considered to have identical origins, gene expression, and function, recent advances have revealed important distinctions in all three categories and have caused a paradigm shift in view of their unique identity and roles. This has important consequences for understanding their individual contribution to neurological function and therapeutic targeting of these populations in diseases like MS. Here, we address the differences between CNS endogenous and exogenously-derived macrophages with a particular focus on myelin damage and regeneration.

1. Introduction

Macrophages are components of the innate immune system, involved in surveying tissue for signals of damage or infection. Detection of these signals via receptors on their surface such as pattern recognition receptors stimulates inflammatory pathways (e.g. nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and type-1 interferon) [1], resulting in the initiation of responses including phagocytosis (of debris/pathogens/apoptotic cells), antigen presentation, and secretion of factors (cytokines, chemokines, growth factors, toxic molecules). These responses are components of both tissue injury and regeneration and must thus be tightly regulated to prevent further damage and to promote resolution. Indeed dysregulation of macrophage activation and function is considered to contribute to disease.

One such example in which this is considered to occur is the autoimmune disease multiple sclerosis (MS), affecting between 2 and 3 million people worldwide and representing the leading neurological disorder among young adults. In MS, immune-mediated destruction of the myelin surrounding axons of the central nervous system (CNS) (termed demyelination) causes axonal dysfunction, damage, and loss, underpinning the clinical deficits in sensation, motion, and cognition. Although macrophages are implicated in induction and exacerbation of demyelination, they are also involved in promoting the regeneration of myelin (termed remyelination) considered to restore axonal function and health [2–5], a process which often fails in progressive MS [6]. Therefore macrophages represent important therapeutic targets for treatment of MS; however their roles in both detrimental and regenerative CNS processes highlight the need to elucidate their heterogeneity in terms of origin, gene expression, and function to determine which subsets of these cells are involved at particular stages of disease.

2. Distinct origins of microglia and monocyte-derived macrophages

Microglia are the resident macrophages of the CNS and make up 5–12% of neural cells [7]. In humans, microglia are generated from myeloid progenitors seeding the CNS during the first two trimesters of gestation, with cells of microglia-like morphology first detectable at 13 weeks and differentiated microglia abundant by 35 weeks [8–10]. Microglia and monocyte-derived macrophages were until recently considered to originate from the same location/process due to shared morphology and expression of markers (e.g. F4/80, CD11b, CD45, CD68, CSF1R, CD200R, CX3CR1, Iba1) [11]. However, fate mapping using mice has shown that microglia are derived from erythromyeloid progenitors in the yolk sac during embryonic haematopoiesis in a PU.1- and IRF8-dependent and Myb-independent manner [12–18]. TGF-β has also been reported to be important for the development and maintenance of microglia in a quiescent state [19]. The microglial precursors arise at E8 in rodents prior to vascularization and blood-brain barrier formation [14,20–21] and penetrate the neuroepithilium at E9–9.5 to form initial

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clusters. These clusters then disperse and expand throughout the CNS [21,22], with ramified microglial morphology, characterised by a small cell body and long processes, appearing at E14. These studies show that microglial seeding in the CNS is conserved across species. The microglial population is long-lived and self-renews [18,23–24].

In contrast, monocyte-derived CNS macrophages are generated from Ly6C^{hi} monocytes; monocytes are derived from Myb-dependent definitive haematopoiesis first in the aorta-gonad-mesonephros at E10.5, fetal liver at E12.5 and bone marrow postnatally [25–27]. Circulating monocytes are short-lived and are continuously replaced via haematopoietic precursor differentiation [23]. As the CNS is immune-privileged, these circulating monocytes will normally only infiltrate the CNS upon infection or injury to differentiate in situ into macrophages [28]. The presence of monocyte-derived macrophages in the CNS is short-lived and these cells do not contribute to the microglia pool [28]. Moreover, the microglial progenitor does not give rise to circulating monocytes [14], further emphasising the distinct lineage of these cell populations.

Although microglia and monocyte-derived macrophages arise from different sources, their expression of the same core surface markers which are dynamically regulated during injury (e.g. CD11b, CD68, Iba1) render distinguishing these different cell types difficult in the CNS. Studies have revealed differential expression of some markers including CCR2, CD39, CD44 [21,29–31] and CD45 (i.e. CD45 is expressed by both microglia and macrophages although the level of expression is greater in monocyte-derived macrophages). Importantly, a recent study has described in detail the exclusive expression of *Tmem119* by most, if not all, microglia during homeostasis and injury/inflammation [32]. Thus, these markers can be used to successfully distinguish between microglia and monocyte-derived macrophages in the CNS following injury.

3. Gene expression profiles of microglia and monocyte-derived macrophages

As microglia and monocyte-derived macrophages reside in different tissues under homeostatic conditions, it has been speculated that they may have different gene expression profiles. Transcriptomic studies of adult mouse brain microglia (CD11b$^+$ CD45^{lo}) and peritoneal macrophages (CD11b$^+$ CD45^{hi}) identified a large number of shared transcripts (1476), indicating similarities between the cell populations [33]. However, recent studies revealed genes highly enriched in microglia (including *P2ry12*, *Tmem119*, *Fcrls*, *Olmfl3*, *C1qa*, *Siglech*, *Sall1*, *Gpr34*, and *Hexb*) [19,33–34] which are distinct from the peripheral macrophage-specific gene signature (including *Fn1*, *Cxcl13*, and *Ednrb*) under non-pathological conditions. Furthermore the core microglial gene signature is conserved among species; genes such as *P2ry12*, *Gpr34*, *C1qa*, and *Mertk* are specifically or highly expressed in human microglia [19]. Microglial transcriptome analysis also revealed a signature for genes involved in endogenous ligand and microbe recognition, termed the 'sensome' [33]. Comparison of microglial and monocyte-derived macrophages indicated that although some sensome genes are also expressed by the latter (*Csf1r*, *Cd53*), 22 genes (out of 100) are exclusively expressed by microglia (*P2ry12*, *Tmem119*, *Siglech*) under homeostatic conditions. In addition, genes involved in microbial killing are highly expressed by microglia relative to peripheral macrophage populations [33].

Inter-regional variability in microglial gene expression is also evident [29,35–37]. Investigation of the microglial sensome revealed that 34 genes have differential expression patterns dependent on brain region [35]. Of these, the majority are enriched in the striatum and cortex and are involved in immune signalling and restraining microglial activation (e.g. *Trem2*, *Siglech*, *Cx3cr1*). Regional differences in adult mouse microglial populations are also observed for genes involved in energy metabolism and immune function [35]. Microglia residing in the cerebellum have increased expression of genes related to immune function, including antigen presentation (MHC-I (*H1-D1*, *H2-K1*) and MHC-II (*H2-Aa*, *H2-Ab1*)), pathogen recognition (*Cd209a*, *Clec7a*, *Fcnb*), and microbial killing (*Camp*, *Ngp*) compared to microglia residing in the cortex, striatum and hippocampus. In addition to the immune function gene signature of microglia, a second brain-region specific microglial signature relates to genes involved in energy production [35]. Microglia of the cerebellum and hippocampus have enriched expression of genes relating to glycolysis, the electron transport chain, and ATP synthesis. Together, these results indicate that the microglia in the cerebellum have a higher state of immune alertness than microglia in the cortex and striatum, and this is associated with co-regulation of genes implicated in energy metabolism. A possible explanation for the increased vigilance of white matter microglia could be that the lower density of microglia present in these regions requires greater immune surveillance, and as cells have to survey larger areas of the brain there are higher associated energy requirements [35]. Gene expression changes in microglia are also induced with ageing. Normal ageing induces a shift towards a neuroprotective microglial phenotype, with increased expression of genes involved in the STAT3 and neuregulin-1 pathways, and upregulation of alternative activation markers [33]. Moreover, microglia from different regions age at different rates [35]. For example, the cerebellum shows an 'aged' expression signature 12 months earlier than forebrain regions, with particular emphasis on genes involved in immune function, including pathogen recognition, cytokine signalling, and the interferon pathway [35,38].

The distinct gene expression profiles for microglia and monocyte-derived macrophages under homeostatic conditions also remains distinct at all stages of disease in a model of immune-mediated demyelination (experimental autoimmune encephalomyelitis; EAE). The recruited monocyte-derived macrophages do not acquire a microglial-like signature [19,31]; subsets of genes enriched specifically in microglia or monocyte-derived macrophages are still detected [39]. At onset of disease, microglia upregulate genes associated with chemoattraction (*Ccl2*, *Cxcl10*, *Ccl5*) and complement (*C1qa*, *C1qb*) [31,39]. They also show a repressed activation profile with downregulation of genes associated with phagocytosis, microtubule and cytoskeletal dynamics, RNA transcription, and synthesis of reactive oxygen species [39]. Contrasting results have been obtained regarding microglial regulation of genes associated with proliferation at disease onset [31,39]. Many genes, including those involved in intracellular signalling, which are enriched in microglia prior to disease onset, are downregulated during onset and peak of disease then return to naïve levels during recovery, indicative of return to homeostasis [39]. With regards to monocyte-derived macrophages, at the onset of EAE these have a signature related to pro-inflammatory responses, including increased expression of genes associated with cell adhesion [31,38], phagocytic activity/cell clearance [39], complement (C3, C1s, Cfb) and chemokines (CCL2, CXCL10, CXCL2) [31]. Conflicting results between studies relate to downregulation of genes in this population at onset, with one study reporting none [39] and another reporting downregulation of select transcription factors (*Pparg*, *Nr4a1*, *Irf4*) [31].

Although comparisons between microglia and monocyte-derived macrophage gene expression during remyelination have yet to be performed, analysis of microglia alone has revealed changes in expression which may reflect regenerative function. In the cuprizone model of demyelination, 6200 genes are expressed by microglia under normal conditions and during demyelination and remyelination [29]. Three patterns of gene expression are observed: i) genes involved in metabolic processes and acute inflammatory responses are downregulated during demyelination and remyelination; ii) cell cycle genes are upregulated during demyelination and downregulated during remyelination; iii) genes involved in immune response, phagocytosis, and antigen processing/presentation are upregulated during demyelination and remain elevated in remyelination [29]. Genes specifically upregulated during demyelination include those involved in cell cycle and p53 pathways, whilst those upregulated specifically during remyelination include