


## REVIEW

Cytokines Focus

# Pathogenic T cell cytokines in multiple sclerosis

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**Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system that is believed to have an autoimmune etiology. As MS is the most common nontraumatic disease that causes disability in young adults, extensive research has been devoted to identifying therapeutic targets. In this review, we discuss the current understanding derived from studies of patients with MS and animal models of how specific cytokines produced by autoreactive CD4 T cells contribute to the pathogenesis of MS. Defining the roles of these cytokines will lead to a better understanding of the potential of cytokine-based therapies for patients with MS.**

## Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating, neurodegenerative disease of the central nervous system (CNS) that affects ~2.3 million people worldwide (Filippi et al., 2018). MS most commonly follows a two-stage disease course. The first stage is referred to as relapsing-remitting MS (RRMS) and is marked by alternating episodes of neurological disability and recovery. Hallmark features of this stage are the formation of inflammatory lesions and plaques of demyelination in the CNS. The second stage is referred to as secondary progressive MS and is marked by a decrease in inflammatory lesions detectable by magnetic resonance imaging while neurological decline and brain atrophy steadily progress. MS is believed to be an autoimmune disease initiated by CD4 T helper cells (Th cells) specific for antigens in the myelin sheath. This perspective is supported by the strong association of MS susceptibility with MHC class II alleles (Hafler et al., 2007) and the fact that experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS, is induced by activation of CD4 T cells specific for myelin antigens (Goverman, 2009). The predominance of CD8 T cells within MS lesions (Salou et al., 2015) and the therapeutic efficacy of eliminating B cells in patients with MS (Bar-Or et al., 2008; Hauser et al., 2008) indicate that other lymphocytes play important roles in this disease. However, the ability to initiate EAE by adoptive transfer of myelin-specific CD4 T cells alone into naive animals suggests that CD4 T cells may trigger both the initial inflammatory cascade and potentially subsequent relapses (Goverman, 2009). Because the major function of CD4 T cells is to orchestrate immune responses via production of cytokines and other soluble mediators, there has been intense effort using the EAE model to identify which cytokines produced by CD4 T cells account for their pathogenic activity.

This is a critical area of investigation as these cytokines could be attractive therapeutic targets.

Research directed toward defining pathogenic cytokines in EAE has focused on determining which of the traditionally defined CD4 T cell effector subsets can induce EAE. CD4 T cell effector subsets have been defined by distinct patterns of cytokine production, requirements for specific cytokine growth factors, and expression of master transcription factors. CD4 T cell subsets with the potential to induce EAE include both Th1 and Th17 cells. Th1 cells produce IFN $\gamma$  as their “signature” cytokine. Differentiation of naive CD4 T cells into the Th1 cell subset is promoted by exposure to IL-12 during the initial priming of CD4 T cells, and the transcription factor Tbet is considered a master regulator responsible for regulating expression of genes associated with the Th1 cell lineage. IL-17 is the signature cytokine for Th17 cells and ROR $\gamma$ t is the master transcription factor controlling their differentiation. IL-6 and TGF $\beta$  promote differentiation of naive CD4 T cells into Th17 cells; however, IL-23 has been identified as an important cytokine that stabilizes the encephalitogenic potential of Th17 cells (Langrish et al., 2005). Despite our increased understanding of the factors that control CD4 T cell lineage commitment and the array of cytokines produced by different T effector subsets, a clear picture of a single pathogenic T cell phenotype required to induce CNS autoimmunity has not emerged. One challenge in defining “the” pathogenic T cell phenotype is that CD4 T cells exhibit plasticity in vivo, and T cells can simultaneously express the signature cytokines associated with different effector subsets. Furthermore, the T cell effector subsets produce multiple cytokines in addition to their signature cytokines, and there is overlap between the T cell subsets in expression of these cytokines. In this review, we will discuss our current understanding of how the

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major cytokines produced by encephalitogenic CD4 T cells contribute to the pathogenesis and regulation of EAE, and how well these insights parallel what is observed in patients with RRMS.

## Pathogenic cytokines in EAE

### IFN $\gamma$

Based on the hypothesis that MS is caused by either a virus or by immunoregulatory defects, clinical trials were initiated in the 1980s in which IFN $\alpha$ , IFN $\beta$ , and IFN $\gamma$  were administered to patients. While IFN $\alpha$  and IFN $\beta$  reduced the frequency and severity of exacerbations in RRMS, a pilot study administering IFN $\gamma$  resulted in an increased exacerbation rate, suggesting that IFN $\gamma$  exerts pathogenic activity in MS (Panitch et al., 1987). Subsequent studies in animal models were consistent with a pathogenic role as encephalitogenic T cells were observed to produce IFN $\gamma$  (Baron et al., 1993), and direct injection of IFN $\gamma$  into the CNS resulted in inflammatory pathology resembling EAE (Simmons and Willenborg, 1990; Vass and Lassmann, 1990; Sethna and Lampson, 1991). Furthermore, treatment of myelin-specific CD4 T cells in vitro with IL-12, which is critical for Th1 cell polarization, enhanced their encephalitogenicity (Leonard et al., 1995; Segal and Shevach, 1996). Mice deficient in IL-12p40, a component of IL-12, were also completely resistant to EAE (Segal et al., 1998). However, many studies point to a more complex role for IFN $\gamma$ , reflecting its pleiotropic activities and ability to act on multiple cell types. Protective effects in EAE are suggested by the exacerbating effect of either administering IFN $\gamma$ -blocking antibodies (Billiau et al., 1988) or introducing genetic deficiency in IFN $\gamma$  or the IFN $\gamma$  receptor (IFN $\gamma$ R; Ferber et al., 1996; Krakowski and Owens, 1996; Willenborg et al., 1996; Tran et al., 2000). IFN $\gamma$  also exerts different outcomes at different stages of disease. Administering IFN $\gamma$  during disease induction exacerbates EAE, while administration at disease onset reduces severity (Naves et al., 2013). IFN $\gamma$  may exacerbate EAE during disease induction via its ability to disrupt tight junctions and induce adhesion molecule expression on endothelial cells of the blood-brain barrier (BBB), allowing transendothelial migration of CD4 T cells into the parenchyma (Sonar et al., 2017). IFN $\gamma$  also triggers astrocyte chemokine production (Ding et al., 2015), enhancing the recruitment and activation of myeloid cells, and increases MHC class I and II expression on CNS resident and infiltrating cells (Miller et al., 2015; Ottum et al., 2015). The ameliorating effect of IFN $\gamma$  following disease onset may reflect the ability of IFN $\gamma$  to inhibit microglia proliferation (Ding et al., 2015) and promote phagocytosis of myelin debris, which would prevent accumulation of neurotoxic lipid peroxidation products (Sosa et al., 2015).

IFN $\gamma$  also appears to influence the localization of lesions within the CNS. In most EAE models, the clinical signs correspond to ascending flaccid paralysis associated with inflammation localized in the spinal cord, referred to as “classic EAE.” However, mice deficient in IFN $\gamma$  or IFN $\gamma$ R exhibit additional symptoms associated with brain inflammation, including body lean and axial rotation, referred to as “atypical EAE” (Abromson-Leeman et al., 2004; Wensky et al., 2005; Lees et al., 2008; Simmons et al., 2014; Stoolman et al., 2014), indicating that IFN $\gamma$  inhibits brain inflammation. IFN $\gamma$ - or IFN $\gamma$ R-deficient mice exhibit inflammatory infiltrates within the brain characterized by a high number of

neutrophils and an increase in neutrophil-attracting chemokines, such as CXCL2 (Lees et al., 2008; Simmons et al., 2014; Stoolman et al., 2014). Neutrophils are essential for brain inflammation in these models as neutrophil depletion or inhibition of neutrophil migration to the CNS via administration of either antibodies against CXCR2 or inhibitors of CXCR2 activity reduced the incidence of brain inflammation (Simmons et al., 2014; Stoolman et al., 2014). Some of these studies showed that IFN $\gamma$  signaling enhances spinal cord inflammation in the same mice in which it is inhibiting brain inflammation, suggesting that IFN $\gamma$  may have opposite effects in the brain and spinal cord (Lees et al., 2008; Simmons et al., 2014). In these mice, IFN $\gamma$  promoted CCL2-mediated infiltration of monocytes and macrophages into the spinal cord (Stoolman et al., 2014). Collectively, these studies demonstrate that IFN $\gamma$  can play both a protective and a pathogenic role in CNS autoimmunity in part by the differential modulation of chemokine production in the brain versus spinal cord.

### IL-17

Observations that the p40 subunit of IL-12, but not IFN $\gamma$  itself, was essential for EAE shifted attention to the cytokine IL-23, which shares the p40 subunit with IL-12 (Oppmann et al., 2000). Mice deficient in p19, the subunit specific to IL-23, were completely resistant to active EAE (Cua et al., 2003), while mice deficient in p35, the subunit specific to IL-12, were similar to mice lacking IFN $\gamma$  in that they developed more severe EAE (Becher et al., 2002; Gran et al., 2002). These observations have been interpreted to indicate that IL-23 and not IL-12 is essential to generate pathogenic T cells. However, adoptive transfer of Th1 cells that were not exposed to IL-23 during priming or following transfer into recipient mice still induced EAE, indicating that IL-23 is also not essential to generate encephalitogenic T cells (Carbajal et al., 2015; Grifka-Walk et al., 2015). Nevertheless, IL-23 is clearly influential in EAE, as it was shown to promote an encephalitogenic T cell population that expresses a pattern of proinflammatory cytokines distinct from T cells differentiated with IL-12, including IL-17A and IL-17F (Langrish et al., 2005), referred to as Th17 cells. While both Th1 and Th17 cells can induce EAE, they promote different patterns of lesion localization. Notably, while IFN $\gamma$  inhibits brain inflammation, IL-17 promotes inflammation in the brain versus the spinal cord in both C3Heb/FeJ and B6 models of EAE (Stromnes et al., 2008; Kroenke and Segal, 2011; Simmons et al., 2014). Several mechanisms have been proposed to account for how IL-17 contributes to CNS autoimmunity. IL-17 increases BBB permeability in vitro, thus enhancing the transmigration of CD4 T cells and monocytes to the CNS parenchyma (Kebir et al., 2007; Huppert et al., 2010). IL-17 also induces chemokine production by CNS resident cells (Kang et al., 2010; Simmons et al., 2014). In C3Heb/FeJ mice, IL-17-stimulated production of CXCL2 by brain astrocytes promoted neutrophil recruitment, which was essential for development of atypical but not classic EAE (Simmons et al., 2014). Additionally, adoptive transfer of Th17 cells induces tertiary lymphoid tissue-like structures within the meninges, and IL-17 directly contributes to the remodeling of meningeal fibroblasts and formation of fibrous networks in vitro and in vivo (Peters et al., 2011; Pikor et al., 2015).

While IL-17 deficiency clearly affects the manifestation of EAE, multiple studies have shown that, like IFN $\gamma$ , IL-17 is dispensable for EAE. EAE was only partially ameliorated by administering IL-17A-blocking antibodies (Hofstetter et al., 2005; Langrish et al., 2005; Park et al., 2005; Chen et al., 2006), and EAE could be actively induced in IL-17A<sup>-/-</sup> mice and by adoptive transfer of IL-17A<sup>-/-</sup> CD4 T cells (Komiyama et al., 2006; Haak et al., 2009). EAE could also be induced in IL-17F-deficient mice that received IL-17A-neutralizing antibodies, although the disease course was relatively mild (Haak et al., 2009). Consistent with the notion that IL-17 is not required for encephalitogenicity, Th17 cells differentiated in the presence of TGF $\beta$  and IL-6 but not IL-23 are not pathogenic, even though they exhibit a high expression of IL-17 (McGeachy et al., 2007). These data suggest that IL-23 confers encephalitogenic potential that is distinct from its ability to promote IL-17 production.

Collectively, these studies demonstrate that IFN $\gamma$  and IL-17 both contribute to EAE pathogenesis. Highly polarized Th1 and Th17 cells that had been exposed to IL-12 or IL-23, respectively, only in vitro can independently induce EAE (Carbajal et al., 2015; Grifka-Walk et al., 2015), and the pathogenic mechanisms are different for Th1- versus Th17-initiated EAE (Kroenke et al., 2008). However, as neither cytokine is required for EAE, and 22% of C3Heb/Fej mice deficient in both IL-17R and IFN $\gamma$ R still exhibited EAE (Simmons et al., 2014), other cytokines have been evaluated for their role in CNS autoimmunity.

#### GM-CSF

Multiple strains of mice deficient in GM-CSF or its receptor demonstrate strong resistance to EAE induction (McQualter et al., 2001; Duncker et al., 2018); therefore, this cytokine has been considered essential in EAE. It remains controversial whether there is a distinct lineage of GM-CSF-producing T cells (Sheng et al., 2014; Komuczki et al., 2019); however, both Th1 and Th17 cells produce GM-CSF (Codarri et al., 2011; El-Behi et al., 2011). Adoptive transfer of Th1- and Th17-polarized cells deficient in GM-CSF strongly reduced the manifestation of EAE (Ponomarev et al., 2007; El-Behi et al., 2011; Duncker et al., 2018) and in some cases completely prevented disease induction (Codarri et al., 2011), demonstrating that GM-CSF contributes to the pathogenicity of both Th1- and Th17-skewed cells. However, the requirement for GM-CSF is not universal across all strains of mice. In SJL/J mice, anti-GM-CSF treatment before disease onset ameliorated clinical signs in Th17- but not Th1-initiated EAE (Kroenke et al., 2008). In C3Heb/Fej mice, GM-CSF synergized with IL-17 to induce atypical EAE but was completely redundant with IL-17 in the induction of classic EAE (Pierson and Goverman, 2017). Collectively, these studies point to an important role of GM-CSF in EAE pathogenesis, especially in promoting brain inflammation, but the relative importance for this cytokine appears model dependent.

Effector T cell priming is compromised in the absence of GM-CSF (McQualter et al., 2001; Sonderegger et al., 2008; King et al., 2009; Pierson and Goverman, 2017; Duncker et al., 2018), which may contribute to the complete resistance of mice deficient in GM-CSF and its receptor in some strains. GM-CSF receptors are not expressed on T cells (Morrissey et al., 1987), suggesting that the effects of GM-CSF on T cell priming is indirect. Specifically,

GM-CSF<sup>-/-</sup> mice lack a subset of CD103<sup>+</sup> dendritic cells (DCs) that are important for Th1 cell differentiation (King et al., 2010), although the importance of GM-CSF in the differentiation of Th1 cells is somewhat controversial (Ponomarev et al., 2007; Sonderegger et al., 2008). In addition, DCs isolated from GM-CSF<sup>-/-</sup> mice produce less IL-6 and IL-23, which are required for the generation and maintenance of Th17 cells (Sonderegger et al., 2008). GM-CSF also contributes to the effector phase of disease as anti-GM-CSF antibodies administered at disease onset ameliorated disease (Codarri et al., 2011). The GM-CSF receptor was shown to be required on hematopoietic cells in both Th1- and Th17-initiated EAE (Codarri et al., 2011; Duncker et al., 2018). In B10.PL mice, adoptive transfer of CD4 T cells deficient in GM-CSF failed to induce EAE because of impaired GM-CSF-dependent activation of microglia (Ponomarev et al., 2007). However, this finding is somewhat controversial as a study in B6 mice found that GM-CSF signaling was not required on microglia (Croxford et al., 2015). Instead, EAE was dependent on GM-CSF signaling on CCR2<sup>+</sup>Ly6C<sup>hi</sup> monocytes, which induces phagocytic and proinflammatory responses in their progeny (Croxford et al., 2015). Another study in B6 mice found that, in the absence of IFN $\gamma$  and IL-17, GM-CSF was required for disease induction, which was inhibited in the absence of neutrophils (Kroenke et al., 2010). In C3Heb/Fej mice, GM-CSF independently promoted neutrophil recruitment to the brain, which is required to induce atypical EAE in this model (Pierson and Goverman, 2017).

#### TNF $\alpha$

TNF $\alpha$  is also expressed by both Th1 and Th17 cells (Langrish et al., 2005). TNF $\alpha$  overexpression in the CNS resulted in demyelination (Probert et al., 1995; Akassoglou et al., 1998; Dal Canto et al., 1999), and blocking TNF $\alpha$  before EAE onset attenuated disease (Ruddle et al., 1990; Baker et al., 1994; Selmaj et al., 1995). However, it is increasingly understood that TNF $\alpha$  signaling is complex due to the fact that TNF $\alpha$  signals through two distinct receptors, TNFR1 and TNFR2, which trigger pathogenic or protective outcomes, respectively (Probert, 2015). In EAE, mice deficient in TNFR1 are either completely resistant (Suvannavejh et al., 2000; Kassiotis and Kollias, 2001) or develop milder disease (Eugster et al., 1999; Steeland et al., 2017). TNFR1 signaling promotes leukocyte migration from the perivascular space into the parenchyma during EAE by promoting adhesion molecules and chemokine expression by CNS-resident cells, including astrocytes (Gimenez et al., 2004, 2006). Signaling via TNFR1 also triggers oligodendrocyte death (Akassoglou et al., 1998; Hövelmeyer et al., 2005). In contrast, TNFR2-deficient mice exhibit more severe disease compared with WT mice (Eugster et al., 1999; Suvannavejh et al., 2000), demonstrating its importance in neuroprotection. TNFR2 signaling contributes to the proliferation and differentiation of oligodendrocyte progenitor cells and subsequent remyelination in the cuprizone model of demyelination (Arnett et al., 2001). TNFR2 signaling may act directly on oligodendrocytes, which express high levels of TNFR2 (Brambilla et al., 2011), as specific deletion of TNFR2 on oligodendrocytes exacerbated EAE and impaired remyelination (Madsen et al., 2016). TNFR2 is also expressed on a subset of activated regulatory T cells (T reg cells; Chen et al.,



2008), and specific deletion of TNFR2 on T reg cells resulted in loss of suppressive functions and exacerbated EAE (Atrekhany et al., 2018). Together, these data demonstrate that TNF $\alpha$  signaling is complex and exerts opposing effects in CNS autoimmunity.

### Pathogenic cytokines in MS

#### IFN $\gamma$

The demonstration that administration of recombinant IFN $\gamma$  exacerbated MS launched many studies analyzing the expression of IFN $\gamma$  in patients with MS, which collectively have shown that IFN $\gamma$  is increased in the blood, cerebrospinal fluid, and brain lesions of MS patients compared with healthy controls (HCs). Elevated serum levels of IFN $\gamma$  have been reported in MS patients relative to HCs (Beck et al., 1988; Arellano et al., 2017), and a longitudinal study showed that increases in IFN $\gamma$  occurred just before a relapse (Beck et al., 1988). Studies have reported an increased frequency of T cells producing IFN $\gamma$  in response to myelin antigen stimulation of peripheral blood monocytes (PBMCs) from patients with RRMS versus HCs (Arbour et al., 2003; Hedegaard et al., 2008). Increases in myelin-specific IFN $\gamma$  protein-secreting and IFN $\gamma$  mRNA-expressing mononuclear cells have also been detected in the blood (Olsson et al., 1990; Sun et al., 1991; Link et al., 1994; Pelfrey et al., 2000; Wallström et al., 2000; Huber et al., 2014) and cerebrospinal fluid (Olsson et al., 1990; Sun et al., 1991; Wallström et al., 2000) of MS patients relative to HCs. IFN $\gamma$ -expressing cells are also enriched in the cerebrospinal fluid compared with blood within individual MS patients (Olsson et al., 1990; Sun et al., 1991; Wallström et al., 2000). IFN $\gamma$  protein and IFN $\gamma$  mRNA have also been detected in active areas of MS lesions (Woodroffe and Cuzner, 1993; Cannella and Raine, 1995; Mycko et al., 2003). Furthermore, increases in IFN $\gamma$  levels or IFN $\gamma$ <sup>+</sup> myelin-specific T cells derived from blood have been correlated with the clinically active phase of disease compared with remission (Correale et al., 1995; Arbour et al., 2003) and with increasing disability (Link et al., 1994; Arbour et al., 2003; Moldovan et al., 2003).

IFN $\gamma$  may also contribute to MS via its expression in T reg cells. A higher frequency of IFN $\gamma$ -secreting Foxp3<sup>+</sup> T reg cells are observed in untreated MS patients relative to HCs, and these T reg cells exhibit impaired suppressive activity *in vitro* compared with T reg cells that are not IFN $\gamma$ <sup>+</sup> (Dominguez-Villar et al., 2011). The defect in suppressive activity of these Th1-like T reg cells could be partially reversed by IFN $\gamma$ -specific antibodies. Interestingly, IFN $\beta$  treatment of patients with RRMS restored the levels of these Th1-like T reg cells to that seen in HCs, suggesting that modulation of Th1-like T reg cells may be a mechanism underlying the therapeutic benefit of IFN $\beta$  (Dominguez-Villar et al., 2011). In sum, these studies support a pathogenic role for IFN $\gamma$  in MS.

#### IL-17

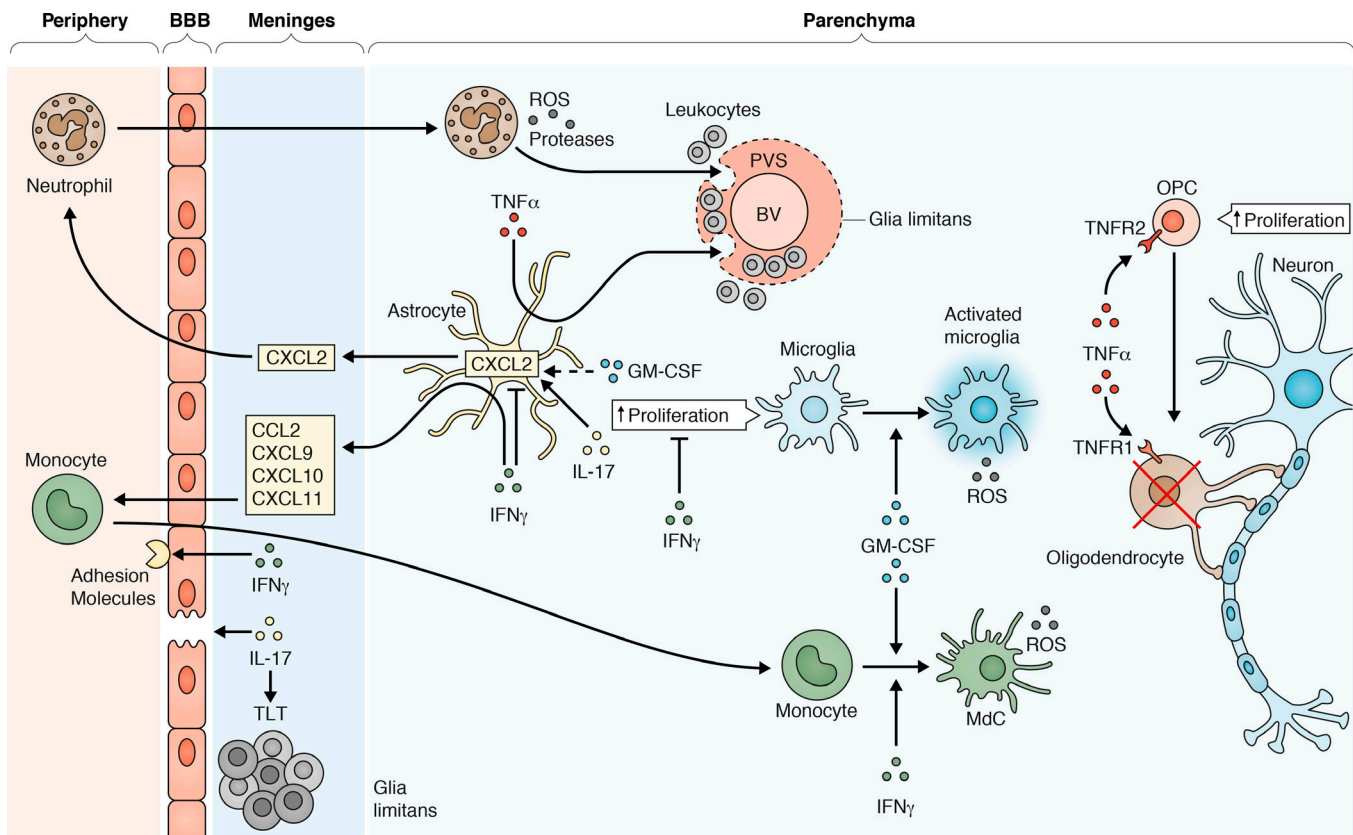
Following the discovery that IL-17 is an influential cytokine in the pathogenesis of EAE, substantial evidence has accumulated implicating a pathogenic role for IL-17 in MS. Expression of IL-17 transcripts and protein was enhanced in MS lesions compared with normal-appearing white matter and brain tissue of those

with nonneurological diseases and HCs (Lock et al., 2002; Tzartos et al., 2008). IL-17<sup>+</sup> T cells primarily localized to active areas of lesions and active borders of chronic/active lesions, and, surprisingly, similar frequencies of IL-17-producing T cells in active lesions expressed CD8 as CD4 (Tzartos et al., 2008). Additionally, as was the case for IFN $\gamma$ , the frequency of PBMCs expressing IL-17 mRNA was reported to be higher in the blood and cerebrospinal fluid of patients with MS relative to HCs, and was enriched in the cerebrospinal fluid compared with the blood within individual patients with MS (Matusevicius et al., 1999). Importantly, the frequency of PBMCs expressing IL-17 transcripts increased during clinical exacerbation compared with remission (Matusevicius et al., 1999), and a higher frequency of Th17 cells was also observed in the cerebrospinal fluid of RRMS patients in relapse compared with remission (Brucklacher-Waldert et al., 2009). The frequency of PBMCs producing IL-17 in response to MBP stimulation also correlated with the number of gadolinium-enhancing lesions in a cohort of patients (Hedegaard et al., 2008). Consistent with this observation, a clinical trial assessing the efficacy of secukinumab, an IL-17A-neutralizing antibody, in patients with RRMS demonstrated a significant reduction in the number of cumulative new gadolinium-enhancing T1 lesions, although the reduction in the cumulative number of combined unique active lesions did not quite achieve significance (Havrdová et al., 2016).

Adding complexity to our understanding of the role of individual cytokines in MS is the fact that human T cells producing IL-17 can also express IFN $\gamma$  and other cytokines. T cells expanded from PBMCs of RRMS patients preferentially adopted an IFN $\gamma$ <sup>+</sup>IL-17<sup>+</sup> phenotype and were readily detected in brain tissue of MS patients, consistent with the observation that these dual cytokine-producing T cells preferentially crossed a human BBB *in vitro* (Kebir et al., 2009). One study of T cell libraries expanded from patients with MS showed that myelin-reactive, CCR6<sup>+</sup> T cells have enhanced production of IFN $\gamma$ , IL-17, and GM-CSF, as well as reduced production of IL-10 when compared with healthy individuals (Cao et al., 2015). A later study supported the importance of the relative expression of IL-10 versus pathogenic cytokines. These investigators identified different transcriptional signatures for human IFN $\gamma$ <sup>+</sup>IL-17<sup>+</sup> versus IFN $\gamma$ <sup>-</sup>IL-17<sup>+</sup> T cells. The IFN $\gamma$ <sup>+</sup>IL-17<sup>+</sup> T cell signature resembled that of pathogenic murine Th17 cells, and, in patients with MS, IFN $\gamma$ <sup>+</sup>IL-17<sup>+</sup> exhibited reduced expression of IL-10 while IFN $\gamma$ <sup>-</sup>IL-17<sup>+</sup> T cells exhibited a higher IL-10 expression in clinically stable versus active patients (Hu et al., 2017). Together, these studies implicate both IL-17 and IFN $\gamma$  as playing a pathogenic role in MS. However, in a phase II clinical trial to test the efficacy of blocking the p40 subunit shared by IL-23 and IL-12, no beneficial effect was observed (Segal et al., 2008). While there are many potential explanations for why this trial failed, this finding encourages further examination of other cytokines that may play a pathogenic role in MS.

#### GM-CSF

The observation that levels of GM-CSF are elevated in the cerebrospinal fluid of relapsing compared with stable MS patients suggested a potential role for GM-CSF in MS pathogenesis before



**Figure 1. Myelin-specific CD4 T cells that cross the BBB and are reactivated within the meninges produce pathogenic cytokines that orchestrate the inflammatory response in EAE.** IL-17 contributes to loss in the integrity of the BBB by breaking down tight junctions between endothelial cells, and acts on astrocytes in the brain to produce CXCL2, a chemokine that recruits neutrophils. Neutrophils produce ROS that can damage axons. They also produce proteases that help break down the glia limitans surrounding blood vessels (BV), allowing leukocytes to escape from the perivascular space (PVS). IL-17 also promotes stromal cell remodeling within the meninges, which results in the formation of tertiary lymphoid tissue (TLT). IFN $\gamma$  promotes recruitment of leukocytes from the peripheral circulation by increasing the expression of adhesion molecules on the endothelial cells of the BBB. IFN $\gamma$  also induces astrocytes to secrete chemokines that attract monocytes, e.g., CCL2, CXCL9, CXCL10, and CXCL11, and facilitates the maturation and activation of monocytes into inflammatory monocyte-derived cells (MdCs) that produce ROS. Additionally, IFN $\gamma$  exerts inhibitory effects on CNS inflammation by decreasing microglial cell proliferation. In the brain, IFN $\gamma$  inhibits expression of CXCL2 in astrocytes, which decreases recruitment of neutrophils. In contrast, IFN $\gamma$  promotes neutrophil recruitment to the spinal cord (not shown). GM-CSF enhances neutrophil recruitment to the brain and spinal cord, promotes microglia activation, and activates monocytes to acquire an inflammatory phenotype. TNF $\alpha$  acts on astrocytes to produce factors that promote migration of leukocytes from the PVS into the parenchyma. TNF $\alpha$  also enhances oligodendrocyte cell death via signaling through TNFR1 but promotes oligodendrocyte progenitor cell (OPC) proliferation via TNFR2 signaling.

studies of this cytokine in EAE (Carriero et al., 1998). Since this early observation, evidence supporting a role for GM-CSF in MS has steadily accumulated. Numerous studies have shown that the number and frequency of GM-CSF-producing T cells in peripheral blood and cerebrospinal fluid is increased in MS patients relative to those with other neurological diseases or HCs (Hartmann et al., 2014; Noster et al., 2014; Cao et al., 2015; Rasouli et al., 2015; van Langelaar et al., 2018; Ghezzi et al., 2019). Additional studies have shown that GM-CSF production by T cells is reduced in patients on immunomodulatory therapies (Hartmann et al., 2014; Rasouli et al., 2015). GM-CSF-producing T cells have also been identified in active MS lesions (Rasouli et al., 2015). Among patients with MS, those with higher frequencies of GM-CSF-producing T cells exhibited increased levels of biomarkers of MS progression in their cerebrospinal fluid and a greater number of T2 lesions (Hartmann et al., 2014). Finally, IL-2 is a strong inducer of

T cell GM-CSF production (Hartmann et al., 2014), and a genetic polymorphism in the IL-2 receptor  $\alpha$ -chain is a known MS risk allele (Hafler et al., 2007; Sawcer et al., 2011; Beecham et al., 2013). Naive T cells from healthy individuals expressing this risk allele exhibited increased IL-2 receptor  $\alpha$ -chain expression on T cells and increased secretion of GM-CSF after IL-2 stimulation compared with T cells with the protective allele. Higher frequencies of GM-CSF-producing memory T cells are also found in PBMCs of healthy individuals with the risk allele compared with those with the protective allele. These findings suggest that some of the risk conferred by this allele may be associated with increased T cell production of GM-CSF. Together, these findings support the notion that GM-CSF produced by T cells exerts pathogenic activity in MS and point to GM-CSF as a potential therapeutic target that is currently being tested in clinical trials (Constantinescu et al., 2015).

## TNF $\alpha$

Early observations indicating that TNF $\alpha$  levels in the blood and cerebrospinal fluid of patients with MS correlated with disease activity (Beck et al., 1988; Hauser et al., 1990; Sharief and Hentges, 1991; Rieckmann et al., 1995) suggested that TNF $\alpha$  antagonists may be an effective therapy in MS. However, infliximab, an anti-TNF $\alpha$  monoclonal antibody, increased gadolinium-enhancing lesions in two patients with MS during a phase I safety trial (van Oosten et al., 1996), and lenercept, a nonselective TNF $\alpha$  inhibitor, exacerbated disease in an MS phase II clinical trial (The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group, 1999). TNF $\alpha$  inhibitors have also induced demyelinating lesions in patients with rheumatoid arthritis (Mohan et al., 2001; Kaltsonoudis et al., 2014) and irritable bowel disease (Colombel et al., 2004; Thomas et al., 2004; Freeman and Flak, 2005; Hare et al., 2014). Additionally, a single-nucleotide polymorphism in the gene that encodes TNFR1 is associated with MS (De Jager et al., 2009; Sawcer et al., 2011) and directs the expression of a soluble form of TNFR1 that blocks TNF $\alpha$  signaling (Gregory et al., 2012), further confirming a protective role of TNF $\alpha$  in MS. However, EAE studies demonstrated that signaling through TNFR1 and TNFR2 have opposing outcomes in CNS autoimmunity, with TNFR1 signaling promoting pathogenic activity and TNFR2 signaling enhancing remyelination via effects on oligodendrocyte precursors. Thus, the adverse effects seen with administration of infliximab or lenercept may be a result of targeting the beneficial as well as detrimental signaling pathways. Specifically inhibiting TNFR1 and/or enhancing TNFR2 signaling rather than broadly targeting TNF $\alpha$  in patients with MS may be a beneficial treatment strategy.

## Concluding remarks

The cytokines discussed above clearly play a key role in CNS autoimmunity. However, MS and EAE studies demonstrate that disease is not governed by any one particular cytokine but instead involves a complex interplay between pro- and anti-inflammatory cytokines (Fig. 1). MS is a heterogeneous disease that involves variations in interactions between genetic and environmental factors in individual patients. Depending on which interactions are at play within an individual, different cytokines may assume a greater or lesser role in the pathogenesis of human disease, and the roles may vary over time. This notion is consistent with EAE studies that have demonstrated that myelin-specific CD4 T cells with diverse cytokine profiles have encephalitogenic potential, suggesting that one cytokine may predominate in some MS patients, or multiple cytokines may contribute in an additive or synergistic manner. Due to this heterogeneity, targeting individual cytokines may have limited efficacy in patients with MS. Instead, targeting multiple cytokines or tailoring therapies to individual patients may be attractive avenues for future MS therapies.

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