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·Review·

Polarization of macrophages and microglia in inflammatory demyelination

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system, and microglia and macrophages play important roles in its pathogenesis. The activation of microglia and macrophages accompanies disease development, whereas depletion of these cells significantly decreases disease severity. Microglia and macrophages usually have diverse and plastic phenotypes. Both pro-inflammatory and anti-inflammatory microglia and macrophages exist in MS and its animal model, experimental autoimmune encephalomyelitis. The polarization of microglia and macrophages may underlie the differing functional properties that have been reported. In this review, we discuss the responses and polarization of microglia and macrophages in MS, and their effects on its pathogenesis and repair. Harnessing their beneficial effects by modulating their polarization states holds great promise for the treatment of inflammatory demyelinating diseases.

Keywords: macrophage; microglia; polarization; demyelination; remyelination

Introduction

Multiple sclerosis (MS) is a chronic autoimmune inflammatory and demyelinating disease characterized by inflammation, demyelination, axonal damage, gliosis and destruction of the blood-brain barrier[1-3]. Although the T-helper (Th) cells Th-1 and Th-17 were thought to be the main response effectors for autoimmune inflammation, macrophages and microglia do play an important role in the pathogenesis of MS. The pathology of newly-forming lesions in relapsing-remitting MS shows only microglial activation and macrophage infiltration in demyelinating areas, with rare lymphocyte infiltration[4,5]. Moreover, macrophage depletion and microglial paralysis significantly suppress the progress of experimental autoimmune encephalomyelitis (EAE), the animal model of MS[6,7].

Macrophages and microglia play important roles in bridging the innate and adaptive immune reponses. Under normal physiological conditions, macrophages monitor the tissue environment for pathogens, maintain tissue homeostasis, phagocytose dead and dying cells, and respond rapidly to perturbations in the local environment. Microglia share many phenotypic and functional characteristics with macrophages. In the adult central nervous system (CNS), microglia are on constant surveillance for perturbations resulting from injury or disease[8]. In MS, in which pro-inflammatory, neurotoxic and myelin-attacking microglia and macrophages predominate, some microglia and macrophages with anti-inflammatory, neuroprotective and remyelination-promoting properties are also present[9]. These conflicting lines of evidence have led to confusion and considerable debate regarding the harmful *versus* the beneficial roles of macrophages and microglia in MS.

The polarization of macrophages and microglia may underlie their differing functional properties. Taking advantage of their beneficial effects by modulating their polarization states holds great promise for the treatment of CNS demyelinating diseases[10-13]. In this review, we discuss the characteristics of various polarized states of macrophages and microglia, the factors that drive such polarization and

the functional features of these polarized macrophages in demyelinating diseases. We emphasize the roles of polarized microglia and macrophages in demyelination and remyelination in MS.

Macrophage Polarization: Phenotypes of Activated Macrophages

One of the most prominent characteristics of the monocyte–macrophage system is phenotypic plasticity. Work in non-neural systems has revealed important insights into the different types of macrophage activation, referred to as macrophage polarization, that result in cells with either pro- or anti-inflammatory properties[8].

The M1, or classically-activated macrophages, are induced by the prototypical Th-1 cytokine interferon-γ (IFN-γ) or lipopolysaccharide (LPS). They are IL-12^{high}, IL-23^{high} and IL-10^{low}. M1 macrophages mainly secrete pro-inflammatory cytokines such as TNF-α, IL-12, IL-23, IL-1β, IL-6 and chemotactic factors, as well as inducing cytotoxic mediators (reactive oxygen and NO). Macrophages activated in this way are involved in the acute pro-inflammatory response and have an increased antigen-presenting capacity. By contrast, the alternatively-activated macrophages (M2) are generally IL-12^{low}, IL-23^{low}, and IL-10^{high}, and are usually induced by the Th-2 cytokines IL-4 or IL-10, and IL-13. Currently, M2 macrophages are divided into M2a, M2b, and M2c subtypes, which have different functions. M2a macrophages are activated by IL-4 and/or IL-13, secreting large amounts of the anti-inflammatory cytokine IL-10, enhancing arginase-1 (Arg-1) activity and specifically expressing mannose receptor (MR; CD206) and macrophage chitinase 3-like protein 3 (Chi3l3; YM-1). They are involved in killing extracellular pathogens, debris removal, angiogenesis, tissue remodeling and wound healing[14]. Macrophages activated by immune complexes or the TLR or IL-1R ligand are type 2-activated macrophages (M2b), which secrete low levels of IL-12 and large amounts of IL-10, TNF-α, IL-6, and IL-1β, inhibiting bacterial endotoxin-induced acute inflammation and promoting Th-2 differentiation and humoral immune responses. M2c macrophages are activated by IL-10, TGF-β and glucocorticoid, and are also known as inactivating macrophages, secreting high levels of IL-10 and TGF-β, and regulating and suppressing inflammation[15,16] (Table 1).

The presence of M1/M2 phenotypic polarization has also been suggested for microglia[17-21]. What lies between is a full spectrum of activation states which share some properties with the poles – either M1 or M2[9].

Heterogeneity and Polarization of Macrophages and Microglia in the Demyelinating CNS

There are three types of macrophages (CD11b^{+}, CD45^{high}) in different locations in the normal CNS: the perivascular macrophages, the choroid plexus macrophages and the meningeal macrophages[22]. These cells are reported to play a role in the early stages of EAE. The ED2 expression on these cells is up-regulated during EAE before lymphocyte infiltration and the onset of clinical symptoms. Moreover, a slight but measurable suppression of EAE clinical score occurs after selective and complete depletion of these ED2-positive macrophages[23].

During inflammatory demyelination, blood-derived infiltrating macrophages are thought to differentiate from two types of monocytes, the “inflammatory monocytes” (Ly6C^{high}, GR1^{+}, CCR2^{+}, CX3CR1^{low} and CD62L^{+}) and the “resident monocytes” (Ly6C^{low}, GR1^{-}, CCR2^{-}, CX3CR1^{high} and CD62L^{-}), and show important migratory and functional differences in mice and humans[24,25]. These two types of cells arise from a common progenitor called the “macrophage dendritic cell precursor”[26]. The Ly-6C^{high} monocytes are pro-inflammatory and are recruited early into inflammatory sites, whereas Ly-6C^{low} monocytes are patrolling cells that replenish resident macrophages. Recently, it was shown that Ly6C^{high} cells are the predominant population at the demyelinating lesions of EAE. They are rapidly recruited into the CNS and play a pathogenic role during autoimmune demyelinating disease[27-29].

The polarization of macrophages has been analyzed during EAE. In acute EAE, more M1 (iNOS^{+}, ED1^{+}) than M2 (Arg-1^{+}, ED1^{+}) macrophages are found at the early stage. The proportion of M2 macrophages increases and more M2 cells are seen at the EAE peak and in the recovery stage, indicating that they are associated with disease remission[30]. In a relapse-remitting EAE model, similar numbers of M1 and M2 lead to mild EAE, while more M1 cells promote relapse. In severe-relapsing EAE, the M1/M2 ratio is constantly high and especially higher during relapse[11,31,32].

Table 1. Characteristics of M1 and M2 macrophages

Macrophage Subtype	Stimulus	Receptors	Signal transducers	Transcription factors	Polarization markers	Cytokines	Other differentially expressed molecules
M1	IFN-γ	IFNγR	JAK1/ JAK2	STAT1	iNOS, CD16, CD32, CD86, MHC-II	TNFα, IL-1β, IL-6, IL-12, IL-15, IL-23	IL-2Ra, IL-15Ra, IL-7R, CSPG
	LPS	TLR4	Myd88, P38, MSK1/MSK2	NF-κB p65, AP1, IRF3			
M2a	IL-4 or IL-13	IL-4Rα IL-13Rα1	JAK1/JAK2/ JAK3, PI3K	STAT6, PPARγ, c-Myc, JMJD3/ IRF4, KLF4	Arg1, YM1, Fizz1, CD163, CD204, CD206	IL-10, TGFβ, IGF1, PDGF, Fibronectin 1	IL-1Ra, Cox3, CD302, CD209, SOCS1, PGC-1, CLEC4A, MAOA, ENPP2, ALOX15, MS4A4A, 6A
M2b	Immune complexes and IL-1β or LPS	FcγR, IL-1R, TLR	PKC, MAPK, PI3K, JNK Myd88	NF-κB p50, PPARγ	CD163, MHC-II, NO, O_2^-, CD86	IL-10 (high), TNFα, IL-1β, IL-6	SPHK1, downregulation of IL-12 and Arg1
M2c	IL-10	IL-10R1,2	JAK1, Tyk2	STAT3, c-Maf, NF-κB p50	Arg1, Arg2, CD163, CD204, CD206	IL-10, TGFβ	IL-1Ra, IL-4Ra, TLR8, TLR2, IL-21R, iNOS
	TGF-β Glucocorticoids	TGFβR1 GR		SMAD3,4			

ALOX15, arachidonate 15-lipoxygenase; AP1, adaptor protein complex-1; Arg1, arginase 1; CD16, Fc-gamma receptor III (Fcgr3); CD32, Fc-gamma receptor IIb (Fcgr2b); CD204, scavenger receptor class B (SCARB1); CD206, mannose receptor, C type 1 (Mrc1); CLEC4A, C-type lectin domain family 4 a; c-Maf, avian musculoaponeurotic fibrosarcoma oncogene homolog; c-Myc, myelocytomatosis oncogene; CSPG, chondroitin sulfate proteoglycan; ENPP2, ectonucleotide pyrophosphatase/phosphodiesterase 2; FcγR, Fc receptor for immunoglobulin G; Fizz1, resistin-like-α (Retnla); GR, glucocorticoid receptor; IFN-γ, interferon gamma; IFNγR, interferon gamma receptor; IGF1, insulin-like growth factor I; IL, interleukin; IL-R, interleukin receptor; iNOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; JAK, Janus kinase; JMJD3, jumonji domain containing 3; JNK, c-JunN-terminal kinase; KLF4, Kruppel-like factor 4; LPS, lipopolysaccharide; MAOA, monoamine oxidase A; MAPK, mitogen-activated protein kinase; MHC-II, major histocompatibility complex II; MS4A, membrane-spanning 4-domains subfamily A member; MSK1/MSK2, mitogen- and stress-activated kinase; Myd88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor of kappa light chain gene enhancer in B-cells; PDGF, platelet-derived growth factor; PGC-1, peroxisome proliferator-activated receptor gamma, coactivator 1; PI3K, phosphatidylinositol 3-kinase; PPARγ, encoding peroxisome proliferator-activated receptor γ; SMAD, drosophila mothers against decapentaplegic protein; SOCS, suppressor of cytokine signaling; SPHK1, sphingosine kinase 1; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor β; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; Tyk2, TYK2 tyrosine kinase; YM1, chitinase 3-like protein 3.

Injecting M2 microglia into a ventricle or M2 monocytes into a blood vessel significantly suppresses the clinic symptoms of EAE, indicating that enhancing the M2/M1 ratio might be a useful therapeutic strategy in promoting remission[10,11].

Ponomarev *et al*. found that resident microglia ($CD11b^+$, $CD45^{low}$) in the normal CNS produce IL-4. In addition, these microglia express YM-1 (a marker for M2a polarized cells) but do not produce NO (a marker for M1 polarized cells) *in vivo*. Thus, these data indicate that microglia in the normal CNS exhibit an M2-like phenotype[33]. Using chimeric mice

that express GFP in peripheral blood-derived macrophages, they further showed that, in contrast to infiltrating macrophages, activated resident microglia still fail to produce NO. Interestingly, the expression of IL-4 and YM-1 in activated microglia is further enhanced in EAE[33]. These facts led us to postulate that the M2-like phenotype of microglia is induced during EAE. Nevertheless, injecting M2 microglia into a ventricle suppresses the clinical symptoms of EAE, indicating that M2 cells are still not sufficient to overcome the detrimental effects of M1s during inflammatory demyelination[10] (Fig. 1).

Roles of Microglia and Macrophages in MS

Demyelination

Oligodendrocyte loss and axonal degeneration, which may lead to demyelination, are usually observed in demyelinating areas in MS[34]. Many neurotoxic molecules have been reported to be present within the MS lesions. These molecules include NO, reactive oxygen species, glutamate, activated complement components, and pro-inflammatory cytokines such as TNF-α[35], which are known to be highly expressed in M1 macrophages and microglia[36-40]. *In vitro* experiments demonstrated that the M1 microglia, activated

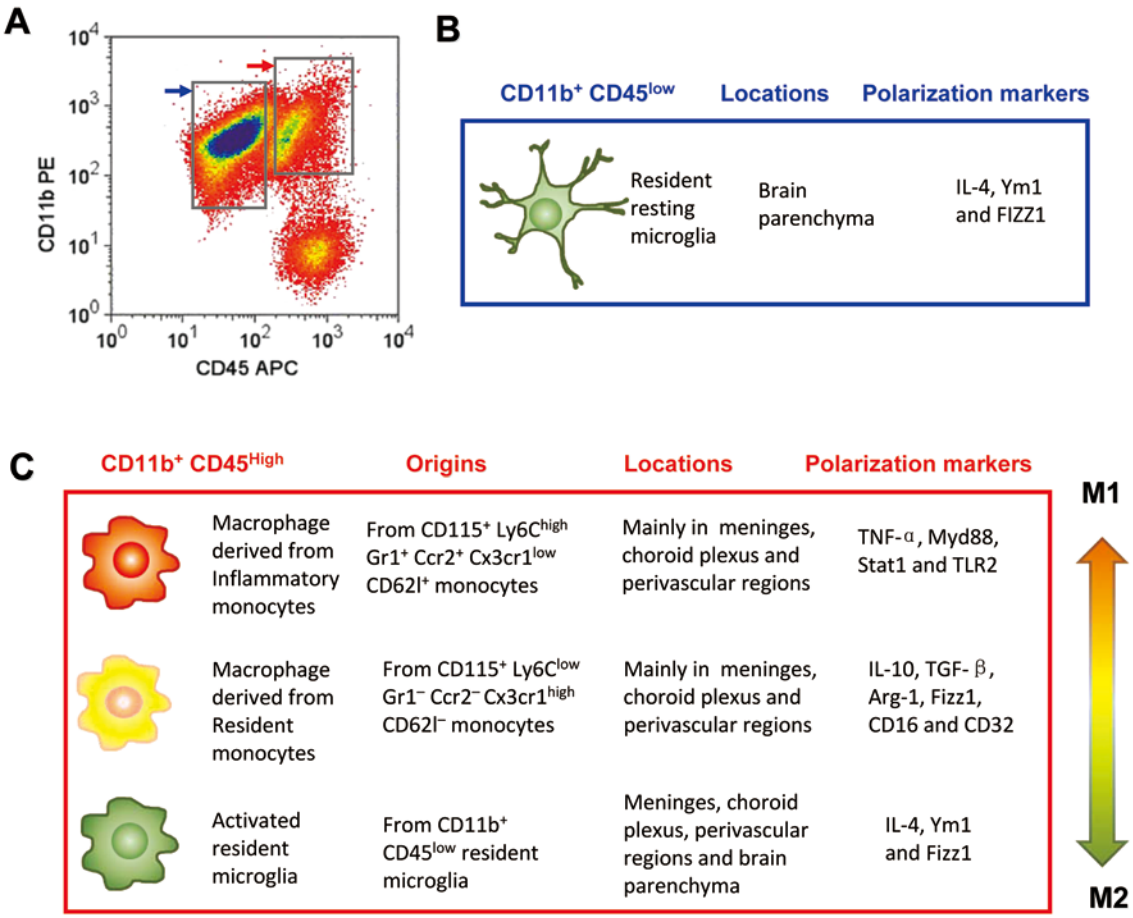

Fig. 1. Macrophage and microglia heterogeneity in an animal model of MS. A: Flow-cytometric analysis of CNS mononuclear cells isolated from EAE mice on the day of peak disease. The cells were stained for expression of CD45 and CD11b and then sorted into two populations: (1) macrophages and activated microglial cells ($CD11b^+/CD45^{high}$) and (2) resting microglial cells ($CD11b^+/CD45^{low}$). The lymphocytes are in the $CD11b^-/CD45^{high}$ population. The subtypes, origins, locations and polarization markers of the $CD11b^-/CD45^{low}$ and $CD11b^-/CD45^{high}$ populations are described in B and C, respectively. Macrophages and microglia shown in orange have an M1-like phenotype, while those in green are shifting toward M2-like activity. Please consult Table 1 for abbreviations.

by LPS or IFN-γ, are toxic to mature oligodendrocytes through a cell contact-dependent mechanism. TNF-α and NO partly mediate the detrimental effects[37,38]. Moreover, neurotrophic factors, including IGF-1 and PDGF, have been reported to be secreted by M2 macrophages[8].

Remyelination

The failure of remyelination in MS might be mainly due to the impairment of oligodendrocyte maturation and formation of myelin sheaths. Autopsy of MS patients shows that numerous OPCs are commonly found in chronic demyelinating lesions. However these OPCs could not form myelin sheaths in the center of chronic activated lesions[41,42]. Moreover, premyelinating oligodendrocytes were observed in some of the lesions. These cells express important myelin sheath proteins such as proteolipid protein and myelin basic protein. However, although they are associated with demyelinated axons, they fail to myelinate them[43,44] (Fig. 2).

Another important issue is dysfunction of endogenous neural progenitor cells (NPCs) in the subventricular zone (SVZ)[43]. Time-course studies indicated that in relapsing-remitting EAE, responses of SVZ-resident NPCs only occur in the acute stage and are completely lost in the chronic disease after repeated relapses[45,46]. Moreover, persistent inflammation has been reported to change the function of the SVZ-resident NPCs in both MS patients and a chronic targeted EAE model, which leads to the accumulation of non-migratory NPCs within the SVZ[47,48].

Experimental evidence shows that microglia contribute in part to the failure of remyelination. *In vitro* studies demonstrated that M1 microglia (activated by high, but not low concentrations of IFN-γ) impair oligodendrogenesis of NPCs through TNF-α[10,49]. Pretreatment of microglia with the M2 inducer IL-4 promotes oligodendrogenesis through up-regulation of IGF-1[49]. Pretreatment with IL-4 significantly decreases the production of typical toxic M1 molecules such as TNF-α and NO, and increases the survival of differentiating OPCs[50]. In both acute and chronic EAE models, injection of M2 microglia into the cerebrospinal fluid results

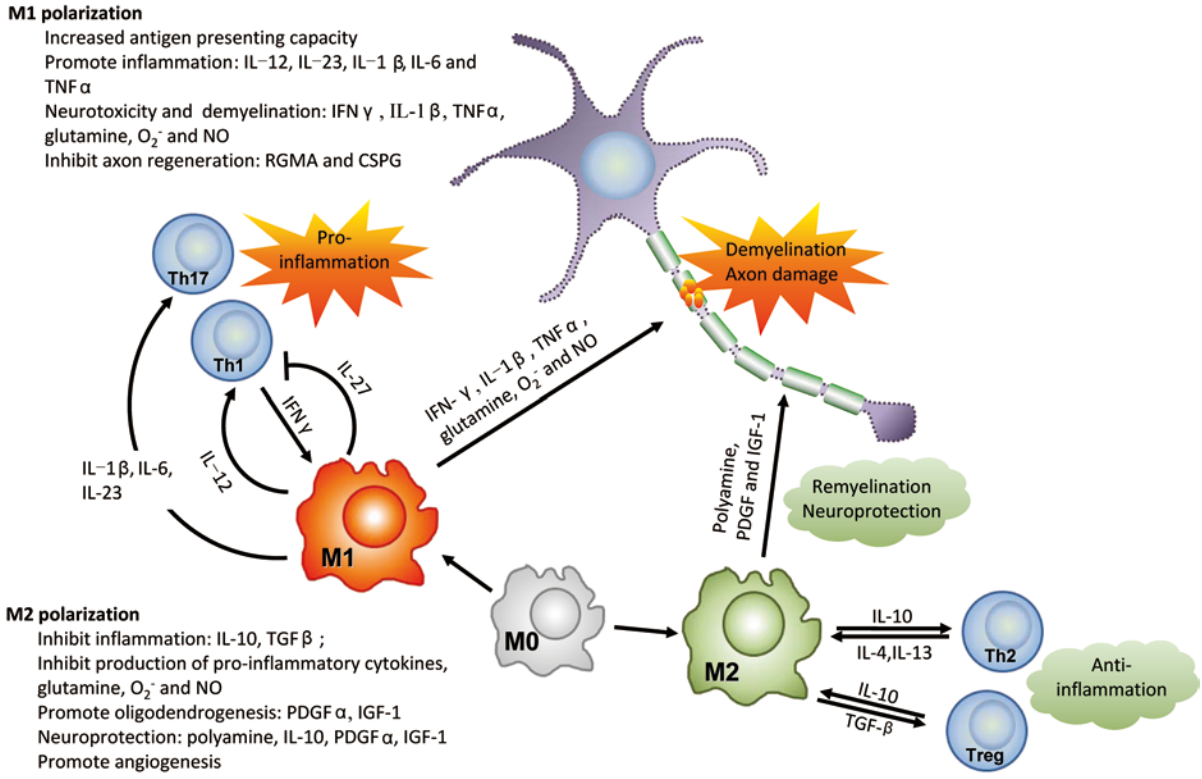

Fig. 2. Schematic of macrophage polarization in CNS demyelination. RGMA, repulsive guidance molecule A. Please consult Table 1 for the other abbreviations.

in increased remyelination in the spinal cord and improved clinical symptoms[10,45]. This indicates that the application of M2 microglia could reverse the detrimental effects of M1 microglia, promote remyelination, and improve clinical symptoms[9].

Neurotoxicity and Neuroprotection

Inflammation-mediated neurodegeneration, including neuronal degeneration, axonal loss and synaptic alteration, has been reported not only in EAE, but also in the acute and chronic stages of MS. Microglia and macrophages are key players in this process. *In vitro* studies demonstrated that medium from M1 macrophages is toxic to cortical neurons[51-53]. NO, glutamine and reactive oxygen species produced by M1 macrophages or microglia are generally accepted to lead to neuronal degeneration in MS[54-56]. Sodium channel blockade can rescue axons from NO-mediated degeneration in EAE[57, 58]. Recently, it was reported that the Nav1.6 sodium channel is upregulated in activated microglia and macrophages in EAE and MS, and blocking this channel can deactivate M1 microglia and decrease pro-inflammatory cytokine release *in vitro*. This indicates that sodium channel blockade may also regulate EAE development *via* microglia and macrophages[59-61]. The glutaminase inhibitor 6-diazo-5-oxo-L-norleucine (DON) and gap junction blocker carbenoxolone (CBX) protect neurons from M1 microglia-mediated cell death by decreasing glutamate release. Systemic application of DON or CBX ameliorates the clinical symptoms of EAE mice[62]. Other molecules, such as galectin-1, an endogenous glycan-binding protein, deactivate M1 microglia and protect against EAE-induced neurodegeneration by suppressing downstream pro-inflammatory mediators, such as iNOS and TNF[12,63]. The M1 and M2 macrophages also play different roles in axonal growth. M1 macrophages promote short but branched neurite growth from dorsal ganglion neurons; however M2 macrophages promote relatively long-distance axon regeneration, probably by secreting neurotrophic factors[51]. Synaptic alteration often develops from the early stage of EAE. Co-culture of Th-1 cytokine-activated microglia (M1) and brain slices leads to synaptic dysfunction through glutamine release and TNF-α signaling[52].

Phagocytosis

Macrophages and activated microglia are responsible for the phagocytosis of cell debris and pathogens during CNS damage, inflammation and infection. It has been reported that in remyelinated areas, the lipid-containing macrophages or microglia are usually in contact with remyelinating fibers[64,65], suggesting a positive role of phagocytosis in remyelination. However, uncontrolled phagocytosis in MS might promote disease severity. It has been reported that CD47, which protects healthy cells from phagocytosis, is significantly down-regulated in MS lesions. Moreover, application of monoclonal antibody against CD47 at the peak of EAE makes the disease more serious[66-68].

The effects of myelin internalization on macrophages and microglia are controversial. Several studies have reported that myelin debris can trigger the release of pro-inflammatory cytokines and NO from macrophages and microglia[69-71]. In contrast, other studies suggest that macrophages and microglia adopt anti-inflammatory characteristics following the internalization of myelin[72-74]. The inducers of M1 and M2 have different effects on macrophage phagocytosis. M1 macrophages activated by IFN-γ or LPS show a decreased ability to phagocytose myelin; whereas application of an M2 inducer, such as IL-4 or IL-10, increases phagocytic activity by macrophages[75,76]. Microglia show a regulation pattern different from macrophages. Both M1 and M2 inducers promote phagocytosis by microglia, probably because microglia need to be activated before acquiring phagocytic function[77].

Modulating Leukocyte Function

Macrophages and activated microglia play important roles in modulating T-cell functions during EAE[31,78,79]. M1 macrophages express high levels of MHC class II and CD86, which are important in antigen presentation. M1 macrophages and microglia stimulated by IFN-γ or LPS produce high levels of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, IL-12p40, IL-18, and IL-23p19. Some of these are involved in Th-1 and Th-17 cell differentiation[80,81]. M2 macrophages and microglia have been reported to express IL-4, IL-10 and TGF-β, which promote anti-inflammatory Th-2 or T-reg differentiation[45,82,83].

Activated microglia and macrophages can also modulate the trafficking of activated T-cells through regulation of the chemokine environment. Chemokines released from both M1 (CCL8, CCL15, CCL19, CCL20, CXCL9, CXCL10, CXCL11 and CXCL13) and M2 (CCL13, CCL14, CCL17, CCL18, CCL22, CCL23, CCL24 and CCL26) cells contribute to leukocyte migration and infiltration into the CNS[8,84-86]. It was reported that CXCR3, the receptor of CXCL9, CXCL10

and CXCL11, promotes the infiltration of T-reg cells into the CNS and ameliorates the severity of EAE[87]. Neutralization of CCL22 promotes the M2 polarization of macrophages and microglia, with decreased TNF and increased IL-10 production, and decreasing clinical symptoms of EAE[88].

Concluding Comments

Macrophages and microglia are crucial in the pathogenesis of MS. Polarization of microglia and macrophages may explain the functional diversity of these cells. M1-polarized microglia and macrophages usually have a greater antigen-presenting ability, leading to demyelination and neurodegeneration and increasing T-cell differentiation toward Th-1 and Th-17 (pro-inflammatory phenotypes) fates. M2 macrophages and microglia increase Th-2 and T-reg (anti-inflammatory phenotypes) differentiation, protect oligodendrocytes and neurons from damage, and ameliorate disease severity.

Recent studies suggest that the microenvironment of severe-relapsing EAE favors M1 polarization. Increasing the proportion of M2 cells would be useful in the treatment of this disease. It should be noted that the effects of M1 microglia or macrophages depend on the extent of their activation; not all M1 cells are detrimental. Only microglia activated by high concentrations of IFN-γ inhibit oligodendrogenesis from NPCs; low concentrations have the opposite effect[10,49]. On the other hand, excessive or prolonged M2 polarization may lead to unwanted fibrotic responses and scarring that might hinder remyelination and functional recovery[8]. Therefore, balancing M1/M2 in an appropriate ratio, and modulating the levels of cytokines and other factors in the microenvironment of the demyelinated CNS to reduce excessive or prolonged M1 polarization and enhance M2 polarization may therefore be a desirable therapeutic goal.

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