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Chapter

Myeloid Cells in Multiple Sclerosis

Marilyn Wang, Sofia Caryotakis, Nagendra Kumar Rai, Alan Nguyen and Athena M. Soulika

Abstract

In steady state, the central nervous system (CNS) houses a variety of myeloid cells, such as microglia, non-parenchymal macrophages and dendritic cells (DCs), and granulocytes. Most of these cells enter the CNS during embryogenesis and are crucial for proper CNS development. In adulthood, these resident myeloid cells exert crucial homeostatic functions. In neuroinflammatory conditions, like multiple sclerosis (MS), both lymphoid and myeloid cells from the periphery infiltrate the tissue and cause local damage. Although lymphocytes are undeniably important players in MS, CNS-resident and CNS-infiltrating myeloid cells have recently gained much-deserved attention for their roles in disease progression. Here, we will review significant advances made in recent years delineating myeloid cell functions within the CNS both in homeostasis and MS. We will also discuss how these cells are affected by currently employed therapeutics for MS patients.

Keywords: microglia, macrophages, dendritic cells, monocytes, granulocytes, disease modifying treatments, multiple sclerosis, EAE

1. Introduction

Myeloid cells are crucial for central nervous system (CNS) tissue function both in development and adulthood. Other than microglia, which are found in the parenchyma, CNS meningeal and perivascular spaces along with the choroid plexus, are populated by special subsets of macrophages and dendritic cells [1–3]. Additionally, granulocyte cells are also present in the homeostatic CNS [4]. Studies in rodents have elucidated mechanisms by which these cells promote tissue physiology.

In multiple sclerosis (MS), myeloid cells play a dominant role. Studies in mice and human patient samples show that myeloid cells from the periphery enter the tissue through a compromised blood-brain barrier (BBB) and together with CNS-resident cells perpetuate the inflammatory environment through secretion of inflammatory cytokines and reactivation of primed T cells. However, myeloid cells may also exhibit anti-inflammatory and pro-reparative functions. The exact contribution of each myeloid subset to disease progression is currently the focus of thorough investigation.

Here, we will provide an overview of myeloid cell types and functions in homeostasis and how these populations evolve in neuroinflammation. In addition, we will review the effects of therapeutics currently employed for MS patients on myeloid cell populations and functions.

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2. CNS-resident myeloid cells in homeostasis

The CNS houses a variety of myeloid cell subsets that exert multiple functions crucial to homeostasis such as BBB maintenance, sampling of the local milieu, synaptic pruning, and control of neuronal populations in development and adulthood. In this section, we will elaborate on the developmental origin and known functions of these subsets in the CNS.

2.1 Microglia

Microglia are resident immune cells within the CNS parenchyma proper. They derive from Runx1+ erythromyeloid precursors in the extraembryonic yolk sac and enter the brain early in embryonic development [5–7]. Before migrating out of the yolk sac, these progenitors acquire CD45 and CX3CR1 expression [8] and seed the brain parenchyma around embryonic day 9.5 [7, 9, 10], through a process that is mediated largely by the metalloproteinases MMP8 and MMP9 [8].

Microglia development relies on transcription factors PU.1, IRF8, and colony-stimulating factor 1 receptor (CSF1R) signaling [11], whereas transcription factors such as MYB, BATF3, and ID2 are not necessary, suggesting that microglia are transcriptionally distinct from bone marrow-derived myeloid cells [6, 10]. Moreover, the microglial transcriptional profile changes at each developmental stage, roughly divided into early microglia (microglia that seed the brain from E10.5 to E12.5), pre-microglia (microglia found in the CNS from E12.5 up to P9), and adult microglia [10, 12]. Early microglia are highly proliferative, pre-microglia exert functions on synapse pruning [10] and excess neuron elimination [13], and adult microglia perform immune surveillance but also synaptic refinement [11, 14–16]. During development, microglia control the numbers of neural progenitors via phagocytosis. This was shown by clodronate-mediated microglia deletion in organotypic brain cultures [13] or in CSF1R knockout mice, which lack microglia [17]. However, CSF1R is also expressed in other cells including peripheral myeloid subsets and neurons. Specific deletion of CSF1R in nestin+ cells recapitulated some of the observed effects in the global CSF1R knockout [17].

Complement components C1q and C3 tag extra synapses which are then removed by microglia via CR3 receptor-mediated phagocytosis [15, 16]. This process is known as synaptic pruning [15]. Neuronally derived CX3CL1 acting on microglial CX3CR1 is one of the cues that guide microglia to the synapses [15]. Mice deficient in microglia or CX3CR1 exhibit neuronal connectivity and behavioral deficits similar to those observed in autism spectrum disorders [4, 10, 18, 19]. Developing microglia also control neural cells in the cerebellum and were shown to induce Purkinje cell death via NADPH activity [5, 20]. On the other hand, developing microglia also secrete trophic factors that promote neuronal circuit formation and neuronal survival. Microglial-derived insulin-like growth factor 1 (IGF-1) promotes survival of cortical layer V neurons in postnatal development. In addition, it induces the fate of many cell lineages, such as oligodendrocytes, and also protects them from glutamate-mediated apoptosis [5]. Basic fibroblast growth factor, hepatocyte growth factor, epidermal growth factor, platelet-derived growth factor, nerve growth factor, and brain-derived neurotrophic factor are all also secreted by microglia and contribute to neuronal development, maintenance, and function throughout life [21–23].

As microglia mature, they adopt a ramified morphology characterized by a small body and thin, long processes. Interestingly, recent studies suggest that adult microglia are not a homogeneous population and their activation state is the result of region-specific cues [24–30]. They are self-renewing via a local progenitor [31, 32],

but in certain instances and when microglia are depleted for prolonged periods of time via genetic or pharmacologic methods, peripheral myeloid cells can enter and engraft in the CNS for long periods but remain functionally distinct [33]. Microglia in the steady-state CNS depend on CSF1R signaling for survival. Both CSF1R ligands, CSF1 and IL-34, are found in the normal CNS and their expression is regionally controlled [34]. Interestingly, in the absence of CSF1, microglia numbers decrease by 30%, while in the absence of IL-34, microglial numbers decrease by 70%. IL-34 in particular controls the migration of microglial precursor cells in the CNS via CSF1R signaling in development [35]. TGF-β signaling is also necessary for homeostatic microglial functions, and in its absence, they assume a transcriptome that is similar to that of peripheral macrophages [36].

Defining microglial markers that are distinct from those of peripheral monocytes has been the focus of investigation for many years. New RNA-Seq techniques yielded a number of genes that are preferentially expressed by microglia but not peripheral myeloid cells in homeostasis [1, 10, 18, 37, 38]. Lately, the most commonly employed markers are the purinergic receptor P2Y, G-protein coupled receptor 12 (P2RY12), the transmembrane protein TMEM119, and the transcriptional regulator Sal-like 1 (SALL1) [1, 10, 18, 37, 38]. Both P2RY12 and TMEM119 are expressed by the vast majority of microglia within the healthy CNS. The function of TMEM119 in microglia has not been yet elucidated, but in other cell types, it has been implicated with differentiation and proliferation [39–42]. P2RY12 serves as a chemotactic receptor that guides microglia to sites of injury [26]. SALL1 is a microglia fate-determining factor, vital for expression of essential microglial genes and normal microglial morphology [26, 36]. Whether these markers are still able to differentiate between microglia and infiltrating myeloid cells in neuroinflammation, when all these cells undergo major transcriptional changes, is still under investigation. However, SALL1 and TMEM119 are emerging as the most reliable microglial markers.

Adult microglia exert multiple roles in tissue maintenance: they phagocytose debris or dead cells, clear toxic amyloid-β, shape neural circuits via phagocytosing inappropriate or inactive connections [16], provide trophic support to neurons by producing growth factors, and regulate neurogenesis in the hippocampus and the subventricular zone (SVZ). Interestingly, microglial-derived CX3CL1 increases with exercise and confers a protective effect on neuronal cells, while CX3CR1 deletion results in activated microglia with an inflammatory phenotype, leading to decreased rates of adult neurogenesis in the hippocampus [43–45]. In addition, microglia phagocytose neuronal progenitors in the adult SVZ, thus controlling the local pool of neurons [18, 44, 46]. Microglia also influence oligodendrocyte development and myelinogenesis both during development and in adulthood. In the adult CNS, microglia are necessary for myelin homeostasis and maintenance of adult oligodendrocyte progenitor cells [47, 48], promote BBB function [49, 50], and in case of injury, they migrate to the affected site to promote repair [51].

Microglial malfunction is associated with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and neurodevelopmental and psychological defects such as Rett syndrome and obsessive-compulsive disorder [10]. Furthermore, the lack of phagocytosis by microglia results in excess synapses which is associated with impaired memory formation [16].

2.2 Tissue-resident macrophages

In addition to microglia, the healthy CNS houses three types of non-parenchymal tissue-resident macrophages. They are named based on their location and are currently categorized as perivascular macrophages, meningeal macrophages,

and macrophages in the choroid plexus [1, 52]. These macrophage populations are optimally placed to regulate and interrogate peripheral cell entry, act as sentinels by sampling their environment, and quickly respond in the event of an insult. Previously thought to be derived from bone marrow (BM) monocytic progenitor cells, it is now established that the majority of CNS-resident macrophages are long-lived and transcriptionally similar to microglia. As such, most of these cells are derived from erythromyeloid progenitors found in the extraembryonic yolk sac or the fetal liver, and their generation is dependent on PU.1 and independent of MYB and BATF3 [1, 7]. Choroid plexus macrophages are the most distinct among these types of CNS non-parenchymal macrophages and originate from either embryonic precursors or BM.

Perivascular macrophages are located between the blood vessel endothelium (of BBB) and the glia limitans, which form the barrier for the CNS parenchyma. They are wrapped around endothelial walls with their elongated cell bodies and monitor the perivascular space [2]. Perivascular macrophages provide nutrients to endothelial cells, regulate vascular permeability, maintain BBB integrity, clear toxic amyloid-β from the CNS, sample debris to assess the local milieu, and communicate with surrounding cells [52]. Their location is ideal to simultaneously sample both the CNS interstitial fluid and the blood [1, 52]. Perivascular macrophages infiltrate the CNS at the same time as microglia (E 9.5) and populate the abluminal spaces of the newly developed vasculature. Together with microglia, these macrophages play significant roles on the refinements of the developing vasculature [53]. In adulthood and in response to injury, perivascular macrophages promote anastomoses and the repair of vasculature [54].

Meningeal macrophages have a very similar origin and transcriptional control as perivascular macrophages. They are located in between meningeal vasculature and ER-TR7+ fibroblast-like cells that line the meninges. They also express similar markers to those of perivascular macrophages and are also long-lived with negligible contribution from the periphery [1, 2].

The choroid plexus macrophages reside on the apical side of the epithelium facing the cerebrospinal fluid (CSF) in the stroma. The stroma of the choroid plexus is highly vascularized and surrounded by a monolayer of cuboidal epithelial cells, which are joined together by tight junctions forming the blood-CSF barrier. The choroid plexus is located in all four ventricles of the brain and is responsible for producing CSF [52]. It allows trafficking of a variety of immune cell types and is an area with an anti-inflammatory environment [55, 56]. In addition, the choroid plexus is the gateway to the CNS and is an area through which pioneering T cells gain access into the CNS in preclinical stages of the MS murine model experimental autoimmune encephalomyelitis (EAE) [57]. Unlike the other types of CNS macrophages, these macrophages are partially replenished from the bone marrow [1].

All of these brain-resident macrophages express the mannose receptor CD206 and scavenger receptor CD163, along with CD11b, CX3CR1, and MHC-II. Perivascular and meningeal macrophages also express the lymphatic vessel endothelial hyaluronan receptor LYVE1, which is not expressed in choroid plexus-associated macrophages [1, 2, 58].

2.3 Dendritic cells

At steady state, dendritic cells (DCs) are sparsely distributed within the non-parenchymal CNS spaces. They are more numerous in the leptomeninges and dura mater, less prominent in the choroid plexus, and mostly absent from perivascular spaces [3].

DCs develop from committed DC or monocyte progenitors in the BM and are dependent upon FLT3 signaling [59]. They are relatively short-lived and are replenished roughly every 1–2 weeks [60]. Mature DCs are divided into conventional DCs (cDCs), plasmacytoid DCs (pDCs), and monocyte-derived DCs (moDCs). cDCs are further subdivided into cDC1 and cDC2. cDC1s are associated with Th1 responses [61, 62], while cDC2 with Th2 and Th17 [63]. cDC1s are also able to cross present antigens and activate CD8+ T cells. cDCs leave the BM in the form of committed precursors, while pDCs mature in the BM before entering the circulation. In addition, moDCs are not usually found in steady state but are crucial mediators on inflammatory responses [64].

IRF4 and IRF8 are transcription factors differentially expressed in the various DC subsets. cDC1s are IRF8+IRF4lo/−, cDC2s are IRF8loIRF4+, pDCs are IRF8+IRF4+, and moDCs are IRF4loIRF8lo [65]. cDC1s do not express CD11b. Within the mouse CNS, the majority of DCs are cDC2 and are mostly located in the leptomeninges and dura mater, while in the choroid plexus, the majority of DCs are cDC1s [3].

2.4 Granulocytes

Although their presence within the CNS at steady state is commonly ignored, various types of granulocytes such as neutrophils, mast cells, basophils, and eosinophils are found within perivascular and meningeal spaces and the choroid plexus [4]. Mast cells in particular are also found within the parenchyma [66, 67].

Neutrophils exit the bone marrow in a mature state and are thought to be short-lived. However, subsets of neutrophils live much longer than previously thought and, more importantly, some have been found in various organs likely as a local reservoir [68]. It is now acknowledged that neutrophils or neutrophil subsets may have different functions. Other than the well-documented inflammatory functions, pro-reparative CD206+ neutrophils, VEGF-responding angiogenic neutrophils, and CD11c+Ly6G+ "hybrid" cell types have been identified [69–72]. Interestingly, neutrophils were recently detected in the normal murine CNS localized within the subdural meningeal spaces, but their contribution to tissue homeostasis is still not known [73].

Mast cells (MCs) are derived from CD34+ bone marrow progenitor cells, enter the circulation in an immature state, and mature once they reach the tissue in response to local cues. They are mostly known for their effects during allergic/atopic responses mediated by cross-linking of their FcεRI receptor by IgE. MCs are a heterogeneous population, and depending on the types of proteases they carry within their granules, they are broadly categorized into at least three subtypes: MCs that contain only tryptase (MCT), MCs that contain only chymase (MCC), and MCs that contain tryptase, chymase, carboxypeptidase, and cathepsin G (MCCT) [74, 75]. MCs are loaded with granules containing preformed mediators and can synthesize mediators de novo. They are found in many tissues and usually associated with vascular epithelial cells and nerves. CNS MCs are constitutively active and degranulate in response to homeostatic or inflammatory stimuli [67, 76–78]. Their preformed granules are released immediately upon activation and contain various mediators such as histamine, serotonin, and TNF-α in addition to proteases. They can quickly synthesize lipid mediators such as prostaglandins and leukotrienes and growth factors. A late-phase MC activation results in de novo production of inflammatory cytokines such as IL-6 and TNF-α [74, 79].

Within the healthy CNS, MCs are found within the thalamus, hypothalamus, entorhinal cortex, hippocampus, meninges, and perivascular spaces in proximity to the BBB. They interact with neurons and microglia, and their granules contain

a plethora of mediators including neurotransmitters. Their location allows them to modulate BBB permeability, and genetically modified mice that lack MCs display decreased BBB permeability both in homeostasis and neuroinflammation [67, 77, 78].

MC activity in stress has been associated with migraines [78, 80]. Moreover, histamine released from MCs was shown to promote wakefulness in adult mice [81] and microglial synaptic pruning in the developing CNS, which then regulates sexual behavior in adulthood [82].

3. Myeloid cells in multiple sclerosis

Pathologically, MS is characterized by focal demyelinating lesions disseminated in space and time and neuronal and axonal damage. MS lesions are rich in myeloid cells (microglia, infiltrating monocyte-derived macrophages, and DCs), which outnumber lymphoid cells [83]. Below we will discuss current knowledge on myeloid cells in MS, which are now emerging as crucial players in disease pathogenesis and progression. Some of this knowledge is derived from studies on the animal model of MS, experimental autoimmune encephalomyelitis (EAE). Although this model has been criticized [84], it mimics most of the CNS pathology observed in MS such as tissue infiltration by immune cells, formation of lesions, local inflammation, and progressive axonal loss [85, 86].

Monocytes are not found in the healthy CNS but are regularly found in the CNS and CSF of MS patients. Once they enter the CNS, monocytes mature into macrophages and participate in disease progression. There are three well-characterized monocyte subsets categorized based on expression patterns of the LPS receptor CD14 and the Fce (greek) RIII receptor CD16: the classical CD14++CD16- (similar to the inflammatory monocyte Ly6ChiCCR2+ in mice), the nonclassical CD14+CD16++ (similar to the anti-inflammatory CX3CR1+Ly6Clo in mice), and the intermediate CD14++CD16+. CD16+ monocytes have been associated with inflammation and promoting the generation of Th17 cells. MS patients with active disease show increased CD14+ cells both in the blood and the CSF. These cells also contribute to BBB disruption [87, 88].

Both conventional and plasmacytoid DCs are increased in the blood and CSF of MS patients. cDCs are usually found early in disease, and pDC numbers are highly increased in the CSF during relapses. Circulating cDCs in MS patients upregulate CCR5 which is a receptor for CCL3 and CCL5, both of which are upregulated in MS lesions. However, cDCs in primary progressive MS display an immature phenotype [89]. Interestingly, although pDC numbers increase in MS, these cells are found to be phenotypically similar to those of healthy controls. Although the data on circulating pDCs are still conflicting, imbalances in DC populations may result in significant changes in T-cell functionally in MS [90].

3.1 Myeloid cells in MS lesions

MS lesions are found both within the brain and spinal cord and can be formed within the white and the gray matter [91, 92]. The most commonly employed classification is the four types of lesions described by Lucchinetti and colleagues [93]. Type I is characterized by macrophage products, and type II is characterized by antibody and complement deposition, while type III lacks complement and antibody deposition. Types I and II have clearly demarcated borders, while type III is characterized by diffuse demyelination and lacks clear demarcation. Type IV is characterized by dystrophic apoptotic oligodendrocytes. In most of these lesions, the major cell types are myeloid cells [83].

Although more pronounced during relapses, infiltrating myeloid cells and activated microglia are found within the CNS of MS patients throughout the disease and are associated with demyelination, oligodendrocytic loss, and axonal damage [92, 94, 95]. With the exception of rapid progressive MS, in which the CNS is intensely infiltrated [94], in progressive forms of the disease, the tissue is not massively infiltrated; however, myeloid cells (microglia and/or infiltrating myeloid) remain activated [92, 96]. During progressive stages of the disease, axonal loss is prominent leading to tissue atrophy in both MS and EAE [86, 92]. These processes are likely mediated via the production of oxygen radicals produced by either microglia or infiltrating myeloid cells [84].

3.1.1 Microglia/macrophages

The contribution of microglia to MS is still debated. Studies in mice have shown that microglia are poor antigen-presenting cells and not likely to activate infiltrating lymphocytes. Instead, microglia may contribute to the disease process via oxidative stress and production of pro-inflammatory cytokines that may activate astrocytes or cause oligodendrocytic damage. Microglia are highly phagocytic and thus can remove myelin debris and cellular fragments, damaged axons, and dead cells. It is clear that microglia are activated in the CNS of MS patients, but whether they promote disease or facilitate repair is still not well delineated. One of the main hurdles for these investigations is that there is no unique marker to reliably distinguish microglia from infiltrating monocytes in neuroinflammation. Additionally, activated microglia are morphologically indistinguishable from infiltrating monocytes/macrophages. RNA transcriptome analysis has yielded a number of markers that show preferential expression in microglia (see Section 2.1). TMEM119 is the only marker so far examined in MS tissue and seems to be expressed by a subpopulation of myeloid cells within lesions and in cells with microglial morphology in nonlesional areas [97]. However, there is still not a wide breadth of studies examining the specificity of TMEM119 in neuroinflammation, when all myeloid cells undergo major transcriptional changes [2]. Thus, below we will talk about microglia and macrophages as one population in active MS lesions and specify TMEM119-expressing cells within the MS CNS.

Microglia/macrophages (M/Ms) in active MS lesions are heterogeneous and capable of performing a variety of activities that may promote or control inflammation and repair [98, 99]. M/Ms found within active MS lesions usually express markers associated with inflammatory macrophage functions, including inducible nitric oxide synthase (iNOS), co-stimulatory molecules CD40 and CD86, the Fc receptors CD32 and CD64, phagocytosis marker CD68, and p22phox, a subunit of NADPH oxidase [100, 101]. In addition, M/Ms may also express anti-inflammatory markers such as the mannose receptor CD206 and the scavenger receptor CD163 [100]. Approximately half of the myeloid cells within active lesions express TMEM119, suggesting these cells may be microglia. Interestingly, PY2R12, which is usually expressed in homeostatic microglia, is not expressed in these cells, suggesting it is downregulated upon activation [97].

MS lesions are not static and over time grow outward, eventually becoming chronically active. These lesions are slowly expanding and have a thin border of M/Ms. The center of these lesions appears quiescent and populated by lipid-laden (foamy) macrophages, many of them expressing CD206 and CD163 [98, 102]. However, M/Ms lining the rim of these lesions express iNOS and HLA-DR, suggesting they are inflammatory and promote T-cell functions [103]. M/Ms at the rims of either active or chronically active plaques contain iron which has been suggested to promote MS pathology [104, 105]. In the normal CNS, most iron is found within

oligodendrocytes or myelin. When iron is released after oligodendrocytic death and demyelination, it is internalized by ferritin+ M/Ms which acquire a dystrophic phenotype [106]. Interestingly TMEM119+ cells that express low or no P2RY12 (likely activated microglia) are found within chronically active or slow-expanding lesions, and their density decreases inward. Strikingly, there are no differences between overall M/M density and levels of activation between lesion types [97, 100, 103].

Areas of the CNS that are far from the demyelinating lesions and often appearing normal (normal-appearing white matter; NAWM) are also characterized by scattered microglial activation. Interestingly, ramified microglia were shown to express iNOS and were often close to injured axons [107]. However, microglia have also been documented to exhibit a suppressed and anti-inflammatory character [108]. Clusters of microglia or macrophages, known as microglial nodules, have been found in NAWM in close proximity to degenerating axons. These nodules appear in the absence of extensive inflammation, astrogliosis, or demyelination, and their formation has been argued to be one of the early events in MS pathology [109]. Furthermore, P2RY12+ TMEM119+ microglia in the NAWM also expressing activation markers CD68 and p22phox are found in both MS and healthy controls' brains, suggesting that certain microglial populations are in a pre-activated state [97].

In addition to white matter, demyelination is also observed within the gray matter. MS gray matter is characterized by less infiltration by immune cells and less activation of M/Ms compared to white matter. This type of demyelination has been mostly attributed to aberrant microglia functions such as ROS production via the NADPH oxidase activity. This mechanism seems to be more prominent in the gray matter compared to white matter lesions. In addition, cortical microglial activation can be observed via PET imaging by administering the traditional PK11195 and more recently the novel PBR28 ligand [110, 111].

In progressive forms of MS, M/Ms are activated both within the lesions and in the normal-appearing white and gray matter, and this has been linked to inflammatory cytokines produced in the meninges, likely by infiltrating B cells [112, 113]. Activated complement component 3 fragments (C3d) are found within microglia clusters of slowly expanding lesions in progressive but not acute MS [114] and in close proximity of damaged axons. This suggests that C3 activation and deposition are not likely associated with lesion initiation but rather a mechanism that facilitates the removal of axonal and cellular debris. Furthermore, the activation/phagocytosis marker CD68 is significantly increased in the NAWM in progressive forms of MS compared to that of relapsing–remitting MS and healthy controls [97].

3.1.2 Dendritic cells

Both cDCs and pDCs accumulate in the leptomeninges and lesions in MS patients. MoDCs, which are not present in the homeostatic CNS, differentiate from infiltrating inflammatory monocytes after these reach the CNS of MS patients. Studies in murine EAE showed that both cDCs and moDCs are found within the CNS infiltrates. cDCs express CD26 and ZBTB46, a transcription factor also expressed in human cDCs, while moDCs express CD88 and CD64 [3, 103, 115]. Although these markers may be expressed by other cell types, they are useful markers for identification of DC subsets. cDCs are the most efficient antigen-presenting cells and are able to process larger myelin fragments to activate naive and effector T cells. Both cDCs and moDCs progressively expand during the onset and peak of EAE in every CNS compartment. pDCs are not efficient antigen-presenting cells but are equipped to secrete inflammatory cytokines and promote an inflammatory environment to support cDCs and moDCs [116].

3.1.3 Granulocytes

Neutrophils are relatively rare in established MS lesions; thus, their contribution to disease course has long been debated. Studies in EAE show that neutrophils are part of the inflammatory lesions, appear early in disease process [86, 117], and are increased in peripheral lymphoid organs and blood [117]. Neutrophils may promote early disease progression by increasing permeability of the BBB, possibly through secretion of matrix metalloproteinases or the release of neutrophil extracellular traps (NETs) [118, 119]. Inactivation of neutrophil products, such as myeloperoxidase or neutrophil elastase, results in milder EAE course and associated optic neuritis [120, 121]. In agreement with the EAE data, CSF of newly diagnosed patients shows elevated neutrophil counts [122], and the CSF of patients with established disease contains increased levels of the neutrophil chemoattractant CXCL8 [123, 124]. Neutrophil elastase and chemokines that promote neutrophil recruitment, such as CXCL1 and CXCL5, are systemically elevated in relapsing MS patients and correlate with lesion burden and clinical disability [125]. Transcripts of the granulocyte colony-stimulating factor (G-CSF) which promotes the proliferation and differentiation of neutrophils (and other granulocytes) are found within lesions but not in NAWM [126], and treatment with G-CSF worsens MS symptoms [127, 128]. Thus, lack of neutrophil detection in MS lesions may be due to incorrect sampling timing.

Interestingly, mast cells are found in close proximity to MS lesions and were initially observed in 1890 by Neuman [129] and later by other groups [66, 130–132]. Their numbers are very low compared with those of the other myeloid subsets; thus, not much is known about their contribution to disease progression. However, the ability of mast cells to secrete histamine and proteases may facilitate disease onset or relapses by promoting vascular permeability and tissue infiltration. In EAE, mice with spontaneous c-Kit mutations that lead to deletion of mast cells have shown that these cells may prevent, promote, or have no effect on disease onset and progression [133]. These conflicting data are likely due to the fact that none of these mouse strains are specific and efficient mast cell knockouts.

4. Effect of MS therapeutics on myeloid cells

MS therapies are designed to dampen immune system activation. Although most of these therapies target lymphocytes, myeloid cells can also be affected directly or indirectly. This section will explore how current MS therapies affect myeloid cells.

4.1 IFN-β

IFN-β, the first FDA-approved biologic therapeutic for MS, is a pleiotropic cytokine exerting a plethora of effects on a variety of cells [134, 135]. Monocytes isolated from MS patients treated ex vivo with IFN-β exhibit impaired inflammatory responses when stimulated with LPS/alum compared to monocytes isolated from healthy donors [136]. Ex vivo treatment of DCs derived from MS patients or healthy donors with IFN-β reduced the expression of IL-1β and IL-23 and upregulated the expression of IL-12p35 and IL-27p28, which resulted in reduced generation of Th17 cells [137]. Additionally, studies in EAE showed that deletion of IFN-α/β receptor (IFNAR), the receptor of IFN-β specifically on myeloid cells, resulted in aggravated EAE disease [138].

IFN-β is one of the most common first-line MS treatments; however, a large proportion of patients is not responsive. Interestingly, non-responders exhibit

exaggerated upregulation of type I IFN-responsive genes either at baseline or in response to IFN-β treatment compared to responders [139–141]. MS patients that upregulated the death-associated receptor TRAIL on monocytes were responsive to IFN-b treatment, but those who did not upregulate TRAIL were not responsive to IFN-b treatment. than patients that did not [142]. Additionally, monocytes isolated from MS patients treated with IFN-β for prolonged periods of time (9 months to 6 years) upregulated the co-stimulatory molecules CD80, CD86, and CD40 [143], and were associated with responsiveness to treatment [144]. A different study, however, showed a positive association between monocytic CD40 upregulation, early after IFN-β injections (9–12 h) and relapses [145].

About 30% of MS patients treated with IFN-β also develop antidrug antibodies and thus are not responsive to treatment. Antidrug antibody generation was associated with decreased NOTCH2 signaling. NOTCH2, which promotes the conversion of patrolling inflammatory monocytes to anti-inflammatory phenotype [146], was markedly reduced in CD14+ monocytes of untreated MS patients that developed antidrug antibodies 12 months after IFN-β therapy initiation [147].

All the above suggest that defining myeloid cell subset propensities in MS before and after treatment initiation will be useful in determining whether IFN-β is a suitable treatment for specific patients.

4.2 Glatiramer acetate

Glatiramer acetate (GA) is a synthetic random copolymer, composed of glutamic acid, alanine, lysine and tyrosine, employed as a treatment for relapsing-remitting MS. MS patients treated with GA show a shift toward Th2 responses and produce anti-inflammatory/pro-repair mediators, likely due to GA effects on myeloid subsets [148, 149]. Initial studies showed that GA binds to MHC-II, altering the myelin antigen presentation capabilities resulting in impaired activation of autoreactive T cells [150, 151]. However, it was later shown that GA can also exert its anti-inflammatory effects independently of MHC-II [152]. Instead, GA was shown to promote the generation of anti-inflammatory monocytes which support regulatory T-cell functions [152].

In support of this, monocytes isolated from the blood of GA-treated MS patients produced significantly higher amounts of IL-10 and lower amounts of IL-12, and the levels of CD16+ anti-inflammatory monocytes were restored to those of healthy controls [153, 154]. DCs from GA-treated MS patients exhibit reduced IL-12 production [155] and express lower levels of CD40, upregulation of which is associated with relapses [156]. Furthermore, the activity of myeloid-derived suppressor cells, a population that suppresses inflammatory responses, is augmented in GA-treated MS patients [157], and GA-treated human microglia express IL-10 and reduce production of pro-inflammatory TNF-α [153]. Increased levels of circulating IL-27, a regulatory cytokine produced by myeloid cells in inflammatory conditions, was recently linked to better GA therapeutic outcomes [158]. Another study showed increased levels of IL-27 in blood, CSF and lesions of MS patients. however, there was no association with treatments [159].

4.3 Fingolimod

Fingolimod is the first oral therapy approved to treat relapsing-remitting MS and is more effective in reducing relapses than IFN-β [160]. Fingolimod (FTY720) is phosphorylated by sphingosine kinase, and its phosphorylated metabolite (FTY720-P) binds to the G-protein-coupled sphingosine-1-phosphate (S1P) receptors. S1P receptors are expressed on a variety of cells including neural, glial,

and endothelial cells in the CNS and most of the immune cells in the CNS and the periphery [161]. One of the mechanisms by which fingolimod reduces disease severity and relapses in MS is that it binds S1PR1, a type of S1P receptor, on lymphocytes and prevents their egress from lymphoid tissues [162].

Fingolimod's immunosuppressive effects are also exerted on myeloid cells. Incubation of murine macrophages or human monocytes with either S1P (the natural ligand of S1PR1), or fingolimod, respectively, reduced inflammatory responses after LPS exposure [163–165]. Although microglia, DCs, and peripheral macrophages express similar patterns and levels of S1P receptors, fingolimod downregulated ERK phosphorylation only in DCs and macrophages. Fingolimod also downregulated expression of the pro-inflammatory cytokine IL-12 and upregulated anti-inflammatory IL-10 in DCs and macrophages but not in microglia [164]. Fingolimod crosses the BBB [168], and therapeutic administration of fingolimod reduced TNF-α production by microglia and monocytes in EAE [163]. Flow cytometry analyses of DCs and monocytes isolated from MS patients before and during fingolimod treatment showed decreased levels of activation markers (CD83, CD150, and HLA-DR). Furthermore, fingolimod treatment reduced pro-inflammatory cytokine production, phagocytic activity of DCs and monocytes, and impaired priming of Th1 and Th17 cells [166]. Interestingly, monocytes isolated from fingolimod-treated MS patients exhibited reduced expression of pro-inflammatory micro-RNA miR-155 but also of antioxidant genes HMOX1 and OSGIN1 compared to untreated patients [167]. When monocyte-derived macrophages and microglia were examined in vitro, fingolimod reduced LPS-induced inflammatory cytokines and increased expression of antioxidants. These data suggest that the effects of fingolimod on myeloid cells in vivo may be an indirect effect.

4.4 Dimethyl fumarate

Dimethyl fumarate (DMF) was approved as an oral first-line therapeutic for relapsing-remitting MS in 2013. It is a methyl ester of fumaric acid, quickly metabolized to active monomethyl fumarate which activates transcription factor nuclear factor erythroid-derived 2 (Nrf2) and suppresses NF-κB to modulate oxidative stress [169]. DMF exerts its effects on multiple immune subsets [170].

Monocytes from DMF-treated RRMS patients express reduced levels of the pro-inflammatory micro-RNA miR-155, and DMF-treated human microglia and monocyte-derived macrophages had reduced production of pro-inflammatory cytokines after LPS stimulation, indicating direct regulatory effects [167].

DMF reduces neuroinflammation levels and cognitive deficits induced by systemic LPS administration in mice [171]. In EAE, DMF promoted the generation of anti-inflammatory monocytes and decreased macrophage infiltration into the CNS resulting in milder clinical deficits. Interestingly, these effects were exerted independently of Nrf2 [172, 173].

4.5 Teriflunomide

Teriflunomide is a reversible inhibitor of dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme active in proliferating cells [174]. Teriflunomide impairs proliferation of lymphocytes, but exerts nebulous effects on myeloid cells [175]. In EAE, teriflunomide reduced T-cell and myeloid cell infiltration of the CNS [176]. In cultured primary microglia, teriflunomide downregulated expression of CD86 and mildly upregulated of IL-10 [177]. Ex vivo, teriflunomide treatment decreased production of IL-6 and CCL2 in activated monocytes from healthy individuals [178].

Furthermore, MS patients after 6 months of treatment showed increased IL-10 production and PD-L1 expression in monocytes, implying that teriflunomide induces anti-inflammatory and regulatory responses in these cells [179].

4.6 Monoclonal antibodies

Several recently developed antibody-based MS therapies target lymphocytes. Below, we will discuss whether and how these therapies affect myeloid cells.

4.6.1 Natalizumab

Natalizumab (NTZ) is an immunomodulatory antibody that limits immune cell infiltration into the CNS by blocking the interaction between the very late activation antigen-4 (VLA-4), an integrin expressed on lymphocytes and myeloid cells, and vascular adhesion molecule-1 (VCAM-1) [180]. As a result, fewer cells are able to migrate and infiltrate the CNS [181]. NTZ reduces relapses and lesion load but increases the risk for progressive multifocal leukoencephalopathy [182]. NTZ reduced the frequencies of mature activated pDCs; however, this activation was not a direct effect of NTZ on pDCs [183].

Triggering receptor 2 expressed on myeloid cells (TREM2) is an innate immune receptor associated with inflammatory responses and within the CNS expressed by microglia [184]. In neuroinflammation, microglia shed TREM2, which can be detected in CSF [185, 186]. NTZ reduced CSF-soluble TREM2 to baseline levels, indicating dampened microglial activation, which is associated with improved clinical outcome after 12 months of treatment [187]. It is not clear however whether there is a direct effect of NTZ on microglia.

4.6.2 Anti-CD20 antibodies

There are multiple anti-CD20 monoclonal antibodies shown to ameliorate relapses in relapsing-remitting MS including rituximab, ocrelizumab, and ofatumumab [188]. However, ocrelizumab is the only anti-CD20 antibody that exerts beneficial effects in relapsing-remitting and also in primary progressive MS [189]. A subset of GM-CSF-producing memory B cells, more prevalent in MS patients than healthy controls, was shown to activate pro-inflammatory myeloid cells in vitro [190]. Following B-cell-depleting therapy in MS patients, the inflammatory myeloid response is diminished [190].

4.6.3 Alemtuzumab

Alemtuzumab is a monoclonal antibody that binds CD52 and effectively depletes CD52-expressing lymphocytes through antibody-dependent cell-mediated cytolysis. Both lymphocytes and myeloid cells express CD52; however, myeloid cells are more resistant to alemtuzumab-mediated cytolysis. Thus their numbers are not affected by treatment [191]. Neutrophils, however, express CD52 and are subject to lysis during alemtuzumab treatment [192], occasionally leading to severe neutropenia [193].

5. Conclusion

The contribution of myeloid cells to MS progression is now widely appreciated. Their persistent elevated presence in lesions and activated phenotype, regardless

of tissue infiltration load, in both relapsing and progressive MS, suggest they play crucial roles in disease progression and chronicity. Although gaps in knowledge still exist, recent advances faciltated the efforts by researchers and clinicians to dissect the roles of each myeloid subset in the disease process.

Current therapeutics have broad activities or specifically target lymphocyte functions. In many instances, however, their efficacy stems from their direct or indirect effects on myeloid cell functions. Future research focusing on modulation of myeloid populations and their activities will prove useful for the design of novel therapeutics for MS patients.

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Conflict of interest

The authors declare no conflicts of interest.

Author details

Marilyn Wang[1,2], Sofia Caryotakis[1], Nagendra Kumar Rai[1,3], Alan Nguyen[1,3] and Athena M. Soulika[1,3]*

1 Shriners Hospital for Children—North America, Sacramento, USA

2 Department of Neurology, University of California, Davis School of Medicine, Sacramento, USA

3 Department of Dermatology, University of California, Davis School of Medicine, Sacramento, USA

*Address all correspondence to: asoulika@ucdavis.edu

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