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The contribution of neutrophils to CNS autoimmunity

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Abstract

Multiple sclerosis (MS) is believed to be initiated when myelin-specific T cells infiltrate the central nervous system (CNS), triggering subsequent recruitment of inflammatory leukocytes to the CNS. The contribution of neutrophils to CNS autoimmune disease has been underappreciated, but several studies in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, indicate that neutrophils have an important role in inflammation. Neutrophils are hypothesized to contribute to the pathogenesis of EAE by producing cytokines and promoting breakdown of the blood brain barrier. Neutrophils may also influence the manifestation of EAE by facilitating parenchymal brain inflammation. This review summarizes evidence supporting a functional role for neutrophils in EAE and MS, highlighting the differential regulation of neutrophil recruitment in the brain and spinal cord.

Keywords

Neutrophils; EAE; MS; IL-17; CXCR2

1. Introduction

Multiple sclerosis (MS) is the most common cause of non-traumatic neurological disability affecting approximately 2.5 million people worldwide [1]. The onset of MS occurs in early to mid-adulthood and symptoms persist and typically worsen throughout life, resulting in significant health and socioeconomic problems [2]. Although the pathogenic pathways leading to MS are not well understood, it is believed to be an autoimmune disease in which self-reactive T cells specific for myelin proteins initiate an inflammatory cascade resulting in demyelination and axonal damage. The extensive heterogeneity in both disease course and pathological features seen in patients with MS suggests that multiple pathogenic pathways contribute to the disease development. The majority of MS patients exhibit a relapsing-remitting disease course (RRMS) in which recurring episodes of neurological symptoms are followed by periods of clinical stability. This stage of MS is characterized by the occurrence

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of focal inflammatory lesions within the central nervous system (CNS) that are detectable by MRI. Inflammation in the CNS leads to plaques of demyelination, which are a hallmark feature of RRMS. The majority of patients with RRMS have lesions disseminated in the brain and spinal cord; however, the focal lesion burden is typically heavier in the brain than in the spinal cord [3]. Interestingly, a small subset of spinal cord-dominant patients (~2-3%) have lesions primarily localized in the spinal cord with comparatively less lesion burden in the brain [4, 5]. The mechanisms underlying these distinct neuroinflammatory patterns are not known. RRMS can persist for many years; however, approximately 80% of RRMS patients eventually convert to the secondary progressive stage of disease (SPMS) in which the extent of recovery after each episode of neurological deficit diminishes [1]. In contrast to RRMS, gadolinium-enhancing MRI lesions are less common during SPMS, despite the steady increase in brain atrophy and disability that occurs during this stage of disease [6, 7].

MS has been extensively studied using the animal model experimental autoimmune encephalomyelitis (EAE), which is induced by the activation or adoptive transfer of CD4+ myelin-specific T cells [8]. Both Th1 (IFN-γ-producing) and Th17 (IL-17-producing) CD4+ T cell subsets are capable of inducing EAE. While studies in EAE have emphasized the role of myelin-specific CD4+ T cells, the importance of other immune cell types in MS pathogenesis became clear when anti-CD4+ treatment exhibited no clinical benefits [9]. In contrast, treatment targeting all leukocytes reduced relapses and the development of lesions [10], suggesting other effector cells may be at play.

Recent studies in both EAE and MS patients suggest a role for neutrophils in disease pathogenesis. In EAE, neutrophils comprise a significant percentage of CNS-infiltrating cells prior to disease onset and relapse [11-15]. Interestingly, disease was ameliorated when neutrophils were depleted prior to, but not after, disease onset or relapse, suggesting that neutrophil function is important during the initial formation of lesions [12, 16, 17]. Recent evidence also implicates neutrophils in the pathogenesis of MS. In contrast to healthy control subjects, neutrophils in the blood of RRMS patients exhibit a primed phenotype, and both neutrophil number and biomarkers of neutrophil activity increase during relapses [15, 18]. Similarly, MS patients have a higher neutrophil-to-lymphocyte ratio in the blood compared to healthy controls, and the ratio is reported to increase with the occurrence of relapse and worsening disability [19]. However, neutrophils are not a pronounced feature of pathology in CNS tissue sections from MS patients. One potential explanation for this is that neutrophils have a short half-life and, as highlighted above, may only contribute to disease at specific stages, i.e., prior to onset or relapses. Therefore, neutrophils may not be prominent in tissue sections from MS patients with established disease. In support of this hypothesis, the percentage of neutrophils in the cerebral spinal fluid (CSF) is higher in early-onset pediatric MS as compared to later-onset pediatric MS and the percentage of neutrophils in CSF decreases while the percentage of lymphocytes and monocytes increases over time [20]. In this review, we discuss potential pathogenic functions of neutrophils in EAE and MS (primarily RRMS) and the role these cells may play in promoting inflammation in the brain versus the spinal cord.

2. Neutrophil Function

Neutrophils perform an essential role in pathogen clearance by exerting a broad array of functions, including phagocytosis, release of lytic enzymes, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs), activation of antigen presenting cells (APCs) and T cells, and secretion of proinflammatory mediators, such as cytokines and chemokines [21, 22]. While the pleiotropic effects of neutrophils are important during an infection, they could cause aberrant tissue damage that, if not resolved, could contribute to autoimmunity [23]. The specific neutrophil functions that contribute to the development and propagation of CNS autoimmunity are unknown. In EAE, neutrophils are reported to play a key role in the effector stage of disease but do not contribute to the initial priming or differentiation of myelin-reactive T cells [12, 16, 17]. The following sections discuss the multiple functions that neutrophils could exert during MS and EAE pathogenesis.

Neutrophil Cytokine Production

Neutrophils can secrete a diverse array of cytokines that may influence MS and EAE. Neutrophils isolated from the CNS at onset of EAE produce TNF-α, IL-6, IL-12/23p40, IL-1β and IFN-γ, which are cytokines thought to contribute to the inflammatory cascade within the CNS [12, 24]. One mechanism by which proinflammatory cytokines may contribute to disease is by promoting maturation of CNS APCs that reactivate CNS-infiltrating T cells via presentation of myelin antigen. Steinbach *et al* found that soluble factors produced during co-culture of bone-marrow derived dendritic cells (DCs) with neutrophils isolated from the CNS during EAE resulted in maturation of the DCs, and that neutrophil depletion prior to disease onset impaired the differentiation of microglia and monocytes/macrophages into professional APCs within the CNS [12]. This suggests that although neutrophils are not important for the initial activation of T cells, they may contribute to their reactivation within the CNS indirectly through the production of cytokines and subsequent APC maturation. Neutrophils have also been found to produce pro-IL-1β upon transendothelial migration during EAE. Myeloid cell IL-1β production has been shown to exacerbate EAE severity by inducing endothelial cells to produce cytokines and chemokines that recruit and activate other myeloid cells [24].

Blood brain barrier breakdown

Leukocyte trafficking into the CNS is restricted by the blood brain barrier (BBB) and blood spinal-cord barrier (BSCB) (referred to here collectively as the BBB). The BBB encompasses an endothelial basement membrane composed of vascular endothelial cells connected by tight junctions, and the glia limitans, which consists of a parenchymal basement membrane formed by laminin and dystroglycan, and a layer of astrocyte feet [25]. A perivascular space separates the two basement membranes. During inflammation, activated T cells can cross the endothelial basement membrane but additional signals are required to break down the glia limitans to allow leukocytes to infiltrate the CNS parenchyma [26]. In EAE, an increase in the permeability of the BBB is associated with the early influx of neutrophils into the CNS. Depletion of neutrophils helps preserve BBB integrity, suggesting that neutrophils play a role in the breakdown of the BBB [17, 27]. Neutrophils have also been correlated with BBB leakage in an acute MS lesion [27].

Moreover, our laboratory found that when neutrophils were eliminated from the periphery prior to EAE induction, inflammatory cells still entered the perivascular space, but did not enter the brain parenchyma [14]. This suggests that neutrophils facilitate leukocyte trafficking from the perivascular space into the brain parenchyma by enhancing the permeability of the glia limitans, although other mechanisms may also contribute to permeability and cell infiltration.

Although the exact mechanisms of neutrophil-mediated BBB breakdown are still unknown, it is hypothesized that matrix metalloproteinases (MMPs) secreted by neutrophils contribute to this step in the pathogenesis of MS [28]. In particular, the activity and expression of MMP-9 is increased in serum, CSF, and active lesions in MS and has been associated with BBB breakdown in mice [29, 30]. Interestingly, MMP-9 can cleave dystroglycan, which anchors astrocyte end-feet to the parenchymal basement layer, suggesting a mechanism by which neutrophils may specifically alter the glia limitans [31]. Additionally, neutrophils can produce ROS, which are known to disrupt junctional proteins of the BBB endothelium, leading to increased permeability [21, 32].

Neutrophil extracellular traps (NETs)

Following stimulation, neutrophils can undergo an active form of cell death, termed NETosis, and release neutrophil extracellular traps. NETs are extracellular matrices composed of fibers containing DNA, decondensed chromatin, histones, and antimicrobial proteins that immobilize and kill microbes [33, 34]. Although beneficial during infection, NETs have been associated with pathophysiological conditions, including MS. A subset of RRMS patients have higher circulating levels of NETs in their sera, although higher levels of NETs did not correlate with disease activity [18, 35]. Interestingly, *in vitro* studies demonstrated that transendothelial migration of neutrophils across brain endothelium triggered the release of NETs that contributed to neuronal cell death [36]. However, there is no reported evidence for NET formation in EAE. The contribution of NETs in EAE and their presence in the CNS requires further investigation in order to implicate a role for NET production in MS.

3. Neutrophil Mobilization and Recruitment

Neutrophils have the capacity to be highly destructive but cannot discriminate specific targets once their functions have been activated. Therefore, neutrophil recruitment and activation is tightly regulated to avoid inappropriate tissue damage. Resting neutrophils are first primed and mobilized from the bone marrow via the activity of proinflammatory cytokines, including G-CSF, GM-CSF, TNF-α, and IL-6 [18, 37]. After exiting the bone marrow, neutrophils are recruited to sites of inflammation via interaction between chemokine receptors expressed on neutrophils and chemokines expressed at the inflamed site. Upon entry into the target tissue, neutrophils become fully activated resulting in the enhancement of their effector mechanisms, including phagocytosis, degranulation, and the release of NETs [18]. As discussed below, a role for neutrophils in MS is supported by EAE studies indicating that many of the factors that contribute to the mobilization, recruitment and activation of neutrophils influence disease pathogenesis.

The role of G-CSF

During an inflammatory response, G-CSF promotes the expansion and indirectly enhances the release of neutrophils from the bone marrow into the circulation by shifting the balance of chemokines produced within the bone marrow [37, 38]. During EAE, the expression of G-CSF is elevated in the periphery and CNS prior to disease onset and correlates with the number of neutrophils in the bone marrow and peripheral blood, suggesting that G-CSF mediates the expansion and mobilization of neutrophils early in disease course [11, 15]. Importantly, G-CSF receptor-deficient (*Csf3r−/−)* mice exhibited lower numbers of circulating neutrophils compared to wild-type (WT) mice at clinical onset and were highly resistant to EAE [15], suggesting that the G-CSF-mediated increase in circulating neutrophils is critical for disease. Consistent with this, administration of G-CSF exacerbated disease in some patients with MS [39, 40]. However, administration of G-CSF during EAE yielded conflicting results. When administered after immunization but prior to disease onset or during remission, G-CSF exacerbated disease [41]. In contrast, when treatment was initiated prior to immunization or at onset, mice were protected from EAE [42, 43]. While the protective effect of administrating G-CSF at onset is not understood, these observations suggest that G-CSF is critical for disease prior to the onset of clinical signs, potentially through mobilization of neutrophils. Consistent with this hypothesis, analyses of MS lesions found that G-CSF is upregulated in acute lesions compared to chronic lesions [44].

ELR+ CXC Chemokines

Neutrophils are typically mobilized and recruited to sites of inflammation by ELR+ CXC chemokines, such as murine CXCL1, CXCL2 and CXCL5, and human CXCL5 and CXCL8, which bind to the neutrophil receptor CXCR2 [38]. During EAE, expression of CXCL1 and CXCL2 is upregulated in the CNS prior to disease onset [14, 17, 45]. These chemokines are primarily produced by endothelial cells, astrocytes, microglia, and infiltrating myeloid cells [14, 24, 46]. All ELR+ CXC chemokines bind to the neutrophil receptor CXCR2; in humans, a few ELR+ chemokines also bind to CXCR1 [38]. In SJL mice, preventing CXCR2 signaling during EAE induction either by administering a blocking antibody or by using CXCR2−/− mice results in resistance to EAE. Susceptibility to disease is partially restored upon transfer of WT neutrophils into CXCR2−/− mice [17], indicating that signaling via CXCR2 on neutrophils is critical for EAE induction. ELR+ CXC chemokines are also implicated in the pathogenesis of MS as serum levels of CXCL8 are higher in patients with MS compared to healthy controls [18, 47]. CXCL8 levels are also higher in the CSF of patients with MS as compared to healthy controls, and expression of this chemokine is significantly increased during relapse [48, 49]. Furthermore, the plasma levels of CXCL1, CXCL5, and neutrophil elastase correlate with the extent of lesion burden and clinical disability [15].

4. The role of neutrophils in shaping patterns of neuroinflammation

The mechanisms responsible for determining where lesions occur within the CNS have not been defined for MS patients. This question has been difficult to investigate because most EAE models are characterized primarily by spinal cord inflammation with few lesions seen in the brain parenchyma. Spinal cord inflammation manifests clinically as ascending flaccid

paralysis, known as classic EAE. However, brain inflammation is seen in a few EAE models using certain mouse strain/myelin antigen combinations [50, 51].

For example, we developed an EAE model in C3Heb/Fej mice in which immunization with MOG resulted in extensive inflammation in both the brain and spinal cord [52]. Mice with brain inflammation exhibit distinct clinical signs such as ataxia, proprioception defects, leaning and in some cases an unusual axial-rotary clinical presentation, collectively known as atypical EAE. Additionally, introducing genetic deficiency in IFN-γ signaling into many strains that normally exhibit predominantly spinal cord lesions causes most inflammation to shift to the brain [53-55]. A few other genetically-engineered models have been described that predispose mice to brain inflammation, i.e., overexpression of IL-6 in astrocytes or deletion of SOCS-3 in myeloid cells [56, 57]. A common feature of atypical EAE models is the predominance of neutrophils among the brain-infiltrating cells. In several models, including the C3Heb/Fej model, neutrophils outnumber both CD4+ T cells and monocytes/macrophages in the brain [14, 58, 59]. While significant neutrophil infiltration in the spinal cord has also been seen in a few models [14], particularly those lacking IFN-γ signaling [12, 45, 59], most classic EAE models exhibit a monocyte/macrophage-dominant infiltrate in the spinal cord at peak disease [45, 58]. Interestingly, models in which neutrophils are a major constituent of the CNS-infiltrating population typically exhibit more extensive infiltration of inflammatory cells into the tissue parenchyma compared to models with fewer infiltrating neutrophils, regardless of whether the infiltration occurs in the brain or spinal cord [14, 45, 59], supporting a role for neutrophils in the breakdown of the BBB.

We demonstrated an important role for neutrophils in promoting brain inflammation by showing that depletion of neutrophils prior to disease induction in C3Heb/Fej mice prevented the development of inflammation in the brain and atypical EAE clinical signs [14]. Parenchymal infiltration of leukocytes in the brain was significantly reduced; instead, inflammatory cells accumulated in the perivascular spaces (referred to as cuffing). A decrease in brain parenchymal infiltration following neutrophil depletion was subsequently confirmed in a separate atypical EAE model [57]. We did not see a reduction in the incidence or severity of classic EAE with neutrophil depletion, although we did observe increased cuffing and a modest decrease in tissue damage in the spinal cord [14]. We concluded that neutrophils play a greater role in promoting parenchymal inflammation in the brain than in the spinal cord. Some classic EAE models have shown a stronger effect of neutrophil depletion on spinal cord inflammation, causing a reduced incidence and/or severity of classic EAE signs depending on the timing of the depletion [12, 16, 17]. However, the spinal cord appears to be more permissive to inflammation induced by a diverse set of leukocytes compared to the brain, and may be less dependent on neutrophil recruitment to achieve a similar degree of tissue damage. This increased “permissiveness” of the spinal cord to leukocyte infiltration may in part reflect differences between the BBB and BSCB in the expression levels of certain tight junction proteins that may lead to increased permeability of the BSCB [60]. Therefore, if a potential function of neutrophils is to promote parenchymal infiltration via disruption of the BBB and BSCB, the greater requirement for neutrophils to facilitate brain versus spinal cord inflammation may reflect the need to traverse a more impenetrable barrier in the brain. Collectively, these studies highlight the key observation that the brain and spinal cord function as distinct

microenvironments that appear to have different requirements for initiating inflammation and clinical disease.

Several groups, including ours, have investigated the mechanisms regulating the localization of inflammation to the brain versus the spinal cord. We previously found that the development of lesions in the brain versus the spinal cord in C3Heb/Fej mice was determined by the relative abundance of Th17 cells compared to Th1 cells within the CNS-infiltrating T cell population. Specifically, when the Th17:Th1 ratio of the myelin protein-specific $CD4^+$ T cells infiltrating the brain was ≥ 1, mice developed parenchymal brain inflammation. In contrast, when the Th17:Th1 ratio of T cells isolated from the brain was < 1, only meningeal inflammation was seen in the brain and parenchymal inflammation was largely restricted to the spinal cord [52]. Spinal cord inflammation appeared less sensitive to the Th17:Th1 ratio of CD4+ T cells entering the CNS. Together, these observations suggest that IL-17 plays a critical role in promoting brain inflammation, potentially by overcoming an inhibitory signal mediated in the brain by IFN-γ. The following sections will review the more recent developments on how the interplay of IL-17 and IFN-γ signaling influences neutrophil recruitment and inflammation in the brain and spinal cord.

Neutrophil recruitment to the brain

We confirmed our initial observation that IL-17 is an important cytokine for promoting brain inflammation by demonstrating that IL-17RA−/− mice exhibited a very low incidence of atypical EAE, while the incidence and severity of classic EAE was unchanged [14]. Similarly, Kroenke et. al found that the transfer of myelin-specific IFN-γ−/− T cells into WT recipients induced brain inflammation, but transfer of the same IFN-γ−/− T cells into IL-17RA−/− recipients induced only spinal cord inflammation [59, 61]. We found that reduced numbers of neutrophils were recruited to the brain in IL-17RA−/− compared to WT mice, correlating with decreased induction of CXCL1, CXCL2, and CXCL5 [14]. These neutrophil-attracting ELR+ chemokines are known to be induced by IL-17 [62]. Kroenke et. al also noted decreased numbers of neutrophils and diminished production of CXCL1 and CXCL2 in the absence of IL-17 signaling [59]. In C3Heb/Fej mice, we found that CXCL2 was induced in the brain to a much greater extent than CXCL1 and CXCL5, and that the vast majority of CXCL2 in the brain was produced by astrocytes in an IL-17-dependent manner [14]. To confirm that the upregulation of ELR+ chemokines was the main driver of neutrophil recruitment and subsequent brain inflammation, we inhibited ELR+ chemokine signaling in vivo using a CXCR2 small molecule antagonist. Blocking CXCR2 signaling resulted in a loss of atypical EAE, similar to that seen with neutrophil depletion [14]. Two other studies also found that CXCR2 blockade suppressed atypical EAE by selectively inhibiting neutrophil infiltration into the CNS [57, 58]. Taken together, these studies support a model in which IL-17 produced by CD4+ T cells infiltrating the brain induces ELR+ chemokine production by astrocytes, resulting in CXCR2-dependent neutrophil recruitment and subsequent infiltration of the parenchyma by leukocytes. In contrast to IL-17, IFN-γ inhibits inflammation in the brain, as evidenced by the development of atypical EAE in IFN-γ−/− and IFN-γR−/− mouse strains that normally develop classic EAE [63]. Importantly, two studies demonstrated increased CXCL2 expression and corresponding infiltration of neutrophils in the brain in the absence of IFN-γ signaling [14, 58]. IFN-γ has also been

shown to downregulate CXCR2 expression on granulocytes [64]. The mechanism by which IFN-γ suppresses ELR+ chemokine induction is not known. These observations suggest that IFN-γ acts antagonistically to IL-17 by suppressing ELR+ chemokine induction and subsequent neutrophil recruitment, thus inhibiting parenchymal brain inflammation. Therefore, the balance of cytokines produced by infiltrating CD4+ T cells likely determines whether neutrophils are recruited to the brain to permit parenchymal inflammation.

Two studies have investigated the ability to induce EAE by adoptive transfer into IFN-γR−/− × IL-17RA−/− recipients. In one study using B6 mice, wildtype T cells were capable of inducing atypical EAE with a similar incidence in IFN-γR−/− × IL-17RA−/− recipients as in IFN-γR−/− recipients, suggesting that IL-17 is not essential for brain inflammation in the absence of the inhibitory effect of IFN-γ signaling [58]. In contrast, we observed a very significant decrease in overall incidence of EAE in IFN-γR−/− × IL-17RA−/− recipients compared to both IFN-γR−/− and IL-17RA−/− recipients in C3Heb/Fej mice [14]. Interestingly, among the few IFN-γR−/− × IL-17RA−/− mice that did exhibit clinical signs, some exhibited atypical signs and some classic signs (unpublished observations), again suggesting that IL-17 signaling may be less important for promoting brain inflammation in the absence of IFN-γ signaling. Neutrophil infiltration was not examined in IFN-γR−/− × IL-17RA−/− recipients in either study; however, the development of brain inflammation in the absence of IL-17 signaling raises the possibility that other factors may contribute to the recruitment of neutrophils to the brain.

Neutrophil recruitment to the spinal cord

Although neutrophils are required for brain inflammation, they are not required for spinal cord inflammation in every model. Nevertheless, neutrophils are abundant among cells infiltrating the spinal cord in many EAE models (often more abundant than in the brain) and have been shown to contribute to the severity of spinal cord parenchymal inflammation [12, 14, 16, 17]. CXCL1 and CXCL2 are upregulated in the spinal cord, especially in EAE models in which neutrophils comprise a large part of the spinal cord infiltrate [14, 17, 45]. Anti-CXCL1 treatment was found to reduce neutrophil infiltration in the spinal cord and decrease classic EAE severity in B6 mice [46]. ELR+ chemokine signaling through CXCR2 has been described as critical to recruit neutrophils to the spinal cord, analogous to neutrophil recruitment to the brain [17, 58]. However, ELR+ chemokine expression appears to be regulated differently in the spinal cord compared to the brain. While IL-17 is responsible for most of the increase in CXCL1, CXCL2 and CXCL5 seen in the brain in C3Heb/Fej mice, only the small increase in CXCL1 observed in the spinal cord is promoted by IL-17, while the induction of CXCL2 and CXCL5 in the spinal cord is independent of IL-17 expression. Thus, in this mouse strain, CXCL2 is strongly upregulated during EAE in spinal cord astrocytes in an IL-17-independent manner, even though astrocytes in the brains of the same mice fail to upregulate CXCL2 in the absence of IL-17 signaling [14]. Consistent with the lack of involvement of IL-17 signaling in CXCL2 induction, IL-17RA−/− mice exhibit no defect in neutrophil recruitment to the spinal cord [14]. However, IL-17 may contribute to spinal cord inflammation in some situations, as one study reported that anti-IL-17 treatment reduced the severity of Th17-driven, neutrophilic spinal cord inflammation [45].

The contrasting effect of IFN-γ signaling on neutrophil recruitment to the spinal cord highlights the regional differences between the brain and the spinal cord in their responses to inflammatory signals. When EAE is induced by adoptive transfer of WT T cells into C3Heb/Fej mice, the induction of CXCL2 is impaired and fewer neutrophils are recruited to the spinal cord in IFN-γR−/− mice relative to WT recipient mice [14]. Thus, IFN-γ exerts completely opposite effects on neutrophil recruitment in the brain versus the spinal cord in this model by inhibiting CXCL2 expression in the brain but inducing CXCL2 in the spinal cord. Induction of CXCL1 is also impaired in the spinal cord of IFN-γR−/− C3Heb/Fej mice [14]. IFN-γ is not typically associated with induction of ELR+ chemokines; however, it may act to increase expression of these chemokines indirectly via induction of other inflammatory mediators such as IL-1β, as IL-1β induction was also significantly reduced in the spinal cord of IFN-γR−/− mice [14]. Another group also found that the absence of IFN-γR led to decreased CXCL2 expression by spinal cord astrocytes [65]. However, IFN-γ did not directly induce CXCL2 in purified astrocytes; IL-17 and TNF-α induced CXCL2 expression in WT astrocytes, and this induction was reduced in IFN-γR−/− astrocytes [65]. The mechanism by which IFN-γ signaling might enhance CXCL2 expression that is induced by other cytokines has not yet been defined. The studies described above reveal how IL-17 and IFN-γ play very different roles in the ELR+ chemokine-mediated recruitment of neutrophils to the brain versus the spinal cord. IL-17 strongly induces ELR+ chemokines in the brain but only minimally in the spinal cord, while IFN-γ suppresses ELR+ chemokine signaling in the brain and induces it in the spinal cord. It is still unclear why IFN-γ has such different effects in the different regions of the CNS. However, neutrophil recruitment to the brain (and atypical EAE) is not completely abolished in the absence of IL-17; likewise, neutrophil recruitment to the spinal cord is only partially dependent on IFN-γ. Thus, other factors likely contribute to neutrophil recruitment to both regions of the CNS.

Conclusion

The role of neutrophils in the pathogenesis of MS and EAE represents a growing area of investigation, with recent studies implicating multiple mechanisms by which neutrophils may contribute to CNS autoimmune disease. Activities associated with neutrophils, such as release of degradative proteases, myeloperoxidase and NETs are consistent with neutrophil-mediated tissue destruction; however, it remains to be shown that neutrophils themselves influence EAE or MS by exerting these specific activities. Nevertheless, it now seems clear that neutrophils play a role in promoting parenchymal infiltration of leukocytes. Unexpectedly, recent studies in EAE suggest that there may be a greater requirement for neutrophils to promote parenchymal leukocyte infiltration in the brain compared to the spinal cord. These studies also provided new mechanistic insight into the opposing roles for IL-17 and IFN-γ in promoting brain inflammation by demonstrating that IL-17 promotes and IFN-γ inhibits the induction of a major neutrophil chemoattractant in the brain. Future investigation into the differing requirement for neutrophils in the brain versus the spinal cord could help elucidate the differences in these two microenvironments. The role of neutrophils in promoting infiltration of inflammatory cells suggests that neutrophils may be particularly important early in disease and in lesion formation during relapses; however, their contribution at later stages of disease is unknown and should be explored. Neutrophil

production of cytokines such as IL-1β may help continuously fuel the inflammatory response that propagates CNS autoimmune disease. There is not yet evidence for secretion of NETS within the CNS, but this issue also warrants further study as generation of NETs could also sustain CNS autoimmunity by enhancing production of reactive oxygen species. In MS, we are beginning to see hints that neutrophils are associated with disease activity. However, these studies are correlative and it is not known whether neutrophils are associated with promoting inflammation in the brain. While it is extremely challenging to study the role of neutrophils directly in patients with MS, further understanding of the regulation and functions of neutrophils in the brain and spinal cord could have potential therapeutic implications.

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Highlights

- Neutrophils are particularly pathogenic in EAE models involving brain inflammation
- Neutrophils promote parenchymal infiltration of leukocytes in the CNS
- Neutrophil recruitment is regulated differently in brain versus spinal cord in EAE