Protective roles for myeloid cells in neuroinflammation

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Protective roles for myeloid cells in neuroinflammation

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Abstract

Myeloid cells represent the major cellular component of innate immune responses. Myeloid cells include monocytes and macrophages, granulocytes (neutrophils, basophils and eosinophils) and dendritic cells (DC). The role of myeloid cells has been broadly described both in physiological but also pathological conditions. All tissues or organs are equipped with resident myeloid cells, such as parenchymal microglia in the brain, which contribute to maintaining homeostasis. Moreover, in case of infection or tissue damage other myeloid cells such as monocytes or granulocytes (especially neutrophils) can be recruited from the circulation, at first to promote inflammation and later to participate in repair and regeneration. This review aims to address the regulatory roles of myeloid cells in inflammatory diseases of the central nervous system (CNS), with a particular focus on recent work showing induction of suppressive function via stimulation of innate signaling in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE).

Introduction

Neuroinflammation, involving infiltration of the normally immune quiescent CNS by blood-derived lymphocytes and myeloid cells, is encountered under many clinical circumstances, with CNS-intrinsic and -extrinsic triggers including stroke, tumors, infection and inflammatory demyelinating diseases (IDD). The latter are typified by MS, characterised by an autoimmune T cell-induced inflammatory response associated with demyelination. Infiltration and activation of myeloid cells, both infiltrating and CNS-resident, are also features of MS. The pathology of relapsing remitting MS, the most common type of MS disease, is reminiscent of a Type IV hypersensitivity with prominent infiltration from blood of CD8+ and CD4+ T cells, B cells and macrophages, associated to grey and white matter demyelination and axonal damage [1]. The related but distinct disease neuromyelitis optica spectrum disorders, termed NMOSD, are considered to be mediated by complement-dependent serum-derived autoantibody attack, and the resultant gliopathy and demyelination are associated with granulocytic infiltrates. A further distinct clinical syndrome is associated with autoantibodies targeting myelin oligodendrocyte glycoprotein (MOG) [1].

Immune responses in the CNS are distinguished from responses in other tissues or organs by two aspects. One is that access from blood is more regulated than in most other tissues, the other is a unique and characteristic microenvironment. Blood–CNS barriers include the blood-brain barrier (BBB) localized within CNS microvessels, and blood-cerebrospinal fluid (CSF) barriers e.g. in the choroid plexus. Immune cell traffic from blood is restricted by interaction with elements of the BBB,

notably endothelial tight junctions and the (mostly) astrocytic glia limitans in post-capillary venules, with interest in the choroid plexus as another site of immune cell entry [2]. Common features of neuroinflammation are local activation of resident myeloid cells, and recruitment of peripheral myeloid cells. Both cell compartments may facilitate the inflammatory responses but importantly can play protective and regenerative functions. Given the bone marrow and blood origins of peripheral myeloid cells it becomes important to consider how they might access the CNS. Within a general model of rolling adhesion, chemokine attraction and protease-mediated barrier disruption, distinct signals and mechanisms underly transmigration of leukocyte sub-lineages such as lymphocytes, monocytes and neutrophils [2]. However, all blood-derived cells can migrate into the CSF, under appropriate conditions.

Here we review protective functions of myeloid cells and mechanisms for their induction in MS and EAE, and contrast them with pro-inflammatory effects.

Myeloid cells in the CNS

CNS myeloid cells can be divided into two major categories, corresponding to their location: parenchymal and extraparenchymal.

Microglia are CNS-resident myeloid cells with classical tissue macrophage properties within the CNS parenchyma. They are distinguished from other CNS-resident myeloid cells as well as infiltrating leukocytes by reduced levels of the cell membrane tyrosine phosphatase CD45, as well as other markers (e.g. [3]). Microglia are of particular interest in a CNS-autoimmune context because of their ability to phagocytose and present tissue antigens, and to regulate inflammatory responses.

Extraparenchymal CNS-resident myeloid cell populations are located within the CSF compartment, in the leptomeninges, and subarachnoid and perivascular spaces. These cells include some DCs, as well as macrophages that have been termed border-associated macrophages (BAMs), which include perivascular, meningeal and choroid plexus macrophages. BAMs share developmental yolk sac origin with microglia but differ transcriptionally from them, as well as from each other [4]. Importantly, the location of these cells at the interface between the periphery and the parenchyma is ideal to monitor both environments.

CNS-resident myeloid cells in inflammatory demyelinating diseases

Animal models for MS include EAE, induced by adjuvant-based immunization against myelin proteins, or by transfer of T cells from immunized animals. The resulting Th1- and Th17-dominated

inflammation is associated with demyelination and axonal damage in spinal cord and brain (depending on species, strain and model) with symptoms appropriate to lesion location [5].

Microglial activation is a common feature in neuroinflammatory diseases. They are fully equipped for antigen presentation and may play an important role in inducing T cell responses within the CNS. In particular, expression of MHC II on activated microglia can be detected even before clinical signs of EAE [6]. Pharmacological [7] and genetic inhibition [8, 9] of microglia activation as well as their pharmacological depletion [10] attenuates the course of EAE, suggesting their detrimental role in the initiation, as well as perpetuation of the disease.

However, a growing body of evidence suggests that there are also protective functions for microglia in EAE. A recent study indicated that pharmacological depletion of microglia significantly exacerbated secondary progression of EAE and data suggested that microglia suppressed secondary progression of EAE by inhibiting the proliferation of CD4+ T cells in the CNS [11]. In addition, the study by Nissen et al. showed a shift from an inflammatory to protective phenotype in microglia that escaped the depletion [10]. This fits to the concept of repopulating myeloid cells being protective, as has been shown in other contexts [12]. Microglia death and repopulation physiologically occurs in response to white matter injury. Importantly, such repopulated cells show a regenerative phenotype and actively contribute to remyelination [13]. Microglia have been shown to respond to the anti-inflammatory cytokine interferon (IFN) β, that positively regulates their repopulation, as well as promotes phagocytosis of myelin debris and as a consequence, facilitates remyelination [13, 14].

It has now been well documented that a subset of microglia that can be identified by CD11c expression emerges during neurodevelopment, neuroinflammation as well as neurodegeneration (extensively reviewed in [15]). These CD11c+ microglia express microglia signature genes, as well as typical microglia markers such as IBA1 or CX3CR1. A transcriptomic signature that includes *Itgax, Igf1, Clec7a, Spp1* is shared by microglia in many studies and is particularly associated with the CD11c+ subset [15]. Interestingly, this subset of microglia is necessary for primary myelination and is involved in protection from EAE [16-18]. CD11c+ microglia can be induced by CSF1R stimulation [16], as well as by blocking Sirpα-CD47 signaling [19]. CSF1R stimulation led to EAE suppression [16].

White matter pathology induced by the copper chelator cuprizone is used as a model for demyelination and remyelination in MS. Microglia predominate in demyelinated lesions [20] and it is instructive to review their role. A beneficial role was suggested by amelioration of demyelination by CSF1R stimulation [21]. By contrast, remyelination and motor function recovery were enhanced by

pharmacological microglial depletion [22], and reduction in microglial number using a different CSF1R inhibition attenuated acute demyelination, and promoted remyelination and neuroprotection in a chronic model [23]. It can be speculated that the distinct outcomes of microglial depletion in cuprizone versus EAE models is due to the relative absence of overt inflammation, or differences in environmental factors in cuprizone induced de- and re-myelination.

Similar to microglia, both DCs and macrophages are implicated as antigen-presenting cells (APCs). APCs have been proposed to regulate entry of antigen-specific T cells to the CNS. For instance, interaction with myeloid phagocytic cells in the meninges was shown to be a necessary step for T cell entry in the parenchyma and establishment of pathology [24]. Using selective transgenic deletion strategies two groups have shown that BAMs and microglia are redundant for this APC activity, whereas MHC II-expressing conventional DCs are essential, and their deletion protected against EAE [25, 26]. It has been reported that all subsets of BAMs adapt their phenotype and generate new specialized subsets in case of neuroinflammation [26]. However, the morphological similarities between infiltrating monocytes and resident BAMs have made it quite difficult to study the role of BAMs specifically in this context. Jordao et al. used a combination of single-cell transcriptomics and a fate-mapping system to achieve this and reported *in situ* proliferation of all BAMs during EAE, although at lower rates than microglia [26]. In addition, they noted that although meningeal macrophages were phenotypically indistinguishable from infiltrating myeloid cells, the latter were distinctly smaller than perivascular macrophages. Interestingly, the accumulation of immune cells in the meninges seems to precede and contribute largely to EAE time-course, pointing towards the meningeal compartment as a gateway of entry of the CNS [26].

Immune suppression by peripheral myeloid cells

Research on tumorigenesis revealed the existence of myeloid cell populations which support tumor growth, by suppressing the activity and proliferation of host-protective T lymphocytes that participate in tumor cell eradication, and by stimulating expansion of regulatory T cells [27]. These myeloid cells, termed myeloid-derived suppressor cells (MDSC), have been described both in human and mouse and sub-phenotyped as either granulocytic/polymorphonuclear (G/PMN-) MDSCs or monocytic (M-) MDSCs. MDSCs have also been studied in the context of chronic infection, transplantation and autoimmunity [28]. It remains unclear whether MDSCs represent an immature stage in myeloid cell development or whether they are a separate sub-lineage, and phenotype of MDSCs is not yet explicit [27].

Mechanisms of immune suppression by MDSCs include cell-cell interactions, depletion of L-arginine, oxidative stress, impairment of viability and migration of T cells and production of anti-inflammatory

cytokines such as interleukin-10 (IL-10) [29]. The anti-proliferative effect of arthritis-protective MDSCs was blocked by anti-IL-10 [30]. There is growing interest in so-called checkpoint inhibitors, especially the programmed death-1 (PD-1) receptor which inhibits T cell activation upon ligation by programmed death-ligand (PD-L) 1, that can be induced on myeloid cells [31].

Furthermore, there is growing evidence that among each major myeloid population, cells exhibiting suppressive or regulatory capacity can be found, protecting the host from uncontrolled inflammation caused by either pathogens or self-antigens [29]. Those cells are called myeloid regulatory cells (MRCs). Because MDSCs are considered a subset of MRCs, we will use the term as a default, wherever MDSCs were not explicitly identified. It is important to understand the mechanisms responsible for induction of MRCs since they have therapeutic potential against e.g. autoimmune diseases, including neuroinflammatory diseases such as MS.

Myeloid regulatory cells in MS and EAE

More recently, MDSCs have proven to be of interest in regulating inflammation not associated with cancer. Accumulating evidence points to cytokines or innate receptor ligands including Toll-like receptors (TLRs) ligands as important players implicated in induction of myeloid suppressive phenotype [27]. The principal effector mechanisms of both granulocytic- (arginase-1 enzyme activity) and monocytic- (nitric oxide (NO) production) MDSCs are enhanced by IFNγ [27, 32]. The cytokine IL-10, another candidate mediator of suppression, was induced by IFNβ in the context of EAE-protection [33], as well as by innate ligands [34]. Another cytokine, transforming growth factor-beta (TGFβ), may also be implicated in myeloid suppression. Abrogation of TGFβ signaling in CX3CR1+ monocyte-derived macrophages led to rapid onset of a progressive and fatal demyelinating motor disease characterized by myelin-laden giant macrophages throughout the spinal cord [35].

Innate receptors, e.g. pattern recognition receptors, are widely expressed and can play pathologic and protective roles in MS and EAE [36]. Triggering innate receptors within the CNS may induce the infiltration of MRCs. As a specific example, G/PMN-MRCs were mobilized in EAE by intrathecal administration of a bispecific microparticle called MIS416, which combines ligands for TLR9 and NOD2 to activate phagocytes [34]. MIS416-associated amelioration of EAE was shown to, at least in part, be caused by PD-L1 expressing cells characterized as neutrophils (CD11b+, Ly6Clow, Ly6Ghigh) and functionally equivalent to G/PMN-MRCs, as transfer of such cells ameliorated EAE. This amelioration was further shown to be dependent on type I IFN signaling. These innately-stimulated G/PMN-MRCs were recruited from blood (shown by bone marrow chimera analyses) by a rapidly-induced CNS-endogenous chemokine response, and it is assumed that as-yet unidentified CSF-resident myeloid cells were the initiating source of G/PMN-attracting CXCL1 and CXCL2 [34].

A similar effect on recruitment of myeloid cells and amelioration of EAE by other innate ligands has been shown, including poly-I:C (ligand for TLR3) [37], though it was not shown whether such ligands could drive MRCs or MDSCs transition in a neuroinflammatory context. Although such findings align with the need for innate stimulation for induction of a suppressive MRC phenotype, and may be assumed to reflect a general mechanism, this requires confirmation. Focus to TLRs has been partly due to availability of reagents, extension to other innate receptor families will be informative.

It is known that G/PMN-MDSCs are recruited to the CNS in EAE. Transfer of cells identified as G/PMN-MDSCs that accumulated in the CNS of mice prior to remission from symptoms of EAE, suppressed disease and inhibited Th1 and Th17 responses in recipient animals [32]. G/PMN-MDSCs have been reported to be recruited to the CNS of mice with EAE and to suppress GM-CSF-producing B cells, which are associated to detrimental microglial activation and lack of recovery [38]. The frequency of CD138+ plasma B cells in CSF of MS patients correlated negatively with that of G/PMN-MDSCs [38]. Taken together, these findings point to a natural role for G/PMN-MDSCs in regulating neuroinflammation. Earlier studies described IFNγ-dependent induction of T cell suppression *in vitro* by neutrophils isolated from CNS of mice with EAE. This suppression was NO-mediated [39]. Ioannou et al. showed that PD-L1 expression by granulocytic MDSCs in peripheral lymphoid tissue and in CNS was upregulated by IFNγ, and these G/PMN-MDSC could transfer suppression of EAE [32].

Observation that G/PMN-MRCs suppression was IFNα receptor (IFNAR, receptor for type I IFNs)-dependent resonates with the known role for type I IFNs in EAE. IFNβ treatment ameliorates EAE, and lack of IFNβ or selective defect of its receptor IFNAR1 on myeloid cells results in exacerbation of EAE [40-42]. Microglia are a major source of IFNβ in EAE, and infiltrating myeloid cells also contribute to CNS-endogenous IFNβ [37, 42]. Peripheral treatment of mice with IFNβ at the onset of disease enhanced the presence of EAE-suppressive MDSCs in spinal cord [40]. Together these findings support an important role for myeloid cells in mediating the protective effect of IFNβ in EAE.

Less evidence is available for a role of M-MDSCs in neuroinflammation. In one EAE study, PD-L1 expressing M-MDSCs with suppressive activity were shown in demyelinated areas of the spinal cord [40]. White et al. have shown that intravenous injection of the MIS416 microparticle mobilized cells functionally resembling M-MDSCs in the periphery, with induction of Treg and NO responses and upregulation of PD-L1, leading to amelioration of EAE [43]. Myeloid cell responses and disease suppression were IFNγ-dependent [43]. In our study involving intrathecal administration of MIS416, cells showing the monocytic phenotype were also recruited to the CNS. However, their transfer via

intrathecal injection did not suppress EAE in recipient mice, which may point to the need for other activation pathways [34]. Interestingly, innate stimulation induced PD-L1+ monocytic myeloid cells to migrate to the CNS, even in mice without EAE, where they are presumed to regulate the inflammatory environment, although their suppressive ability was not determined [34, 43].

Validation that findings in EAE reflect the clinical situation in MS includes that neutrophils can play a role in MS and that this may be protective [38], as in other autoimmune diseases [44]. This is consistent with the role for IFNγ-producing Th1 and Th1/17 cells in MS [45]. The activation of STAT3 that led to induction of G/PMN-MDSC in the CNS in the study by Knier et al. was interpreted to be induced by IL-6 [38]. It remains to be determined whether other cytokines or ligands drive MDSC transition in a neuroinflammatory context.

The fact that granulocytes are implicated in mediation of pathology, rather than protection, in NMOSD may reflect a distinctive cytokine milieu in these primarily antibody+complement-mediated autoimmune diseases [1, 46]. These cells are not well-represented in the current generation of animal models for NMOSD [46]. NMOSD patients either show no benefit or indeed worsening after treatment with IFNβ, in contrast to relapsing-remitting MS [47]. Dependence on Type I IFN signaling has been shown in mouse models for NMOSD and anti-MOG-associated encephalitis, mirroring the clinical situation [48, 49]. One study suggests that microglia may play a detrimental role in NMOSD [50], although this and signals that can activate suppressor function for granulocytes remain to be more fully defined.

Synthesis and Conclusions

Like other organs, the CNS is equipped with resident myeloid cells. Moreover circulating myeloid cells can enter the CNS upon stimulation. Parenchymal microglia fulfill tissue macrophage function, via activities such as phagocytosis, and release of inflammatory signals, as well as repair and regeneration-inducing mediators, so they can be deviated to either pro- or anti-pathologic status in a context-dependent manner.

Monocytes and neutrophils are recruited chemotactically from the circulation as a consequence of stimulation of innate receptors and undergo transition to functional MRCs. This is best described for G/PMN-MDSCs which then act to suppress inflammation via arginine metabolites and iNOS, as well as anti-inflammatory cytokines. M-MDSC may be more prevalent in the periphery, where induction of Tregs suppresses inflammatory CD4+ T cell responses. PD-L1 induction is a common mechanism of suppression, triggered by innate stimuli including inflammation-associated cytokines. See Figure 1 for a schematic overview to these points.

Unanswered questions include whether M-MRCs/M-MDSCs can exert functional suppression within the CNS, and whether MRCs can protect against antibody-mediated inflammatory diseases such as

NMOSD. It should also be determined whether MRCs/MDSCs can be switched to being pro-inflammatory and under what conditions. Understanding how to induce and maintain protective roles for microglia and BAMs would have major implications for therapy in MS and other IDDs. These are challenges for the future.

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Figure legend

Major aspects of protective activities of myeloid cells in the CNS

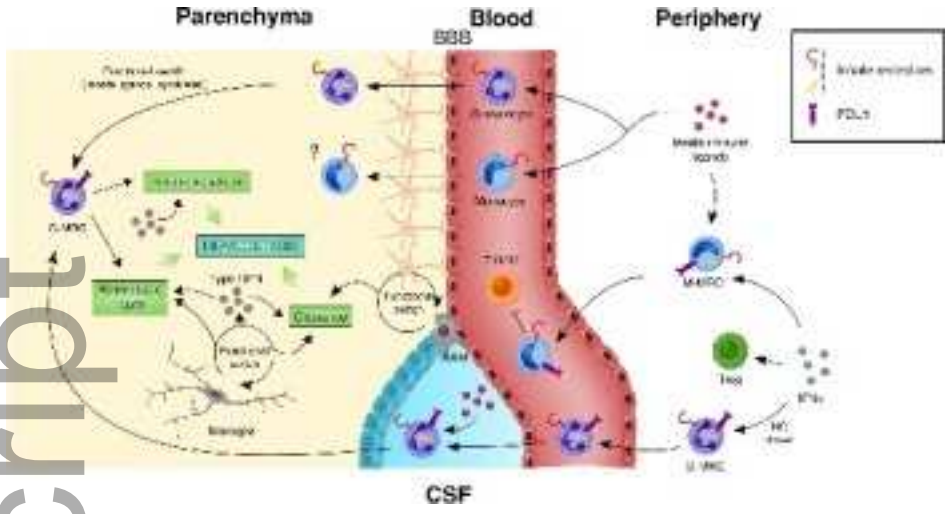
(Suppressive myeloid cells are labelled as MRC, which include MDSC).

Innate signaling triggers suppressive functionality in myeloid cells in the periphery. The Th1 cytokine IFNγ has been shown to be necessary for this *in vitro* and *in vivo* and it can be extrapolated that it plays a similar role within the CNS.

Tregs and NO are both induced but may not be sufficient for suppression of Th1 and Th17. PDL1-expressing monocytic MRC were identified as active suppressors, and shown to migrate to the CNS in mice, with or without EAE. IFNγ-responsive G-MRC were also identified as active suppressors.

Myeloid cells within the CSF are triggered to exert suppressive functions by innate signaling and an associated chemokine response recruits granulocytes and monocytes from blood. Combined effects of

innate signals and cytokines+chemokines induce functionally suppressive G-MRC which contribute to homeostasis via modulation of inflammation. Type I IFN signaling is required for this. Resident parenchymal myeloid cells (microglia), and particularly protective subtypes, promote homeostatic regeneration and repair via myelinogenic mediators as well as myelin clearance that is necessary for remyelination. Extra-parenchymal resident myeloid cells also contribute to this clearance.



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