# Chapter 16
# Multiple Sclerosis

**Yoshifumi Sonobe and Akio Suzumura**

**Abstract** Multiple sclerosis (MS) is an inflammatory disease characterized by demyelination and axonal degeneration in the central nervous system (CNS). Although MS is considered an autoimmune disease against myelin antigens, its pathogenesis still remains unclear. Microglia are macrophage-like cells in the CNS which play a critical role in innate immunity, in addition to activating pathways associated with adaptive immunity. Microglia produce pro-inflammatory and anti-inflammatory mediators, including cytokines and chemokines, and phagocytose various types of cellular debris. In MS, microglia critically contribute to the inflammatory milieu, but also participate in disrupting the blood–brain barrier integrity, thus inducing the migration of various types of immune cells such as T and B lymphocytes, macrophages, and neutrophils into the CNS. In this disease, microglia may additionally behave as antigen-presenting cells and function as effector cells causing demyelination and axonal degeneration. However, recent evidence also indicates that microglia could play a beneficial role in remyelination and neuroprotection in MS. In this chapter, we will discuss about microglial involvement in MS, with an emphasis on the experimental autoimmune encephalomyelitis (EAE) animal model and describe the cellular and molecular mechanisms which could be specifically implicated in the pathogenesis.

**Keywords** Microglia • Inflammation • Cytokine • Chemokine • Blood–brain barrier • Antigen presentation • Demyelination • Neurodegeneration • Multiple sclerosis • Experimental autoimmune encephalomyelitis

### Bullet Points

- Microglia, macrophage-like cells in the central nervous system (CNS), play a substantial role in the pathogenesis of multiple sclerosis (MS).
- In MS, microglia contribute to the development of neuroinflammation by producing both pro-inflammatory and anti-inflammatory mediators.

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- Microglia also promote the migration of peripheral immune cells into the CNS and might additionally behave as antigen-presenting cells in MS.
- Microglia could lastly influence by their effector functions the demyelination and axonal degeneration observed in MS.

## 16.1 Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) resulting from an autoimmune response against myelin antigens. It affects approximately 2.5 million people worldwide, with a predominance in women (ratio of females to males, 2:1). The disease is characterized by a progressive loss of neurological functions caused by the destruction of axonal myelin sheaths throughout the brain and spinal cord white matter. The loss of myelin translates into clinical symptoms ranging from paralysis, muscle spasms, and optic neuritis, to neuropathic pain. Pathological features of MS lesions include increased blood–brain barrier (BBB) permeability, axonal degeneration, glial scar formation, and the prevalence of peripheral immune cells such as T and B lymphocytes, macrophages, and neutrophils within the CNS (Williams et al. 2007; Jadidi-Niaragh and Mirshafiey 2011). The etiology of MS is still unclear. Genetic factors like variations in the HLA-DRB1 gene coding for the major histocompatibility complex (MHC) class II complex (DRB1-9 beta chain), and the IL-7R gene coding for the interleukin (IL)-7 receptor, as well as environmental factors such as exposure to the Epstein-Barr virus, low levels of vitamin D, and smoking, have been associated with an increased risk of developing MS (Haines et al. 1996; Sawcer et al. 1996; Teutsch et al. 2003; Oreja-Guevara et al. 2014). Approximately 85 % of MS patients repeatedly undergo relapse followed by partial or complete recovery periods (or remissions), a form of the disease which is termed relapsing-remitting MS. In more than 50 % of the relapse-remitting MS patients, the disease progressively worsens with minor remissions, reaching a stage of secondary progressive MS. In the remaining 10–15 % of MS patients, however, the disease only advances without remission, a form of the disease which is termed primary progressive MS (Thompson et al. 1997; Haines et al. 2011).

### *16.1.1 Inflammatory Mechanisms in MS/EAE*

Experimental autoimmune encephalomyelitis (EAE) is commonly used as an animal model of MS, being similarly associated with axonal degeneration and chronic demyelination, primarily in the spinal cord, resulting in tail and hindlimb paralysis. However, similar to MS, the disease symptoms reflect the anatomical location of the inflammatory lesions and may also include emotional instability, sensory loss, ataxia, muscle weakness, and spasms. EAE is generally induced in rodents by

immunization with myelin peptides, such as myelin basic protein (MBP), myelin proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), emulsified in an adjuvant (typically complete Freund's adjuvant) to enhance the immune response. EAE is also produced by adoptive transfer of myelin-reactive T lymphocytes expressing CD4 glycoproteins on their surface ($CD4^+$ T cells), isolated from mice immunized with myelin peptides, and further stimulated in vitro with myelin peptides. Depending on the nature of the antigens, and on the background of the animals, an acute stretch of EAE, a relapsing-remitting form, or chronic EAE can be induced (Rangachari and Kuchroo 2013).

In both EAE and MS, the infiltration of T and B lymphocytes, macrophages, and neutrophils is pronounced around the demyelinating lesions in situ, within the perivascular space and/or parenchyma. In addition, oligoclonal IgGs are commonly detected in the cerebrospinal fluid of EAE mice and MS patients (Mehta et al. 1985; Tomioka and Matsui 2014), thus suggesting the presence of an immune response in the CNS. Although the initiation mechanisms of EAE still remain unclear, they were shown to be mediated by Th effector T cells, a phenotype of CD4+ cells resulting from their activation by antigen-presenting cells (APCs) (Montero et al. 2004; reviewed in Kawakami et al. 2012) (see Chap. 5 for further reading on antigen presentation).

### *16.1.2 Microglia in MS/EAE*

It is usually difficult to discriminate microglia from infiltrated macrophages in the postmortem brains of MS patients. However, several lines of evidence have suggested that microglia could play a pivotal role in mediating neuroinflammation in MS. Microglia are macrophage-like cells that reside in the CNS and contribute in various manners to maintaining CNS integrity. In the inflamed CNS, microglia can also function as immunocompetent cells, particularly involved with the production of inflammatory mediators and/or the presentation of antigens, depending on the context (Ransohoff et al. 2003; Tran and Miller 2003; Raivich and Banati 2004; Chastain et al. 2011).

During the development of EAE and MS, microglia display several signs of 'activation' at the morphological and gene expression levels. For instance, microglia have larger cell bodies, accumulate around lesions sites, and show immunoreactivity for MHC class II and CD68, a lysosomal marker also named 'macrosialin' in mouse that is upregulated during inflammation (Minagar et al. 2002; Jack et al. 2005; Marik et al. 2007; also see Chap. 10 for additional reading about CD68). In the postmortem brains of progressive MS patients, demyelination and neuronal damage reportedly correlate with an increased density and clustering of CD68 or MHC class II positive cells, a pathological feature commonly referred to as 'microglial nodules' (Prineas et al. 2001; Singh et al. 2013). In addition, microglia have been proposed to behave as APCs in MS. They were shown to express MHC class II, display antigens on their cellular surface, and colocalize with $CD4^+$ cells before the onset of EAE,

and the infiltration of myeloid cells in bone marrow chimera in vivo (Ponomarev et al. 2005). These observations suggest a possible role for microglia in the activation of T cells in MS, or in their reactivation following antigen presentation in the periphery (see Chap. 5 for more information on both processes), although direct evidence remains to be shown. In addition, microglia and macrophages contained phagocytosed myelin debris around the white matter demyelinating lesions in MS postmortem samples, highlighting their possible involvement, whether detrimental or beneficial, to the demyelination and axonal degeneration (Tanaka et al. 1975; Bauer et al. 1994; also see Napoli and Neumann 2010).

Moreover, it has been reported that preventing microglial activation could repress the development of EAE in vivo (Heppner et al. 2005). In particular, Heppner and colleagues have generated a mouse model expressing the herpes simplex virus thymidine kinase (HSVTK) specifically in microglia/macrophages, under the CD11b promoter, thus rendering these cells susceptible to ganciclovir cytotoxicity. Following transplantation of wild-type bone-marrow cells, to spare the peripheral myeloid cell population from ganciclovir treatment, and the subsequent peripheral injection of ganciclovir, microglial transformation to amoeboid morphologies was found to be arrested, a phenomenon referred to as "microglial paralysis", which resulted in delayed EAE onset and reduced clinical score (Heppner et al. 2005). These findings strongly suggested that microglia could play a significant role in the pathogenesis of EAE and MS. The focus of this chapter is on microglial implication in multiple immunological aspects of the disease pathogenesis, including antigen presentation, inflammation, demyelination, and neurotoxicity.

## 16.2 Microglia as Antigen-Presenting Cells

Dendritic cells (DCs), which are monocyte-derived cells considered as 'professional' APCs, are often encountered in the leptomeninges and white matter lesions of MS patients (Ganguly et al. 2013; Nuyts et al. 2013). However, microglial cells could also behave as APCs in MS as will be discussed below (Smith et al. 1998) (see Chap. 5 for further reading). After the phagocytosis of antigens, such as myelin peptides, APCs become engaged in antigen presentation through MHC class II signaling to $CD4^+$ cells, which express the cognate T cell receptor, leading to their activation (or reactivation) and differentiation into various Th effector T cell subsets, such as the pro-inflammatory, encephalitogenic Th1 and Th17 cells. Costimulatory molecules such as CD80 and CD86 on APCs, or the CD40 ligand (CD40L) expressed on T cells further contribute to activating Th cells via CD28 (member of the B7 family), or to activating APCs via CD40, to promote cellular expansion and survival. Conversely, the costimulatory molecules-programmed cell death-ligand 1 (PD-L1) and PD-L2 suppress Th cell activation by acting on their programmed cell death 1 (PD-1) receptor (Keir et al. 2008; Elgueta et al. 2009).

During normal physiological conditions, microglia express undetectable to low levels of MHC class II molecules (Wong et al. 1984; Suzumura et al. 1987) and constitutively express low levels of CD80 and high levels of CD86 (Satoh et al. 1995; Dangond et al. 1997). Over the course of EAE, however, the expression of MHC class II, CD80 and CD86, was found to be upregulated in $CD11b^{+}CD45^{low}$ microglial cells, isolated from the brains of EAE mice by flow cytometry (Ponomarev et al. 2005; Murphy et al. 2010). Expression of MHC class II is also induced in cultured microglia/macrophages upon stimulation with interferon (IFN)-γ (Suzumura et al. 1987). This pro-inflammatory cytokine produced by Th1 cells, T cells expressing the CD8 glycoprotein ($CD8^{+}$ T cells), macrophages, DCs, and microglial cells in culture, is well known for promoting immune responses against viral and bacterial infection, as well as the development of tumors (Munder et al. 1998; Kawanokuchi et al. 2006; Vremec et al. 2007). Additionally, treatment of cultured microglial cells with the supernatant from IFNγ-producing Th1 cell lines, specific for MBP, particularly induced microglial expression of MHC class II, CD80, CD86, and CD40 in vitro (Seguin et al. 2003). The binding of CD40 to CD40L induces APCs to produce pro-inflammatory mediators such as TNF-α, IL-6, and IL-12 in vitro (Aloisi et al. 1999; Rezai-Zadeh et al. 2008). When cultured in the presence of IFNγ-stimulated microglial cells, the proliferative capacity of Th cells is additionally increased and accompanied by an enhanced production of IL-2 and IFNγ in vitro (Aloisi et al. 1998). On the other hand, IFNγ also induces the expression of PD-L1 on microglia, while suppressing Th cell activation and the production of IFNγ in vitro (Magnus et al. 2005). Together, these findings suggest that IFNγ-stimulated microglial cells may not only activate Th cells via the induction of CD80 and CD86, or CD40, but also suppress T cell activation via the induction of PD-L1 expression, at least in vitro.

The cytokine granulocyte macrophage colony-stimulating factor (GM-CSF), which is secreted by T cells, as well as astrocytes and macrophages in vitro (Ohno et al. 1990; Shi et al. 2006), may also play a critical role in the induction of APCs functions in microglia (Matyszak et al. 1999). During EAE, MHC class II expression is considerably reduced ex vivo in microglial cells derived from GM-CSF-deficient mice, compared to wild-type mice (Ponomarev et al. 2007; Codarri et al. 2011), suggesting that GM-CSF regulates microglial expression of MHC class II. Interestingly, GM-CSF also promotes DCs-like properties in microglia ex vivo and in vivo, enhancing their expression of the DCs marker CD11c, as well as MHC class II, CD80, and CD86 (Schermer and Humpel 2002; Li et al. 2011). GM-CSF-stimulated microglia also have the ability to induce the proliferation and reactivation of $CD4^{+}$ T cells in vitro (Fischer et al. 1993; Aloisi et al. 2000). In GM-CSF-stimulated microglia, however, the levels of MHC class II are lower than in DCs and associated with reduced Th cells proliferation (Lambert et al. 2008), in agreement with the view that microglia have limited antigen-presenting capacity (Ransohoff and Engelhardt 2012). Therefore, GM-CSF-stimulated microglia could be used for stimulating adaptive immune responses, such as antigen presentation, in EAE and MS, albeit with a limited capacity as compared to monocyte-derived DCs.

## 16.3 Microglia as Inflammatory Cells

Activated microglial cells produce a variety of cytokines and monokines (i.e., cytokines mainly produced by monocytes/macrophages) involved in mediating neuroinflammation. In particular, the secretion of IL-1β, IL-6, and tumour necrosis factor α (TNFα) (Fig. 16.1) is upregulated in cultured microglia upon direct contact with MBP-primed T cells (Dasgupta et al. 2005), while their production of TNFα, IL-6, and IL-12 is enhanced by antigen presentation to $CD4^+$ T cells via the CD40-CD40L signaling pathway in vitro (Rezai-Zadeh et al. 2008; Aloisi et al. 1998). These findings suggest that microglial interactions with T cells could influence their contribution to the neuroinflammatory milieu in EAE and MS, specifically by modulating their release of TNFα, IL-1β, IL-6, and IL-12 as discussed below.

1. *TNFα* is produced in the MS/EAE brain by microglia as well as macrophages (Renno et al. 1995) and functions as a pro-inflammatory mediator in the CNS, by inducing the production of chemokines (or **chemo**tactic cyto**kines**; mediating

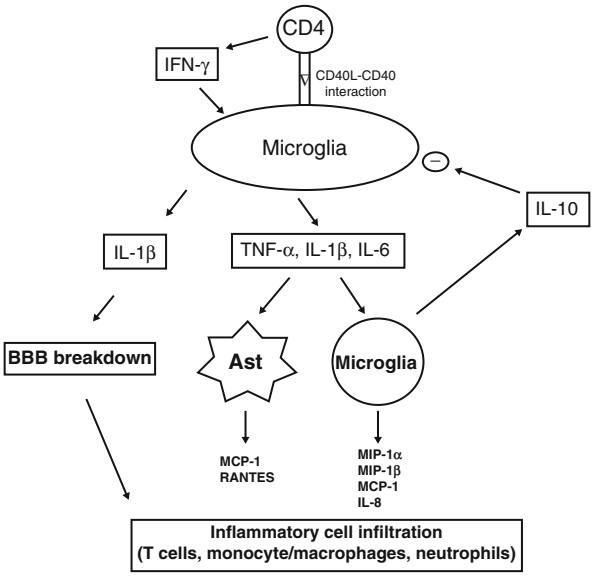


**Fig. 16.1** The role of microglia in neuroinflammation. Microglia produce monokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 when stimulated with CD40 ligand (CD40L) expressed on activated T cells and/or T cell-derived cytokines. In addition, activated microglia produce a variety of chemokines and induce inflammatory cell infiltration. Monokines activate microglia and astrocytes to induce the production of chemokines, leading to the infiltration of inflammatory cells including T cells, monocytes/macrophages, and neutrophils. IL-1β further disrupts blood–brain barrier (BBB) and induces the production of the chemokine monocyte chemoattractant protein (MCP)-1, which contributes to the infiltration of inflammatory cells. However, TNF-α also induces microglial production of IL-10 via the TNF receptor (TNFR)2, thus exerting anti-inflammatory responses as well

the attraction of their responsive cells) such as IL-8, macrophage inflammatory protein (MIP)-1α and MIP-1β in cultured human microglia (Ehrlich et al. 1998; McManus et al. 1998), and the production of monocyte chemoattractant protein (MCP)-1 and regulated on activation, normal T cell expressed and secreted (RANTES) in cultured rat astrocytes (Guo et al. 1998). MIP-1α, MIP-1β, MCP-1, and RANTES are chemotactic for T cells, macrophages, and microglia. Interestingly, in mice devoid of CNS expression of TNF receptor 1 (TNFR1), the recruitment of macrophages and granulocytes is reduced over the course of EAE, induced using MOG-reactive T cells. The levels of MCP-1 and MIP-2, i.e., a chemotactic factor for neutrophils produced by macrophages and microglia, were also found to be reduced in the CNS of these mice (Gimenez et al. 2006). These findings suggest that TNFα could enhance neuroinflammation in EAE and MS by acting on the infiltration of inflammatory cells from the periphery, and the induction of additional chemokines in microglia or astrocytes. Consistently, transgenic mice over-expressing TNFα were shown to spontaneously develop an inflammatory demyelinating disease characterized by the activation of astrocytes and microglial cells, together with the infiltration of $CD4^+$ and $CD8^+$ T cells into the meninges and CNS parenchyma (Probert et al. 1995). TNFα-deficient mice also displayed a delayed EAE onset, but similar to higher levels of EAE severity were observed in later phases of the disease (Kassiotis et al. 1999), thus suggesting that TNFα could exert distinct roles, either detrimental or beneficial, depending on the stage of disease progression.

The functions of TNFα are exerted via either TNFR1 or TNFR2 expressed on various types of cells (Dopp et al. 1997; Baker and Reddy 1998; Tracey et al. 2008; Martin et al. 2014). In the healthy CNS, these receptors are found on neurons, astrocytes, and oligodendrocytes (Yang et al. 2002; Kuno et al. 2006; Faustman and Davis 2013). In EAE/MS, the infiltrated lymphocytes, neutrophils, macrophages, and MHC class II positive cells additionally express TNFR1 and TNFR2 around EAE lesions (Kahn et al. 1999), while oligodendrocytes express TNFR1 around MS lesions (Probert et al. 2000). TNFR1, but not TNFR2, contains a death domain. The affinity of TNFα for TNFR1 is significantly greater than for TNFR2 (Grell et al. 1998). In previous reports, TNFR1-deficient mice were found to be resistant to MOG-induced EAE, whereas TNFR2-deficient mice displayed enhanced $CD4^+$ and $F4/80^+$ cells infiltration in vivo, together with an exacerbated EAE outcome (Eugster et al. 1999; Suvannavejh et al. 2000). In addition, TNFR2 stimulation promotes microglial expression of the anti-inflammatory cytokine IL-10 in vitro (Veroni et al. 2010). The findings suggest that TNFα could mediate neuroinflammation in MS and EAE, through the activation of TNFR1, and anti-inflammatory responses via TNFR2.

2. *IL-1β* is also detected in microglial cells and macrophages during EAE (Bauer et al. 1993; Cash et al. 1994). MBP-primed T cells induce the production of IL-1β from murine microglial cells and macrophages in vitro (Dasgupta et al. 2005). Microinjection of IL-1β into the CNS reportedly increases BBB permeability, accompanied by a pronounced recruitment of neutrophils (Ferrari et al. 2004). In addition, IL-1β induces the expression of genes favoring blood vessel

plasticity, such as the hypoxia-inducible factor 1α (HIF-1α) and its target, vascular endothelial growth factor (VEGF)-A, in cultured human astrocytes. This results in increased BBB permeability through downregulation of the tight junction proteins claudin 5 and occludin in endothelial cells (Argaw et al. 2012). In a mouse model of traumatic brain injury, IL-1β similarly induces the invasion of neutrophils and T cells into the CNS (Clausen et al. 2009). In vitro studies also demonstrate that IL-1β induces MCP-1, IL-8, MIP-1α, and MIP-1β expression in microglial cells (Calvo et al. 1996; Ehrlich et al. 1998; McManus et al. 1998), and that of MCP-1 and RANTES in astrocytes (Hayashi et al. 1995; Barnes et al. 1996). Thus, microglia-derived IL-1β could induce disruption of the BBB, release of chemoattractant mediators from microglia and astrocytes, and infiltration of peripheral inflammatory cells into the CNS. However, the particular involvement of microglia-derived IL-1β as a neuroinflammatory mediator in EAE and MS remains to be tested experimentally.

3. *IL-6* is also a potent inducer of microglia-mediated neuroinflammation. In EAE, IL-6 is produced by microglial cells as well as T cells and macrophages (Diab et al. 1997; Wlodarczyk et al. 2014). Many reports have suggested that IL-6 plays an inflammatory role in the pathogenesis of EAE (Erta et al. 2012). Neuron-targeted expression of IL-6 has been shown to induce reactive astrogliosis and microglial activation in transgenic mice in vivo (Fattori et al. 1995). Accordingly, IL-6 also induces MCP-1 mRNA expression by rat microglia in vitro (Calvo et al. 1996). In transgenic mice where the production of IL-6 is restricted to the cerebellum, MOG-induced EAE additionally resulted in the activation of infiltrated macrophages and microglia, accompanied by severe ataxia, enhanced cerebellum infiltration of neutrophils and B cells, and expression of RANTES (or CCL-5), MCP-5 (or CCL-12), and TNFα in situ (Quintana et al. 2009), suggesting its involvement in EAE and MS.
4. *IL-12*: IFNγ induces the expression of CD40 on microglia (Aloisi et al. 1998), while binding of CD40 to its ligand CD40L induces microglial production of IL-12p70 in vitro (Aloisi et al. 1999). This heterodimeric cytokine, which is composed of the IL-12p35 and IL-12p40 subunits, is a crucial differentiation factor for pro-inflammatory Th1 cells, which can trigger inflammatory responses and activate APCs and cytotoxic T cells to attack their target cells (Knutson and Disis 2005). In addition, microglial cells stimulated with IFNγ in conjunction with the Toll-like receptor (TLR) 4 ligand, bacterial lipopolysaccharide (LPS), produced IL-12p70 and IL-23 in vitro (Suzumura et al. 1998; Sonobe et al. 2005). IL-23 is a heterodimer consisting of p19 and the IL-12p40 subunit. It induces the development of IL-17-producing Th17 cells, another type of pro-inflammatory $CD4^+$ cells, which play a crucial role in the pathogenesis of EAE (Langrish et al. 2005). However, more recent studies have suggested that mice deficient in the IL-12p35 subunit are fully susceptible to EAE (Becher et al. 2002), indicating that factors other than IL-12p70 might be crucial for the induction of EAE. In contrast, p19-deficient mice are reportedly resistant to EAE (Cua et al. 2003), suggesting that IL-23 production is more critical than IL-12p70 production in the CNS. It is important to note that mice in which the IL-23

subunit IL-23p40 is devoid of CNS expression have decreased EAE severity, indicating that IL-23 produced by CNS-resident cells is important for the development of EAE (Becher et al. 2003). Moreover, expression of the IL-23p19 subunit was observed in APCs including microglia and macrophages localized around the lesion sites in MS postmortem brains (Li et al. 2007). Because IFNγ is also involved with the production of IL-23, IFNγ could regulate the differentiation of both Th1 and Th17 cells.

Taken together, these findings suggest that three main monokines secreted by microglia, namely TNF-α, IL-1β, and IL-6, could synergistically 'activate' glial cells and promote infiltration of peripheral immune cells in the CNS, as observed in MS and EAE, while IL-12 and IL-23 could mediate differentiation of the encephalitogenic Th cells. Nonetheless, the specific contribution of microglial cells to the release of these monokines, at different stages of MS and EAE, and their ultimate impact on the disease pathogenesis remain unknown.

## 16.4 Microglia in Demyelination

Microglial cells are recruited to areas of demyelination in MS/EAE, where they transform their morphology and actively proliferate. Activated phenotypes are also observed in rodents upon feeding with dietary cuprizone, a copper chelator which causes demyelination of the corpus callosum (Remington et al. 2007; Groebe et al. 2009). Recent findings suggest that microglia could be directly involved in the mechanisms of demyelination, by their release of excitotoxic glutamate, reactive oxygen species, pro-inflammatory cytokines, nitric oxide, and mediators of apoptosis as described below (see Fig. 16.2).

The expression levels of glutaminase, an enzyme that converts glutamine into glutamate, were shown to be increased in microglial cells localized within the active MS lesions in situ (Werner et al. 2001), suggesting a possible role of excitotoxic glutamate released from microglia in the demyelination process. LPS-activated microglia also increase the extracellular glutamate levels and induce the death of oligodendrocytes in vitro (Domercq et al. 2007). IL-1β reportedly induces apoptosis in cultures of oligodendrocytes, astrocytes and microglia, and this effect is blocked pharmacologically by applying antagonists of the AMPA/kainate glutamate receptors (Takahashi et al. 2003). The combined findings suggest that glutamate released from microglia could be directly involved in demyelination during EAE and MS, by inducing toxicity against oligodendrocytes, although this hypothesis remains to be tested.

In the brains of MS patients, DNA oxidation and lipid peroxidation are mainly observed in the nucleus and cytoplasm of oligodendrocytes, respectively, thus suggesting an ongoing state of oxidative stress (Haider et al. 2011). Accordingly, the expression levels of several enzymes controlling the respiratory burst, including the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) catalytic subunits p91phox, p22phox, and p47phox, were found to be upregulated in MS lesions

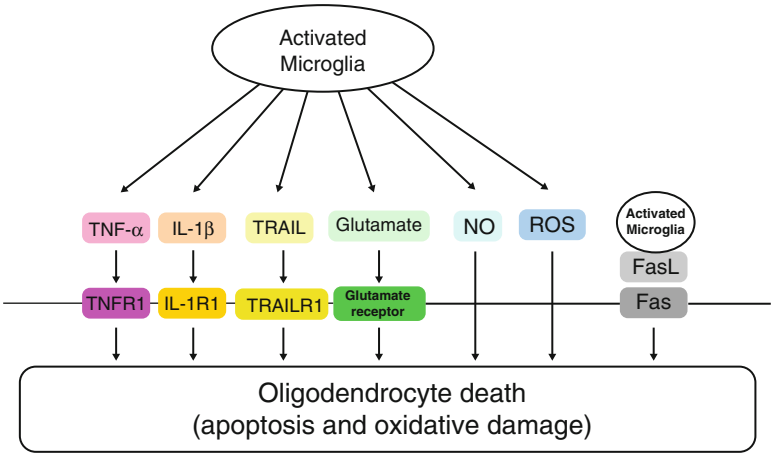


**Fig. 16.2** The role of microglia in demyelination. Activated microglia produce soluble factors including TNF-α, IL-1β, TNF-related apoptosis-inducing ligand (TRAIL), glutamate, nitric oxide (NO), and reactive oxygen species (ROS), which damage oligodendrocytes via the induction of apoptosis and/or oxidative stress. Alternatively, Fas ligand (FasL) expressed on activated microglia interacts with Fas on oligodendrocytes and induces apoptosis of oligodendrocytes. Abbreviations as in Fig. 16.1

(Fischer et al. 2012). IFNγ- or GM-CSF-stimulated microglial cells reportedly produce reactive oxygen species (ROS) in vitro (Hu et al. 1995; Smith et al. 1998), showing cytotoxicity against cultured oligodendrocytes (Schreibelt et al. 2007), thus suggesting a possible role for microglial production of ROS in demyelination.

Microglial release of TNFα and IL-1β could be specifically involved in this process. It has been reported that microglia-secreted TNFα induces the death of oligodendrocytes and their progenitor cells in vitro (Zajicek et al. 1992; Pang et al. 2010). Oligodendrocyte-specific ablation of TNFR1 also attenuates the clinical signs of EAE, suggesting that oligodendrocytic TNFR1 could be involved in demyelination (Hovelmeyer et al. 2005). On the other hand, using the cuprizone-induced demyelinating model, Arnett and colleagues revealed that TNFR2, but not TNFR1, is critical for the regeneration of oligodendrocytes in vivo (Arnett et al. 2001). These observations suggest that microglia-derived TNFα could mediate the death of oligodendrocytes via TNFR1, and their regeneration through TNFR2.

In previous reports, IL-1β was also reported to cause demyelination in vivo and in vitro, while IL-1β-stimulated microglia exerted an increased oxidative activity in vitro (Smith et al. 1998), suggesting that IL-1β could damage oligodendrocytes through the release of ROS. In addition, IL-1β degrades intracellular sphingomyelin to ceramide and induces the apoptosis of oligodendrocytes in vitro (Brogi et al. 1997). Sphingomyelin, which mainly consists of ceramide and phosphocholine, is a component of the myelin sheath. Accordingly, administration of the IL-1 receptor antagonist suppressed EAE in rats, while mice deficient in the IL-1 receptor 1 (IL-1R1) showed ameliorated symptoms of EAE (Martin and Near 1995; Sutton et al. 2009). These findings suggest that IL-1β could contribute to demyelination in MS and EAE via the induction of oligodendrocyte apoptosis. On the other hand, IL-1β-deficient

mice undergoing the cuprizone-induced demyelination failed to remyelinate, following withdrawal of the dietary cuprizone, suggesting that IL-1β additionally contributes to remyelination (Mason et al. 2001). This failure of remyelination also appears to correlate with a lack of insulin-like growth factor-1 (IGF-1) production by microglia and astrocytes, as their mRNA levels were shown to be reduced in brain extracts from IL-1β-deficient mice (Mason et al. 2001). Since IGF-1 is also required for the differentiation of precursor cells into mature oligodendrocytes, it has been speculated that IL-1β could play a crucial role in remyelination through the induction of microglial and/or astrocytic IGF-1 (Mason et al. 2001). Thus, it is possible that microglial IL-1β is similarly required in MS for remyelination.

Furthermore, in vitro studies have shown that microglia stimulated with IFNγ and/or LPS produce nitric oxide (NO) and the TNF-related apoptosis-inducing ligand (TRAIL) (Chao et al. 1992; Zielasek et al. 1992; Genc et al. 2003). NO reportedly causes single-stranded DNA breaks and mitochondrial damage in oligodendrocytes in vitro (Mitrovic et al. 1994), suggesting another mechanism by which microglia could contribute to demyelination in MS and EAE. In addition, TRAIL, which is a member of the death-signaling molecule family, reportedly induces human oligodendrocyte apoptosis via TRAILR1 in vitro (Matysiak et al. 2002). Thus, IFNγ might also promote demyelination via the production of NO and TRAIL by microglia.

In MS lesions, microglia, infiltrated macrophages, and T lymphocytes were lastly found to express the Fas ligand (FasL), which belongs to the TNF family and induces apoptosis upon binding to its receptor FasR (Dowling et al. 1996; D'Souza et al. 1996). Fas ligation with an anti-Fas antibody or the Fas ligand induced oligodendrocyte cell membrane lysis and subsequent cellular death in vitro (D'Souza et al. 1996). In addition, mice lacking Fas in oligodendrocytes exclusively were reported to be partially resistant to EAE (Hovelmeyer et al. 2005). Further research will test the direct contribution of microglia-derived glutamate, ROS, TNFα, IL-1β, IGF-1, NO, TRAIL, and FasL in the processes of oligodendrocyte apoptosis and regeneration, as well as concomitant demyelination and remyelination in MS.

## 16.5 Microglia in Neurodegeneration

MS has long been considered as a chronic inflammatory demyelinating disease of the CNS. However, several lines of evidence also suggest that axonal degeneration in MS and EAE could occur independently from demyelination. Even though axonal degeneration is widespread in the corpus callosum in EAE (Mangiardi et al. 2011), the underlying molecular mechanisms still remain unclear. Howell and colleagues proposed that microglial cells could be involved, since neurodegeneration is accompanied by the presence of activated microglia in MS postmortem brains, showing thicker and shorter processes than observed in control microglia (Howell et al. 2010). The evidence from in vivo and in vitro studies that activated microglia could cause 'inflammation-induced neurodegeneration' in MS and EAE, via the release of NO, ROS, glutamate, and various pro-inflammatory cytokines, will be reviewed in the following section.

Neuronal degeneration is induced in cultures of microglia and neurons upon stimulation with LPS and IFNγ and reduced by the addition of NOS inhibitors (Chao et al. 1992). In addition, heat shock protein (HSP)60 induces microglial production of NO via TLR4, in addition to causing extensive axonal loss and neuronal death in vitro (Lehnardt et al. 2008). Lipoteichoic acid (LPA), an agonist of TLR2 derived from staphylococcus aureus, which is involved in pathogen recognition and has been reported to exacerbate both EAE and MS (Gambuzza et al., 2011), also promotes the production of NO and superoxide by microglial cells. LPA additionally induces neuronal death, and this process is blocked by an iNOS inhibitor and a peroxynitrite scavenger in vitro, suggesting a direct detrimental contribution of microglia-derived NO to neuronal death (Kinsner et al., 2005).

In the EAE model, Nikić and colleagues revealed that ROS and reactive nitrogen species (RNS) released from activated macrophages/microglia induce focal axonal degeneration, using two-photon in vivo imaging (Nikić et al. 2011). Microglial expression of p47 phox, a cytosolic subunit of NADPH oxidase, was further shown to be upregulated in the active lesions of MS postmortem brains (Fischer et al. 2012). Microglia stimulated with the nucleotide ATP also released superoxides via P2X7 receptor, a purinergic receptor for ATP, and elicited toxicity against cultured neurons (Mead et al. 2012). It has been shown that blockade of the P2X7 receptor ameliorates EAE (Matute et al. 2007). Interestingly, microglia activated with thrombin, which induces blood coagulation via converting fibrinogen to fibrin, induce oxidative stress resulting in hippocampal neuronal cell death (Choi et al. 2005). Prothrombin kringle-2, a domain of prothrombin distinct from thrombin, also induces the loss of cortical neurons and this effect is partially inhibited by a NADPH oxidase inhibitor (Won et al. 2009). In addition, the leakage of fibrinogen from blood vessels has been shown to activate microglia and further induce axonal damage in EAE, using two-photon in vivo imaging (Davalos et al. 2012) (see Chap. 4 for additional information on these observations). It is possible that these blood-derived proteins enter the CNS through damaged BBB, thus activating microglia and inducing neurodegeneration through the release of ROS. Furthermore, high mobility group box chromosomal protein 1 (HMGB1), a chromatin-associated nuclear protein, has been detected in active lesions of MS and EAE (Andersson et al. 2008). It reportedly induces p47 phox membrane translocation and microglial production of ROS via MAC1, which consists of integrin alpha M and beta 2 (Gao et al. 2011). These findings strongly suggest that ROS produced by microglia, possibly activated by ATP or thrombin, contribute to axonal and neurodegeneration in MS and EAE.

Moreover, microglial stimulation with Chromogranin A, a marker of neurodegeneration that is released from damaged neurons and elevated in the CSF of MS patients (Stoop et al. 2008), induced their production of NO and glutamate in vitro. The conditioned medium from Chromogranin A-stimulated microglia also killed rat cerebellar granule cells via caspase-3-dependent apoptosis, blocked with an ionotropic glutamate receptor antagonist, thus suggesting that microglia induce neuronal death via glutamate release (Kingham et al. 1999). In addition, in vitro studies have suggested that LPS and TNFα could induce neurotoxicity through the release of glutamate from activated microglia in vitro (Takeuchi et al. 2006; Yawata et al. 2008).

Neuroinflammation, including microglial TNFα production, is associated with neurodegeneration in EAE (Centonze et al. 2009). Thus, it is possible that microglia-derived glutamate exerts toxicity against neurons in EAE and MS.

## 16.6 Conclusion

In EAE and MS, microglial cells function as APCs, albeit possibly at lower levels than professional APCs such as DCs (Fig. 16.3). IFNγ and GM-CSF induce expression of MHC class II and costimulatory molecules of the B7 family in microglia,

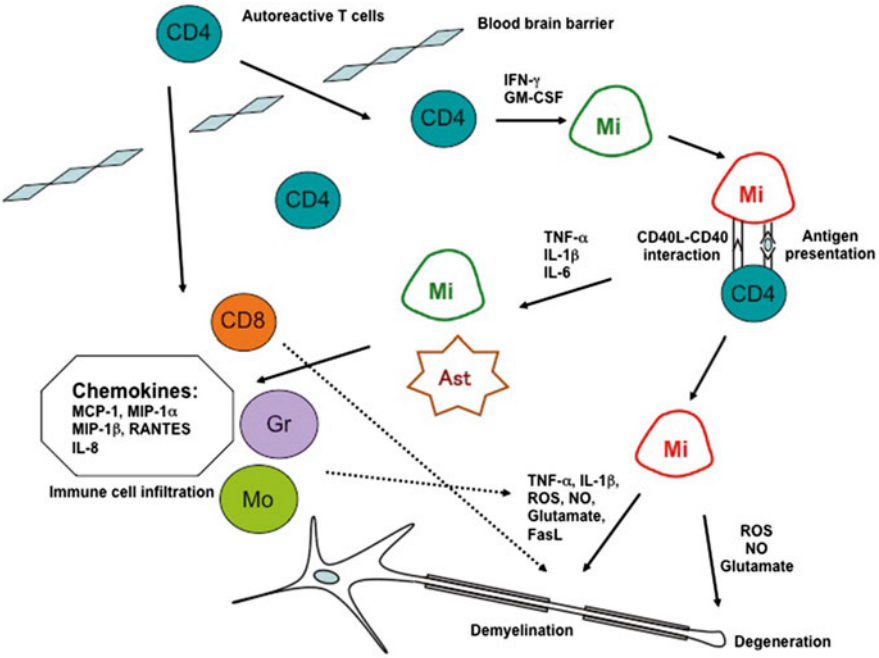


**Fig. 16.3** The role of microglia in neuroinflammatory diseases. Microglia are activated by $CD4^+$ T cells via CD40L–CD40 interactions and/or soluble factors. In some cases, activated microglia express major histocompatibility complex (MHC) class II and costimulatory molecules including CD80 and CD86, and possibly behave as antigen presenting cells (APCs) that restimulate infiltrating $CD4^+$ cells. In another cases, activated microglia produce monokines such as TNF-α, IL-1β, and IL-6, which contribute to the activation of astrocytes and bystander microglial cells. Astrocytes and microglia activated by the monokines produce chemokines including monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation, normal T cell expressed and secreted (RANTES), and IL-8 which induce chemotaxis of various immune cells including monocytes/macrophages, neutrophils, $CD4^+$, and $CD8^+$ T cells. Activated microglial cells could also induce demyelination via FasL-Fas interactions, and the production of TNF-α, IL-1β, NO, glutamate, and ROS. Moreover, activated microglia could further induce neurodegeneration via the production of NO, glutamate, and ROS. Abbreviations as in Figs. 16.1 and 16.2

activate (or reactivate) myelin-specific T cells, and enhance neuroinflammation. In addition, microglia are activated by monokines such as TNFα, IL-1β, and IL-6 early in disease. Activated microglia also produce monokines, which in turn act on microglia and astrocytes to further enhance neuroinflammation via the production of cytokines and chemokines. Microglia-derived inflammatory monokines such as, IL-1β and TNFα, and degenerative factors such as ROS, NO, and glutamate could additionally induce demyelination. Furthermore, microglia-derived degenerative factors, such as ROS, NO, and glutamate, could cause inflammation-induced neurodegeneration. Thus, microglial cells could be considered as conductors that orchestrate a plethora of neuroinflammatory phenomena involved in the pathogenesis of EAE and MS. However, many of the discussed studies were performed in vitro. Further investigation is needed to clarify the direct contribution of microglia versus the other inflammatory cells in this exciting field.

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