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## Mechanisms regulating regional localization of inflammation during CNS autoimmunity

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### Summary

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) characterized by inflammatory, demyelinating lesions localized in the brain and spinal cord. Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS that is induced by activating myelin-specific T cells and exhibits immune cell infiltrates in the CNS similar to those seen in MS. Both MS and EAE exhibit disease heterogeneity, reflecting variations in clinical course and localization of lesions within the CNS. Collectively, the differences seen in MS and EAE suggest that the brain and spinal cord function as unique microenvironments that respond differently to infiltrating immune cells. This review addresses the roles of the cytokines interferon- $\gamma$  and interleukin-17 in determining the localization of inflammation to the brain or spinal cord in EAE.

### Keywords

EAE/multiple sclerosis; autoimmunity; Th17/Th1; cell trafficking; neuroimmunology

### Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by focal inflammatory infiltrates, demyelinating plaques, and axonal damage. The location of these inflammatory plaques within the brain and spinal cord determines the clinical symptoms, which can include sensory or motor impairment, ataxia, spasticity, fatigue, and cognitive impairment (1). MS is a very heterogeneous disease with respect to disease course and pathological features. The majority of MS patients manifest a relapsing-remitting form of MS with full recovery of function between relapses. Relapsing-remitting MS can persist for many years; however, most patients eventually convert to the secondary progressive stage of disease in which the extent of recovery after each episode of neurological deficit diminishes. In contrast to relapsing-remitting MS, 10–20% of MS patients exhibit primary progressive MS in which there is less recovery after each relapse (2).

The composition of lesions within the CNS of MS patients is also extremely variable. Early active plaques in MS patients exhibit extensive immune cell infiltration and profound heterogeneity. A systematic neuropathological study of a large set of tissue sections categorized these lesions into four patterns (3). The different lesion types can be delineated on the basis of specific myelin protein loss, presence or absence of infiltrating T cells and macrophages, the accumulation of immunoglobulin and complement (signaling a role of pathogenic autoantibodies), and the presence or absence of severe oligodendrocyte

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degeneration. Importantly, multiple lesions within individual MS patients all exhibit the same pattern. This suggests that distinct immune-mediated pathways are responsible for generating the different patterns of inflammatory demyelinating lesions, and only one pathway operates in an individual (4). The existence of multiple pathogenic pathways suggests that individual patients may need distinct treatment strategies that target the specific pathway underlying their disease. For example, plasmapheresis is most effective in patients with antibody and complement-associated tissue destruction (5).

The locations that are targeted by lesions within the CNS are the major determinant of clinical signs, and these are also variable among patients. The majority of lesions are found in the brain, particularly in the periventricular white matter, cerebellum, brainstem, and optic nerves. Many patients exhibit lesions in the spinal cord as well as the brain, while 2–10% of patients exhibit inflammation in the spinal cord and optic nerves without extensive involvement of the brain (referred to as opticospinal MS) (6, 7). These different patterns suggest that the different regions in the CNS function as distinct microenvironments whose response to inflammatory cells differs, i.e. certain types of inflammatory cells might be more effective in inducing inflammation in the optic nerve and spinal cord compared to the brain and *vice versa*. Thus, it is important to understand the molecular mechanisms that determine where inflammatory cells localize within the CNS to predict whether specific therapies will be effective in the microenvironment that is targeted in an individual patient. This review focuses on our current understanding of the mechanisms that regulate the localization of inflammation to either the brain or spinal cord that have been defined using animal models of MS. In particular, we focus on the potential roles of interleukin-17 (IL-17) and interferon- $\gamma$  (IFN- $\gamma$ ) in the regulation of CNS inflammation.

## Experimental autoimmune encephalomyelitis

### The pathogenesis of EAE

The pathogenic mechanisms that lead to the development of MS have been widely studied using the animal model experimental autoimmune encephalomyelitis (EAE). EAE is induced by stimulating CD4<sup>+</sup> T-cell-mediated immunity to myelin proteins, either by immunization with myelin antigens in complete Freund's adjuvant or by adoptive transfer of myelin-specific T cells (8, 9). Self-reactive CD4<sup>+</sup> T-cell responses toward myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) have been most extensively studied. EAE has been very useful for investigating the immunological mechanisms that contribute to CNS autoimmune disease. The synthesis of many studies indicates that the following events comprise the pathogenic pathway that leads to EAE and presumably MS (10). CD4<sup>+</sup> T cells are activated in the periphery and express cell-surface activation markers that facilitate their extravasation across the blood brain barrier. Upon entering the CNS, the CD4<sup>+</sup> T cells are re-activated by myelin epitopes presented by dendritic cells (DCs), which are required but may (11) or may not (12) be sufficient for T-cell re-activation. Activation of macrophages and microglia as well as myelin damage occurs during this initial inflammatory response. The number of major histocompatibility complex (MHC) class II<sup>+</sup> antigen-presenting cells (APCs) capable of presenting antigen to CD4<sup>+</sup> T cells then increases dramatically within the CNS, largely due to recruitment of circulating monocytes, which differentiate into inflammatory DCs and macrophages upon entering the tissue (13). The blood brain barrier becomes increasingly permeable, allowing naive T cells to enter the CNS, some of which are specific for myelin epitopes distinct from that of the initial infiltrating T-cell population. Recognition of myelin antigen by these naive T cells leads to a phenomenon known as determinant-spreading in which chronic inflammation is propagated by continuous generation of APCs and diversification of the myelin-specific T-cell response (14, 15).

## Heterogeneity in EAE models

There are many similarities between the pathology of lesions seen in EAE and MS; however, an important difference between most EAE models and MS is the clinical manifestation of disease. Instead of the heterogeneous clinical presentation seen in MS, the clinical signs in rodents with EAE are typically manifested as ascending flaccid paralysis. This clinical presentation is referred to as 'classic' EAE and reflects the fact that inflammatory cells predominantly infiltrate the spinal cord with a relative lack of inflammation in the brain in these models. This dominance of spinal cord inflammation in classic EAE has limited investigation of the mechanisms that localize inflammatory cells to the brain. However, EAE has now been studied in many different strains of mice or rats using different myelin antigens to induce disease, and some of the clinical heterogeneity seen in MS patients has emerged in these animal models (16).

No single model replicates the full spectrum of inflammatory mechanisms and neurodegeneration seen in MS, just as individual patients manifest only a subset of the diverse features of the disease. Therefore, EAE models that exhibit distinct pathology and clinical signs can be valuable tools to investigate different aspects of the disease. There is variation even in classic EAE models as the clinical symptoms follow a monophasic or chronic course, or, in the case of SJL/J mice, exhibit a relapsing-remitting disease (17, 18). NOD and Biozzi ABH mice may recapitulate some of the mechanisms involved in secondary progressive MS, as these mice develop EAE with a relapsing remitting course followed by a chronic progressive course (19, 20). The generation of mice expressing transgenic T-cell receptors (TCRs) specific for myelin antigens has produced a new toolset to study CNS autoimmunity as EAE occurs spontaneously in many of these models (21). In some models, the incidence of spontaneous EAE occurs with varying frequency that is influenced by the microbial exposure in the environment in which the mice are housed (22). Recently, the presence of commensal bacteria has been suggested to influence the development of spontaneous EAE in MOG-specific TCR transgenic mice (23). The frequency of spontaneous EAE is significantly increased in many TCR transgenic models when regulatory T cells are eliminated from the TCR transgenic mice by crossing them to the RAG<sup>-/-</sup> background (24, 25). While the majority of spontaneous EAE seen in TCR transgenic mice develop monophasic or chronic classic EAE, a TCR transgenic model on the SJL/J background develops spontaneous relapsing remitting EAE; these mice also exhibit variable clinical EAE symptoms in each relapse (26).

In addition to the heterogeneity seen in the disease course of EAE among the various models, there is also heterogeneity in the localization of lesions within the CNS. While the majority of mouse models have parenchymal inflammation present only in the spinal cord, a growing number of models exhibit parenchymal inflammation in different parts of the brain, particularly in the brainstem and cerebellum, as discussed below. Interestingly, a combination of two genetically engineered EAE models has led to the development of a model for optospinal MS. T cells expressing a T-cell receptor (TCR) specific for MOG exhibit a very low incidence of spontaneous classic EAE with inflammation localized in the spinal cord (25). However, when these mice are crossed to mice carrying a knockin of the heavy chain gene derived from an antibody specific for MOG, the incidence of spontaneous EAE increased and inflammation localized to the spinal cord and optic nerve (27, 28). This double transgenic model demonstrated that increasing the precursor frequency of MOG-specific T and B cells simultaneously influenced the distribution of lesions within the CNS by a mechanism that has not yet been defined but is believed to be related to the antigen presentation function of B cells. In addition to their role in myelin-specific autoantibody production, B cells are believed to play both pathogenic and regulatory roles in the activation and expansion of myelin-specific T cells (23).

## CD4<sup>+</sup> T-cell subsets and cytokines in EAE and MS

While both CD4<sup>+</sup> and CD8<sup>+</sup> T cells are known to contribute to the pathogenesis of EAE, the roles of CD4<sup>+</sup> T cells have been studied more extensively and are discussed here. The pathogenic T cells mediating inflammation in EAE were originally thought to be Th1 cells, whose signature cytokine is IFN- $\gamma$ . T-cell clones that produce IFN- $\gamma$ , and Th1 cells generated *in vitro* are able to induce EAE upon adoptive transfer (29, 30). Additionally, IFN- $\gamma$  is secreted by CNS-infiltrating T cells in EAE (31, 32). Furthermore, a deficiency in T-bet, a transcription factor that is essential for expression of IFN- $\gamma$ , confers resistance to EAE (33). Based on observations from EAE, it was suggested that MS is also mediated by Th1 cells, and this notion was supported by detection of IL-12 (a growth factor for Th1 cells) and IFN- $\gamma$  in MS lesions and cerebrospinal fluid (34, 35). There are many activities of IFN- $\gamma$  that could be pathogenic in the CNS. IFN- $\gamma$  induces MHC class II expression and activates CNS resident microglia and macrophages. IFN- $\gamma$  also induces production of certain chemokines important for inflammatory cell recruitment (36). However, subsequent observations emerged that undermined the paradigm that IFN- $\gamma$  is the major pathogenic cytokine in EAE, particularly the observations that IL-12<sup>-/-</sup> (37, 38) and IFN- $\gamma$ <sup>-/-</sup> mice (39) remain very susceptible to development of EAE.

The discovery that IL-23<sup>-/-</sup> mice are very resistant to EAE led to the proposal that T cells that produce IL-17 (Th17 cells) may represent the pathogenic T-cell subset in EAE, as IL-23 is a Th17-promoting cytokine (40). Subsequent studies lent considerable support to this idea (41). IL-17-producing cells have also been detected in both EAE and MS lesions (42), and various studies have shown a reduced incidence, severity, and delayed onset of EAE in the absence of IL-17A or its receptors (43–46). However, not all studies are consistent with an essential role for IL-17 in EAE. No major decrease in EAE susceptibility was observed when the activities of both IL-17A and IL-17F were neutralized (47). Additionally, there has been some controversy over the pathogenicity of Th17 cells, with one study reporting that highly purified Th17 cells did not transfer disease (48). It is now clear that studies showing the importance of Th1 or Th17 cells based solely on detection of the respective cytokines in the tissue are complicated by the fact that IL-17 producing cells readily convert to IFN- $\gamma$ -producing cells (49).

Although neither IL-17 nor IFN- $\gamma$  are required for the induction of EAE, another T-cell cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), appears to play a critical role in EAE development. GM-CSF<sup>-/-</sup> mice are resistant to EAE (50), and GM-CSF production by T cells is required for their pathogenicity (51). Recently, two reports strengthened the evidence for the critical role of GM-CSF in EAE by demonstrating that GM-CSF production by both Th1 and Th17 cells was required for EAE induction (52, 53). GM-CSF<sup>-/-</sup> T cells were able to infiltrate the CNS initially, but these cells did not accumulate to levels seen in wildtype mice. T-cell production of GM-CSF seems to be influenced by multiple cytokines. IL-23 and IL-1 $\beta$  upregulated T-cell production of GM-CSF, while IL-12, IL-27, and IFN- $\gamma$  inhibited GM-CSF production. Although ROR $\gamma$ t was implicated as an important transcription factor for GM-CSF expression, the presence of ROR $\gamma$ t was not essential for GM-CSF production under certain conditions (particularly *in vitro*) (52, 53). GM-CSF could promote inflammation in the CNS in many ways. GM-CSF mobilizes Ly6C<sup>hi</sup> monocytes from the bone marrow into the bloodstream, facilitating their infiltration into the CNS during EAE where they differentiate into inflammatory DCs and macrophage (13). GM-CSF is also required during EAE for generation of migratory CD103<sup>+</sup> DCs in lymph nodes that strongly promote the differentiation of CD4<sup>+</sup> T cells into effector T-cell subsets (54). GM-CSF may also upregulate MHC class II and pro-inflammatory cytokine expression in microglia, macrophages, and dendritic cells (52, 53, 55). The studies described above utilized the classic model of EAE and, with the exception of the IFN- $\gamma$ <sup>-/-</sup>

mice (see below), did not address the role of these cytokines in influencing distribution of lesions within the CNS.

## The brain and spinal cord are distinct microenvironments

While most models of EAE report a classic phenotype characterized by ascending flaccid paralysis, reports began to emerge in the 1980s and 1990s of EAE models in which mice showed signs of what was referred to as ‘vestibular-type’ or ‘non-classical’ EAE (56, 57). These non-classical EAE mice were characterized by atypical symptoms of head tilt, spinning, or axial rotation. The significance of these atypical symptoms was unclear until 2000, when Muller *et al.* (57) reported that atypical signs correlate with inflammation in the brain (particularly the brainstem and cerebellum), while classical symptoms correlated with spinal cord inflammation. Atypical EAE (and parenchymal brain inflammation) occurred only in EAE models that utilized specific mouse strains and antigen combinations. These studies suggested that conditions leading to inflammation in the spinal cord were not sufficient to promote inflammation in the brain and were the first indication in animal models of CNS autoimmunity that the brain and spinal cord are distinct microenvironments with different requirements for inflammation. Apart from the influence of antigen/strain combination, specific factors leading to inflammation in one area versus the other were unknown until 2005, when a critical role for IFN- $\gamma$  on determining susceptibility of the brain to inflammation was discovered.

## IFN- $\gamma$ suppresses inflammation in the brain, but not in the spinal cord

As discussed earlier, mice lacking IFN- $\gamma$  or components of the IFN- $\gamma$  signaling pathway were found to be highly susceptible to EAE. Importantly, the inflammatory pattern in the CNS in mice deficient in IFN- $\gamma$  signaling differed from that seen in wildtype mice. The ability of IFN- $\gamma$  signaling to determine lesion localization independent of antigen specificity was clearly illustrated in a TCR transgenic model in which a monoclonal myelin-specific TCR was expressed on a wildtype or IFN- $\gamma^{-/-}$  background (58). Rag $^{-/-}$ -MBP TCR transgenic mice developed spontaneous EAE typified by spinal cord inflammation and classic symptoms of ascending flaccid paralysis. However, when these mice were backcrossed to an IFN- $\gamma^{-/-}$  background, they developed a non-classical EAE characterized by brain inflammation and atypical symptoms. This suggested that IFN- $\gamma$  suppressed inflammation in the brain but not in the spinal cord. In fact, spinal cord inflammation appeared less severe in the Rag $^{-/-}$ -MBP TCR transgenic mice on the IFN- $\gamma^{-/-}$  background, as lower numbers of CD4 $^{+}$  T cells and total cellularity were observed in spinal cords of mice lacking IFN- $\gamma$  compared to wildtype mice (58).

Subsequent studies confirmed the suppressive effect of IFN- $\gamma$  on brain inflammation. Two groups have demonstrated that MOG-specific polyclonal T cells isolated from IFN- $\gamma^{-/-}$  mice induce an atypical disease when transferred into wildtype hosts (59, 60). The study by Lees *et al.* (59) showed that MOG-specific, but not OVA-specific, wildtype Th1-skewed cells were able to suppress the brain inflammation induced by the IFN- $\gamma^{-/-}$ -T cells. This group also showed that the suppressive ability of IFN- $\gamma$  in the brain is dependent on host cells receiving a signal from IFN- $\gamma$ , as wildtype T cells transferred into IFN- $\gamma R^{-/-}$  hosts also induced brain inflammation and atypical EAE. Their results were also consistent with the finding of Wensky *et al.* (58) that IFN- $\gamma$  signaling has a non-redundant, pro-inflammatory effect in the spinal cord, as IFN- $\gamma^{-/-}$ -T cells induced less disease in the spinal cords of wildtype mice compared to wildtype T cells (59). The mechanisms by which IFN- $\gamma$  exerts differential effects in the brain and spinal cord are not known.



### Th17:Th1 ratio affects lesion localization

Our laboratory recently developed a model of EAE that exhibits both brain and spinal cord inflammation that does not involve ablation of IFN- $\gamma$  signaling. We found that immunization of C3HeB/Fej mice with recombinant rodent MOG induces a high frequency of atypical EAE characterized by proprioception defects, rolling, and ataxia, as well as limp tail in some mice (61). Consistent with these clinical signs, extensive parenchymal brain inflammation is observed in addition to spinal cord inflammation. In contrast, only classic EAE is seen when the MHC congenic strain C3H.SW is immunized with MOG, suggesting that the T cells primed in C3HeB/Fej mice are responsible for inducing the distinct inflammatory pattern. CD8<sup>+</sup> T cells and B cells did not contribute to the enhanced ability to induce brain inflammation; therefore, we focused on defining what properties of CD4<sup>+</sup> T cells were responsible for directing preferential localization of inflammatory cells in the brain. The CD4<sup>+</sup> T cells in C3HeB/Fej mice recognize two different epitopes of MOG, MOG<sub>79-90</sub> (I-E<sup>k</sup> restricted) and MOG<sub>97-114</sub> (I-A<sup>k</sup> restricted). Adoptive transfer of each population separately demonstrated that MOG<sub>79-90</sub>-specific T cells induced inflammation localized predominantly in the spinal cord, while MOG<sub>97-114</sub>-specific T cells preferentially induced inflammation in the brain (61). Interestingly, we found that the polyclonal population of T cells primed by immunization with MOG that were specific for MOG<sub>97-114</sub> exhibited a significantly higher Th17:Th1 ratio than T cells that responded to MOG<sub>79-90</sub>. The difference in Th17:Th1 ratio was observed both in the T cells that infiltrated the CNS during EAE and in the periphery before disease induction, suggesting that this might be an intrinsic property of these epitope-specific T cells.

To test the idea that the Th17:Th1 ratio of CNS infiltrating T cells influenced where inflammation localizes in the CNS, we altered the Th17:Th1 ratio prior to adoptive transfer by incubating the T cells in either IL-23 or IL-12. Incubating T cells of either specificity with IL-23 triggered inflammation in the brain and spinal cord, while incubation with IL-12 directed inflammation toward the spinal cord rather than the brain (61). Interestingly, the Th17:Th1 ratio rather than the absolute number of Th17 or Th1 antigen-specific T cells present in either CNS microenvironment appeared to be the major determinant of whether inflammation occurred in the brain or was restricted to the spinal cord. Thus, brain inflammation would not occur even if there were high numbers of Th17 cells in the brain as long as Th1 cells predominated in the infiltrating population. It is not known why polyclonal MOG<sub>97-114</sub>-specific T cells exhibit a higher Th17:Th1 ratio than MOG<sub>79-90</sub>-specific T cells. MOG<sub>97-114</sub>-specific T cells also exhibited a higher functional avidity for their cognate antigen compared to MOG<sub>79-90</sub>-specific T cells based on cytokine response of primed T cells, and this may be one factor in determining the Th17:Th1 ratio.

We analyzed where transferred T cells localized within the brain when a low Th17:Th1 ratio prevented induction of inflammation. Immunohistochemical analyses showed that transferred T cells were confined primarily to the meninges in the brain of mice with classic EAE, while parenchymal infiltration was observed in the spinal cord. When the Th17:Th1 ratio was >1, the T cells invaded the parenchyma in both the brain and spinal cord. We hypothesized that the brain might be more susceptible to an inhibitory effect of IFN- $\gamma$  signaling than the spinal cord. Consistent with this idea, real time polymerase chain reaction (PCR) data showed that the expression of IFN- $\gamma$ Rb is fivefold higher in the brain than in the spinal cord. Together these data suggest that the Th17 cells, and possibly IL-17 itself, may overcome an inhibitory signal mediated by IFN- $\gamma$  that dampens inflammatory responses in the brain.

This work helped illuminate the roles that IFN- $\gamma$  and IL-17 play in the pathogenesis of EAE, demonstrating that their effects vary, depending on the local environment in which they are secreted. The evidence that parenchymal inflammation in the brain but not the spinal cord is dependent on the presence of a high Th17:Th1 ratio supports the emerging concept that

inflammation is regulated differently in the brain and spinal cord. The molecular mechanisms that define these differences in the CNS microenvironments are not understood. Our work provides additional evidence that IFN- $\gamma$  inhibits inflammation in the brain but not the spinal cord, and we have also demonstrated a role for IL-17 in this process. We hypothesize that IL-17, perhaps in combination with GM-CSF, promotes brain inflammation, counteracting the suppressive effects of IFN- $\gamma$ .

### IL-17 signaling promotes inflammation localized to the brain

While IL-17 is not essential for inflammation in the spinal cord, several observations indicate that IL-17 is more important for inflammation in the brain. In the study described above, atypical disease induced by the transfer of Th17-skewed cells was converted to classical disease upon administration of a soluble IL-17 receptor-fusion construct that blocks IL-17 signaling (61). In support of the notion that IL-17 exerts a pro-inflammatory influence in the brain, Domingues *et al.* (62) reported that mice develop atypical EAE symptoms after the transfer of Th17 cells but develop classic symptoms after transfer of Th1 cells. Further evidence of the specific role of IL-17 came from a study that showed that IFN- $\gamma^{-/-}$  T cells, which induce atypical disease when transferred into wildtype hosts, instead induced classic disease when transferred into IL-17RA $^{-/-}$  mice (60). Collectively, these studies support the idea that IL-17 signaling promotes inflammation in the brain but is not required for inflammation in the spinal cord. There are many known inflammatory functions of IL-17, and it is not yet clear which of these functions are important for promoting inflammation in the brain or why these effects appear to differ in the brain versus the spinal cord.

### Potential signaling mechanisms affecting regional localization

Suppressive mechanisms of IFN- $\gamma$  in different inflammatory settings Pro-inflammatory activities of IFN- $\gamma$  have been described in multiple settings (36); however, there is also evidence that IFN- $\gamma$  can suppress inflammation. T cells that were differentiated in the presence of IFN- $\gamma$  and IL-12 produced less GM-CSF, a critical cytokine in the induction of EAE (53). IFN- $\gamma$  also enhances production of IL-27 by DCs (63). IL-27 is a potent anti-inflammatory cytokine that inhibits Th1 and Th17 cell activity, and induces IL-10-producing regulatory Tr1 cells (64–66). IFN- $\gamma$  also inhibits the production of the pro-inflammatory cytokine osteopontin (OPN) (63). OPN expressed by epithelial and immune cells acts as a chemoattractant to macrophages and neutrophils and regulates their phagocytic activity and functions (67). OPN $^{-/-}$  mice have reduced severity of EAE and administration of OPN-blocking antibody suppresses EAE (68–70). OPN appears to promote survival of activated T cells in the CNS via altering the activity of different transcription factors and altering expression of pro-apoptotic proteins (71). Thus, IFN- $\gamma$  could mediate its suppressive effects on EAE in part via suppression of OPN expression.

IFN- $\gamma$  has also been shown *in vitro* to inhibit effects of IL-1 $\beta$  signaling via downregulation of IL-1RI. As a result, IFN- $\gamma$  can suppress IL-1 $\beta$ -mediated induction of certain matrix metalloproteinases such as MMP3 and MMP9, and inhibit IL-1 $\beta$ -mediated production of TNF- $\alpha$ , MIP-1, IL-6, IL-8, and COX-2 by macrophages (72). Finally, IFN- $\gamma$  induces production of nitric oxide (NO) and indolamine 2,3-dioxygenase/indolamine (IDO) by activated macrophages and microglia. NO and IDO inhibit T-cell proliferation and promote T-cell apoptosis, and both NO and IDO downregulate inflammation in EAE (73, 74). In sum, IFN- $\gamma$  exerts many suppressive functions and it remains to be determined whether any of these are of particular relevance in suppressing inflammation in the brain compared to the spinal cord.

## Potential inflammatory mechanisms of IL-17

Although IL-17 does not appear to be required for induction of classic EAE (47), it has been shown to contribute to pathogenicity in various EAE models by promoting brain inflammation. It is not known what function(s) IL-17 signaling mediates that exacerbate brain inflammation. In infection models, IL-17 induces production of ELR<sup>+</sup> chemokines that are important for neutrophil recruitment, including CXCL1 and CXCL2 (75, 76). Accordingly, the infiltrate in mice with EAE induced by adoptive transfer of Th17 cells is more neutrophilic, while EAE induced by transfer of Th1-skewed cells is characterized by a more mononuclear cell infiltrate (77). Th17-induced disease can be ameliorated by depleting neutrophils with a Ly6G-specific antibody or by blocking the function of ELR<sup>+</sup> chemokines CXCL1 and CXCL2 with an anti-CXCR2 antibody, suggesting an important role for IL-17-induced production of these chemokines during EAE (78). Human endothelial cells express IL-17 receptor, and IL-17 disrupts tight junctions in the blood brain barrier to facilitate migration of CD4<sup>+</sup> T cells across the blood brain barrier in humans and mice (42, 79). *In vitro*, human Th17 cells exhibited toxic activity to neurons, which may result from the expression of granzyme B by a fraction of the cells (42). Th17 cells also appear to promote ectopic lymphoid follicle formation in the CNS during EAE, which may be critical for sustaining chronic inflammation (80). IL-17RA<sup>-/-</sup> mice had reduced numbers of ectopic lymphoid follicles throughout the CNS, indicating that ectopic lymphoid follicle formation is partly dependent on IL-17 signaling. Thus, there are multiple mechanisms by which IL-17 could promote and sustain inflammation in the CNS; however, it is not yet clear which IL-17-induced activity is important for overcoming the inhibitory signals mediated by IFN- $\gamma$  in the brain.

## Intersecting pathways of IL-17 and IFN- $\gamma$

The complex interactions between pathogenic IL-17 signaling and suppressive IFN- $\gamma$  signaling in the CNS are not well understood. These pathways may intersect at various points in EAE development and could act on the pathogenic T cells, other infiltrating cells, or on the CNS-resident cells. *In vitro*, IFN- $\gamma$  inhibits T-cell secretion of IL-17 (81), and IL-17 inhibits expression of a variety of Th1-associated factors including T-bet, IFN- $\gamma$ , and IL-12Rb2 (82). In EAE models, IFN- $\gamma$  also inhibits IL-17 production, demonstrated by increased percentage and number of IL-17<sup>+</sup> cells in IFN- $\gamma$ <sup>-/-</sup> mice (44, 59). In addition to suppressing IL-17 production, IFN- $\gamma$  suppresses factors known to be induced by IL-17, such as CXCL2 and MMP9 (83–85).

IL-17 and IFN- $\gamma$  also exert antagonistic activity on CNS-infiltrating leukocytes via their effects on CXCL12 expression. CXCL12 is expressed on the abluminal surface of the blood brain barrier and functions to retain CXCR4-expressing leukocytes within the subarachnoid space (86). However, CXCL12 can be scavenged from the endothelial cell surface by activation of CXCR7, thereby preventing interaction with CXCR4 on the leukocytes and facilitating their entry into the parenchyma. CXCR7 expression was increased by IL-17 but decreased by IFN- $\gamma$ , such that the effect of IL-17 on this pathway is to promote, while the effect of IFN- $\gamma$  is to inhibit leukocyte infiltration into the CNS (87).

Another potential target of IFN- $\gamma$  and IL-17 interaction may be the myelin-producing oligodendrocytes themselves. While IL-17 has been consistently shown to promote oligodendrocyte cell death, IFN- $\gamma$  appears to exert both toxic and protective effects on oligodendrocytes. IL-17 (in combination with TNF- $\alpha$ ) enhances oxidative-stress-mediated oligodendrocyte apoptosis *in vitro* (88), while IFN- $\gamma$  protects against peroxide (and lactacystine)-induced apoptosis *in vitro* (89). However, IFN- $\gamma$  appears to enhance other forms of oligodendrocyte apoptosis, including Fas ligand and staurosporine-mediated cell death (89).



## Differential effects of IL-17 and IFN- $\gamma$ signaling in CNS microenvironments

While there are many potential points of intersection between IL-17 and IFN- $\gamma$  signaling, it is not known which pathways are critical for induction of inflammation specifically in the brain. However, a basic model for the role of these cytokines in brain and spinal cord inflammation can be proposed. In the brain, IL-17 (and potentially GM-CSF) pathogenic signaling may function to counterbalance suppressive signaling of IFN- $\gamma$ . The presence of a higher ratio of Th17:Th1 cells would allow the pro-inflammatory signals to outweigh the inhibitory signals. In the spinal cord, IFN- $\gamma$  does not appear to mediate suppressive effects; therefore, the ability to induce inflammation is not dependent on a high Th17:Th1 ratio. In addition, the pathogenic effects of IFN- $\gamma$  may be more important in the spinal cord than the brain. Collectively, these data suggest that the effects of IFN- $\gamma$  signaling differ in the brain and spinal cord. This could occur if the local environment in each compartment generates different crosstalk between cytokine signaling pathways. For example, IFN- $\gamma$  usually suppresses MMP9 production, but in the presence of IL-1 $\beta$ , IFN- $\gamma$  can upregulate MMP9 production (90). Alternatively, the IFN- $\gamma$ -responsive cells may differ in the brain and spinal cord. There is some evidence that IFN- $\gamma$  activates downstream signals in a cell-dependent manner, such as the activation of STAT3 in neutrophils but not eosinophils (91). We reported (61) that the levels of IFN- $\gamma$ Rb transcript are significantly higher in the brain than the spinal cord, suggesting the possibility that these higher levels may translate into a more suppressive IFN- $\gamma$  signal in the brain that prevents parenchymal infiltration in the absence of a high Th17:Th1 ratio. Further studies are needed to determine how IFN- $\gamma$  signaling interacts with IL-17 and GM-CSF signaling in the brain versus the spinal cord to differentially induce or suppress CNS inflammation.

## Differential trafficking of Th1 and Th17 to the CNS

The correlation of high Th17:Th1 ratios with parenchymal brain inflammation suggests that differential Th17 migration to the brain may be important for the development of brain inflammation. T-cell extravasation into the CNS is mediated by various chemokines and adhesion molecules. T cells in the blood are induced to slow their velocity and 'roll' along vessel walls by interacting with P- and E-selectins as well as interactions mediated by VLA-4. T cells that are slowed to a roll can then interact via their chemokine receptors with chemokines expressed on the luminal side of the vasculature near sites of inflammation. These interactions transduce a signal to activate integrins expressed on the T-cell surface that facilitates extravasation across the endothelium into the inflamed tissue (92).

Both human and mouse Th1 and Th17 cells are known to express different chemokine receptors. The receptors CXCR3 and CCR5 are preferentially expressed on Th1 cells, while CCR6, the receptor for CCL20, is preferentially (though not exclusively) expressed on Th17 cells (93). Adoptive transfer of wildtype and CCR6<sup>-/-</sup>MOG-specific Th17 cells into wildtype recipients showed that the initial migration of T cells into the CNS was CCR6 dependent. However, CCR6<sup>-/-</sup>T cells were able to infiltrate the CNS after inflammation was already established (94). This study also demonstrated strong expression of CCL20 by epithelial cells of the choroid plexus in both healthy and EAE mice, and CD45<sup>+</sup> cells were observed to accumulate at the choroid plexus barrier in the absence of CCR6 expression. These results suggested that CCR6<sup>+</sup> Th17 cells may be required for initial T-cell infiltration, which occurs via the choroid plexus in the brain.

In addition to contributions from chemokine receptors, Th1 and Th17 cells may be differentially recruited to the brain and spinal cord based on their different patterns of T-cell integrin expression (95, 96). Two recent studies showed that the  $\alpha$ 4 integrin (a subunit of VLA-4) was more highly expressed on Th1 compared to Th17 cells. Both groups examined

the role of this integrin in Th1 and Th17-mediated disease using a conditional  $\alpha 4^{-/-}$  in CD4<sup>+</sup> cells and found decreased numbers of IFN- $\gamma$ -producing cells in the CNS in the absence of  $\alpha 4$  integrin. Rothhammer *et al.* (95) observed a switch from classic to atypical EAE in the  $\alpha 4$  conditional knockout mice characterized by brainstem and forebrain infiltrates with infiltrates also observed in the choroid plexus and periventricular regions. Adoptive transfer of  $\alpha 4^{-/-}$ Th1 cells did not induce EAE, while the transfer of  $\alpha 4^{-/-}$ Th17 cells induced atypical EAE with reduced numbers of T cells observed in the spinal cord but not the brain. These observations suggested that  $\alpha 4$  integrin is required for infiltration of the spinal cord by both Th1 and Th17 cells but not for infiltration of the brain by Th17 cells. This study also found that both Th1 and Th17 cells express CD11a ( $\alpha L$  integrin, a subunit of LFA-1), and blockade of CD11a in combination with the  $\alpha 4$  conditional knockout prevented T-cell infiltration of the brain (95). These findings suggest that VLA-4 may be more important for spinal cord migration and that Th1 cells may be more likely than Th17 cells to migrate to the spinal cord due to their higher levels of  $\alpha 4$  integrin expression. Additionally, LFA-1 may be used by Th17 cells to access the CSF and periventricular compartments in the brain via the choroid plexus in a VLA-4-independent manner. Thus, the relative levels of integrin expression on Th1 and Th17 cells may lead to preferential localization of Th17 cells in the brain and Th1 cells in the spinal cord. A recent *in vitro* study that analyzed interactions of Th1 and Th17 cells with adhesion molecules under flow conditions found that Th17 cells interacted more frequently with E-selectin compared to Th1 cells (97). Interestingly, interactions between CCR6 on Th17 cells and CCL20 induced LFA-1 activation, promoting adhesion to ICAM-1 and arrest on the surface. Collectively, these studies suggest that CCL20-CCR6 interactions not only promote migration of Th17 cells to the choroid plexus but also promote LFA-1 integrin activation and attachment to ICAM-1, which may be required for Th17 entry into the brain. The preferential migration of Th17 cells to the brain is consistent with the higher Th17:Th1 ratio that we observed in the brain compared to the spinal cord (61).

## Conclusions

The roles of various T-cell cytokines in the induction of EAE have been debated for some time. It is now clear that GM-CSF is a critical T-cell cytokine for inducing CNS inflammation, and IL-17 and IFN- $\gamma$  play important and complex roles in the localization of inflammation in the brain and spinal cord. We have emphasized here the findings from studies that demonstrate that inflammation is regulated differently in the brain compared to the spinal cord. While the mechanisms responsible for this differential regulation are not yet well understood, it is clear that IFN- $\gamma$  signaling plays an important role in suppressing brain but not spinal cord inflammation, and pro-inflammatory IL-17 signaling may function to counteract this negative signal. From a clinical perspective, understanding these mechanisms in detail is important, because it is possible that some therapeutic strategies designed to reduce inflammation by targeting specific immune mediators (such as IFN- $\gamma$ ) might instead cause relocalization of lesions. Further work is needed to understand how these cytokine signaling pathways intersect in the brain and the spinal cord and what other mediators and mechanisms are involved in promoting or suppressing inflammation in the two microenvironments. To translate this knowledge to humans, it will be important to synthesize the sometimes contradictory results obtained from the multiple models of EAE currently in use to discern the 'general principles' of regulating inflammation within the different regions of the CNS. For example, the disease processes that occur in the active and passive forms of EAE induction are clearly very different, which may explain why different conclusions are sometimes reached when the same question is investigated using different methods of disease induction. Ultimately, understanding the detailed interactions that occur between CNS resident cells in each microenvironment and immune cells activated by both protocols will help elucidate the fundamental mechanisms that regulate autoimmunity in the

CNS. A deeper understanding of these mechanisms will provide insight into the heterogeneity seen in MS and may help to identify biomarkers for the different inflammatory patterns to better target currently available therapies as well as promote development of new therapies for MS patients.

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