Cytokine-Regulated Neutrophil Recruitment Is Required for Brain but Not Spinal Cord Inflammation during Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the CNS thought to be mediated by myelin-specific T cells. Most patients with MS have lesions disseminated in the brain, and some have accompanying spinal cord lesions. Interestingly, a subset of patients have lesions restricted primarily to the spinal cord and optic nerves, termed opticospinal MS. The clinical signs of disease reflect the site of lesion localization, and the nature of these clinical signs can significantly impact the extent of disability and prognosis. It is important to determine whether different pathogenic mechanisms contribute to these distinct inflammatory patterns, as this may suggest a need for individualized therapeutic approaches.

MS is widely studied using experimental autoimmune encephalomyelitis (EAE), which is induced by immunization with myelin Ags or by adoptive transfer of myelin-specific T cells. Unlike most MS patients, lesions are predominantly localized in the spinal cord in most rodent EAE models. Disease in these models is manifested by ascending flaccid paralysis, referred to as “classic” EAE. However, some “atypical” EAE models have been described that exhibit head tilt, body lean, ataxia, spinning, or axial rotation indicative of inflammation in the brain (1–4). Atypical EAE was observed in specific Ag/strain combinations (5–7), and in mice deficient in IFN-γ signaling (2, 4, 8, 9), suggesting that IFN-γ inhibits brain inflammation in EAE. Although it is not known how IFN-γ deficiency promotes brain inflammation, the inflammatory infiltrate in the brain in these mice was often dominated by neutrophils (2, 8). Subsequently, we and others demonstrated that IL-17 signaling preferentially promotes brain inflammation (9–11). Although IL-17 is known to induce ELR+ chemokines that recruit neutrophils, it has not been established that this is the mechanism by which IL-17 promotes brain inflammation. Defining the mechanisms and cell types that promote lesion formation in the brain parenchyma is especially relevant, as most patients with MS have lesions in this region.

We investigated how IL-17 and IFN-γ differentially influence brain versus spinal cord inflammation in C3Heb/FeJ mice that were wild-type (WT) or genetically deficient in IFN-γ. Atypical, but not classic, EAE signs were eliminated by administering an antagonist for CXCR2, the major chemokine receptor for CXCL2, which plays the major role in neutrophil recruitment to this region. Importantly, we showed that neutrophils were required for tissue damage in the brain and development of atypical clinical signs. In stark contrast to their effects in the brain, we found that IFN-γ suppressed, whereas IL-17 promoted, induction of the ELR+ chemokine CXCL2, which played the major role in neutrophil recruitment to the spinal cord. Strikingly, neutrophil depletion ameliorated atypical EAE but had no effect on classic EAE signs. Histochemical analyses confirmed that tissue injury in the spinal cord was much less dependent on neutrophil infiltration than the brain. The therapeutic implications of these findings were further supported by showing that atypical, but not classic, EAE signs were eliminated by administering an antagonist for CXCR2, the major chemokine receptor for CXCL2 expressed on neutrophils.

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Abbreviations used in this article: CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein; IFN-αR, IFN-γ receptor α-chain; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; WT, wild-type.

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ROLE OF NEUTROPHILS IN CNS AUTOIMMUNITY

Materials and Methods

Mice

C3HeB/FeJ mice were purchased from The Jackson Laboratory and bred and maintained in a specific pathogen-free facility at the University of Washington (Seattle, WA). IL-17RA−/− mice generated by Amgen were obtained from Taconic and backcrossed onto the C3HeB/FeJ background for at least 12 generations. IFN-γR−/− and glial fibrillary acidic protein (GFAP)–GFP transgenic mice were obtained from The Jackson Laboratory and backcrossed onto the C3HeB/FeJ background for at least 12 generations. Mice used for EAE induction were between 6 and 10 wk of age. All procedures have been approved by the Institutional Animal Care and Use Committee at the University of Washington.

Protein and peptides

Recombinant rat myelin oligodendrocyte glycoprotein (MOG) protein (rMOG; residues 1–125) was produced in Escherichia coli and purified as previously described (12). MOG35–114 peptide (rat sequence, 5′-TCFDRHDSYQEEAVELK-3′) was purchased from GenScript.

EAE induction

EAE was induced by cultivating splenocytes (1 × 10⁶ cells/ml) from rMOG-immunized mice for 3 d with 5 μg/ml MOG35–114 peptide and 10 ng/ml rIL-23 (eBioscience). Viable cells were isolated from a lympholyte gradient (Cedarlane) and i.p. injected (2 × 10⁶ cells/mouse) into sublethally irradiated (250 rad) mice. The severity of EAE was scored as follows (a grade was assigned when any one of its associated signs was observed): 1, paralyzed tail; 2, hind limb clasping; 3, one paralysed leg, mild body leaning; 4, two paralysed legs, moderate body leaning; 5, forelimb weakness, severe body leaning; 6, hunched, breathing difficulty, body rolling; 7, moribund. Atypical EAE was determined by the presence of one or more of the following symptoms: head tilt, body leaning, or body rolling.

Isolation of CNS cells

Mononuclear cells were isolated from the CNS of perfused EAE mice, as previously described (13). Briefly, brain and spinal cord were dissociated with a 5-mL syringe plunger through a sterile stainless steel mesh and centrifuged for 10 min at 3000 rpm. Cell pellets were resuspended in 30% Percoll, overlaid onto 70% Percoll, and centrifuged without brake for 20 min at 2400 rpm. Cells were collected from the 30–70% Percoll interface. For cell sorting experiments, cells were isolated from the CNS of perfused mice by digesting brains or spinal cords with 0.5 mg/ml papain (Worthington Biochemical) and 20 ng/ml DNase in HBSS for 20 min at 37°C prior to isolating the cells on a Percoll gradient.

Flow cytometry

Cells were incubated with Fc block (clone 2.4G2; eBioscience) in normal mouse serum for 15 min at 4°C, washed, and stained with Abs for 30 min at 4°C. Ab specificity, fluorochrome, clone name, and vendor were as follows: CD4-allophycocyanin (clone GK1.5), CD45–Pacific Blue (clone 30-F11), and CD11b–PE-Cy7 (M1/70) were from eBioscience; IFN-γ–FITC (clone XMG1.2), IL-17–PCP-Cy5.5 (clone TC11-18H10), TCR–allophycocyanin-Cy7 (clone H57-597), Ly6G–FITC (clone 1A8), Ly6C–PCP-Cy5.5 (clone HK1.4), and CD31–allophycocyanin (clone MEC 13.3) were from BD Biosciences. Cells were analyzed using a FACSComp cytometer (BD Biosciences) and FlowJo software version 8.8.7 (Tree Star).

Quantitative PCR

For tissue samples, brain and spinal cord tissues were harvested from perfused naive mice or mice with EAE (1–3 d after onset, grade ≥ 3) and snap-frozen in liquid nitrogen. mRNA was extracted using RNAeasy Midi (QIAGEN) kit from tissue homogenates. cDNA was synthesized using SuperScript II (Invitrogen). Quantitative PCR was performed on an ABI 7300 real-time PCR system (Applied Biosystems). Gene expression was normalized to values for GAPDH.

In vivo treatments during EAE

The neutrophil-depleting Ab anti-Ly-6G (clone 1A8) and isotype control Ab (clone 2A3) were purchased from Bio X Cell (West Lebanon, NH). Beginning on the day of T cell transfer, either 200 μg anti-Ly-6G or isotype control (clone 2A3) was administered by i.p. injection every other day until time of sacrifice (typically 7–8 d posttransfer). SB332235, the small molecule competitive antagonist of CXCR2 provided by GlaxoSmithKline (500 μg dissolved in 200 μl H2O), or vehicle control (H2O) was administered to mice by oral gavage two to three times daily beginning on day of T cell transfer and continued until time of sacrifice (typically 7–8 d posttransfer).

Histochemical analysis

Brains and spinal cords from mice with EAE were preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with H&E. Sections were examined by a board-certified veterinary pathologist who was blinded to group assignments. The total sectional tissue area of multiple brain and cord sections from each mouse and the area of each focal, aggregated inflammatory/necrotic lesion within each section were measured using Nikon NIS-Elements software and expressed as percentage of total brain or spinal cord. Numbers of vessels cuff by inflammatory cells within each section were also counted and normalized to total tissue area. Included in the analysis was a semiquantitative assessment of character and severity of tissue injury (manifested by necrotic and apoptotic cell death coupled with inflammatory cell accumulation graded on an inclusive scale of 1+ [minimal] to 4+ [maximal] injury).

Statistical analysis

Statistical analyses were performed with Prism version 5.0 (GraphPad Software) using an unpaired two-tailed Student t test or χ² test. A p value <0.05 was considered significantly different.

Results

IL-17 and IFN-γ signaling differentially affect clinical manifestation of EAE

We previously showed that WT C3HeB/FeJ mice develop atypical EAE with or without accompanying classic EAE signs following adoptive transfer of Th17-skewed MOG-specific T cells (10). In the present study, we developed genetic models to define the mechanisms by which IL-17 and IFN-γ signaling determines lesion localization and clinical manifestation of EAE. EAE was induced by transferring CD4+ T cells from WT MOG-immunized mice into WT, IL-17RA−/−, IFN-γ receptor α-chain−/− (IFN-γR−/−), or IL-17RA−/− × IFN-γR−/− double knockout mice. The transferred T cells were skewed in vitro by incubation with IL-23 prior to transfer (Th17/Th1 ratio of ~2:1) to predispose the WT recipients toward atypical EAE and to bypass any potential effects of IL-17 or IFN-γ signaling on development or priming of myelin-specific T cells. In mice deficient in both cytokine receptors, EAE incidence was reduced to 22% (6 of 27) compared with 93% (97 of 104) for WT mice. In contrast, EAE incidence was only modestly reduced in both IL-17RA−/− mice and IFN-γR−/− mice (Fig. 1A). Importantly, deficiency in either cytokine receptor alone significantly impacted the clinical manifestation of disease. In IL-17RA−/− mice that developed EAE, both the severity and incidence of atypical signs were sharply reduced compared with WT mice (Fig. 1B, 1C), supporting our previous finding that IL-17 signaling promotes atypical EAE (10). However, the severity and incidence of classic EAE signs among these same mice was unchanged (Fig. 1B, 1D). In contrast, IFN-γR−/− recipients of IL-23-skewed T cells demonstrated similar severity but increased incidence of atypical EAE signs compared with WT mice (Fig. 1E, 1F), consistent with other reports that IFN-γ signaling inhibits the incidence of brain inflammation (2, 4, 9). Interestingly, both the severity and incidence of classic EAE in these same mice

(Applied Biosystems). Gene expression was normalized to values for GAPDH.

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was significantly reduced compared with WT recipients (Fig. 1E, 1G), supporting the notion that IFN-γ promotes inflammation in the spinal cord (2, 4), despite its inhibitory effect in the brain. Collectively, these data indicate that both IL-17 and IFN-γ signaling promote overall EAE development, but do so by differentially influencing the development of atypical versus classic EAE signs.

**IFN-γ and IL-17 differentially regulate neutrophil recruitment to the brain and spinal cord via opposing effects on CXCL2 expression**

To define the mechanisms by which IL-17 and IFN-γ signaling exert opposite effects on induction of brain inflammation, we analyzed the inflammatory infiltrate in the brains of WT, IL-17RA−/−, IFN-γR−/−, and IL-17RA−/− × IFN-γR−/− mice. The number of WT mice used as controls for transfers into recipients of each genetic background is indicated. (B) Representative disease course for 5 IL-17RA−/− and 6 WT mice is shown (top) and clinical characteristics are summarized for all 59 IL-17RA−/− and 97 WT control recipients with EAE (bottom). Clinical data are compiled from eight independent experiments. (C) The percentage of mice with EAE that exhibited atypical clinical signs with or without accompanying classic clinical signs is shown for the 59 IL-17RA−/− and 97 WT control recipients. (D) The percentage of the same mice shown in (C) that exhibited classic EAE signs with or without accompanying atypical EAE signs is shown. (E) Representative disease course for 9 IFN-γR−/− and 13 WT recipients is shown and clinical characteristics are summarized for all 35 IFN-γR−/− and 65 WT control recipients with EAE. Data are compiled from four independent experiments. (F) The percentage of mice that exhibited atypical clinical signs with or without accompanying classic signs is shown for the 35 IFN-γR−/− and 65 WT control recipients with EAE. (G) The percentage of the same mice shown in (F) that exhibited classic EAE signs with or without accompanying atypical EAE signs is shown. *p < 0.05, **p < 0.01, ****p < 0.0001.

FIGURE 1. IL-17 and IFN-γ signaling influence clinical manifestation of EAE. (A) Overall incidence of disease is shown for EAE induced by adoptive transfer of IL-23–skewed WT T cells into WT, IL-17RA−/−, IFN-γR−/−, and IL-17RA−/− × IFN-γR−/− recipients. The number of WT mice used as controls for transfers into recipients of each genetic background is indicated. (B) Representative disease course for 5 IL-17RA−/− and 6 WT mice is shown (top) and clinical characteristics are summarized for all 59 IL-17RA−/− and 97 WT control recipients with EAE (bottom). Clinical data are compiled from eight independent experiments. (C) The percentage of mice with EAE that exhibited atypical clinical signs with or without accompanying classic clinical signs is shown for the 59 IL-17RA−/− and 97 WT control recipients. (D) The percentage of the same mice shown in (C) that exhibited classic EAE signs with or without accompanying atypical EAE signs is shown. (E) Representative disease course for 9 IFN-γR−/− and 13 WT recipients is shown and clinical characteristics are summarized for all 35 IFN-γR−/− and 65 WT control recipients with EAE. Data are compiled from four independent experiments. (F) The percentage of mice that exhibited atypical clinical signs with or without accompanying classic signs is shown for the 35 IFN-γR−/− and 65 WT control recipients with EAE. (G) The percentage of the same mice shown in (F) that exhibited classic EAE signs with or without accompanying atypical EAE signs is shown. *p < 0.05, **p < 0.01, ****p < 0.0001.
We investigated the mechanism by which IL-17 and IFN-γ exert their disparate effects on neutrophil recruitment by analyzing the expression of ELR<sup>+</sup> chemokines in the brains of IL-17RA<sup>−/−</sup> and IFN-γR<sup>−/−</sup> mice. ELR<sup>+</sup> chemokines attract neutrophils to inflamed tissues and are induced in response to inflammatory cytokines such as IL-17 and IL-1β (15). We found that CXCL1, CXCL2, and CXCL5 were induced in WT brains of mice with EAE compared with healthy brains, with CXCL2 induced to the greatest extent (Fig. 2C). As expected, expression of CXCL1, CXCL2, and CXCL5 was significantly decreased in the brains of IL-17RA<sup>−/−</sup> mice (Fig. 2D). Un-
expected, IFN-γ appeared to exert the opposite effect, as CXCL2 expression trended higher in the brains of IFN-γR−/− mice (Fig. 2D), which may account for the greater accumulation of neutrophils in the brains of IFN-γR−/− compared with WT mice. Thus, the differential recruitment of neutrophils to the brain in IL-17RA−/− versus IFN-γR−/− mice correlates with an essential role for IL-17 in promoting CXCL1, CXCL2, and CXCL5 expression as well as a potentially inhibitory effect of IFN-γ signaling on CXCL2 expression.

Our observations that atypical EAE correlated with neutrophil recruitment and that atypical but not classic EAE was decreased in IL-17RA−/− mice suggested that neutrophil recruitment to the spinal cord may not depend on IL-17 signaling. Indeed, we found no difference in neutrophil number in spinal cords of WT and IL-17RA−/− mice with EAE (Fig. 3A), in contrast to our results for the brain. Surprisingly, neutrophil numbers were significantly reduced in IFN-γR−/− compared with WT mice (Fig. 3B). Thus, IFN-γ signaling promotes neutrophil recruitment to the spinal cord but inhibits neutrophil influx into the brain. Analyses of ELR+ chemokine expression in the spinal cord during EAE in WT mice demonstrated that, as seen in the brain, CXCL2 induction was much greater than that of CXCL1 or CXCL5; furthermore, its induction was 10-fold greater in the spinal cord compared with the brain (Fig. 3C). CXCL2 was induced to comparable levels in the spinal cord and brain of WT mice (Fig. 3D), indicating that IFN-γ signaling promotes neutrophil recruitment to the spinal cord but not to the brain. Importantly, CXCL2 induction was significantly decreased in the spinal cord of IFN-γR−/− mice compared with WT mice (Fig. 3D), indicating that IFN-γ signaling promotes CXCL2 induction in the spinal cord in WT mice. Differences between the small induction of CXCL1 and CXCL5 seen in WT EAE mice and their induction in IL-17RA−/− and IFN-γR−/− mice did not correlate with neutrophil recruitment in the spinal cords of these mice. Thus, CXCL2 was induced in the spinal cord via an IL-17-independent but IFN-γ-dependent mechanism, and its induction correlated with neutrophil recruitment to this region in EAE.

We investigated the source of CXCL2 during EAE by sorting different subsets from CNS mononuclear cells at peak disease and analyzing CXCL2 expression by quantitative PCR. Astrocytes were the predominant producers in both the brain and spinal cord and, as observed for whole tissue, CXCL2 induction was much greater in spinal cord compared with brain astrocytes (Fig. 4A, 4B). As our data predicted, CXCL2 induction was reduced in astrocytes from brains but not spinal cords of IL-17RA−/− compared with WT mice (Fig. 4C). The observation that IFN-γ promotes CXCL2 induction by astrocytes was surprising because IFN-γ is more commonly known for inducing chemokines that attract monocytes (16, 17). Therefore, we hypothesized that IFN-γ may induce CXCL2 indirectly via induction of other inflammatory mediators. Because IFN-γ signaling can promote expression of IL-1β, a known inducer of ELR+ chemokines (18), we analyzed IL-1β expression in brain and spinal cords of WT and IFN-γR−/− mice. Although no differences were observed in the brain, IL-1β induction was significantly decreased (~5-fold, p < 0.01) in spinal cords of IFN-γR−/− compared with WT mice (Fig. 5). Thus, IFN-γ may induce CXCL2 in the spinal cord but not the brain via its spinal cord–specific induction of IL-1β.

**Neutrophil depletion and disruption of CXCR2 signaling prevent parenchymal tissue injury in the brain but not spinal cord**

Our finding that neutrophil recruitment to a particular CNS region (brain or spinal cord) correlated with clinical signs reflecting inflammation in that region suggested that neutrophils may play a critical role in promoting CNS tissue injury during EAE. To test this hypothesis, we administered the neutrophil-specific anti-Ly6G Ab beginning on the day of T cell transfer. Neutrophils were reduced by 90% in the blood without affecting either monocytes or lymphocyte number (Fig. 6A). Surprisingly, neutrophil depletion did not affect the overall incidence, onset, or severity of EAE. However, neutrophil depletion had a striking effect on the clinical manifestation of EAE (Fig. 6B). The incidence of atypical signs was dramatically reduced in neutrophil-depleted compared with control mice. In contrast, neither the incidence (Fig. 6B) nor severity (data not shown) of classic EAE signs was affected by treatment with neutrophil-depleting Ab. These data suggest that neutrophils are required for brain, but not spinal cord, inflammation during EAE.
Because our data implicate CXCL2 as a major determinant of neutrophil recruitment to both the brain and spinal cord, we investigated whether blocking ELR+ chemokine signaling in vivo would recapitulate the therapeutic effect of neutrophil depletion by ameliorating atypical EAE. A small molecule antagonist of CXCR2, the main receptor for ELR+ chemokines on neutrophils, was administered daily beginning on the day of T cell transfer. The onset, peak severity, and incidence of classic EAE signs were similar between CXCR2 antagonist–treated and control mice (Fig. 6C). Importantly, the incidence of atypical signs was reduced from 73% in mice treated with vehicle control to 0% in antagonist-treated mice (Fig. 6C). Thus, antagonizing CXCR2 signaling in vivo during EAE is therapeutically equivalent to neutrophil depletion in preventing clinical signs of brain inflammation.

To confirm that the dramatic reduction in atypical clinical signs that was observed upon neutrophil depletion reflected decreased tissue damage in the brain, we performed histopathological analyses of brain sections from neutrophil-depleted and control mice. Loss of blood vessel wall integrity and extensive infiltration of parenchymal brain tissue by inflammatory cells as well as occasional hemorrhage were observed in mice treated with isotype control Ab (Fig. 7A, 7B, left). In contrast, inflammatory cells...
consisting primarily of mononuclear cells were predominantly localized around blood vessels, and significant migration into the surrounding parenchyma was not seen in the brains of neutrophil-depleted mice (Fig. 7A, 7B, right). This distribution of inflammatory cells resulted in a pattern of pronounced perivascular cuffing that was not commonly seen in comparable sections from control mice. These changes caused a significant reduction in the percent area of brain sections populated by inflammatory foci in anti-Ly6G–treated compared with control mice (Fig. 7C, left). We also quantified the extent of tissue damage observed in tissue sections by assigning a tissue injury score based on the extent of necrotic and apoptotic cell death associated with inflammatory lesions. Importantaly, a significant reduction in tissue injury was observed in the brains of neutrophil-depleted versus control mice (Fig. 7C, middle), accompanied by an increase in the number of perivascular cuffs per section (Fig. 7C, right). Despite the impaired parenchymal migration of inflammatory cells in the brains of neutrophil-depleted mice, a diffuse pattern of gliosis was occasionally observed in the parenchyma of anti-Ly6G–treated mice, which may reflect the activity of soluble inflammatory mediators.

Overall, these findings in the brain are consistent with the ability of neutrophils to affect the integrity of the blood/brain barrier and enhance migration of inflammatory cells beyond the perivascular space to promote parenchymal tissue injury.

Our unexpected finding that anti-Ly6G treatment did not ameliorate classic EAE signs despite a >90% reduction in neutrophil number in the spinal cord (Fig. 8A) suggested that the spinal cord may differ from the brain in its dependence on neutrophil infiltration for tissue injury. Indeed, we found that although spinal cords in control mice exhibited extensive parenchymal involvement (Fig. 8B, left) and spinal cords from anti-Ly6G–treated mice demonstrated increased perivascular cuffing (Fig. 8B, right), there was only a modest reduction in the extent of tissue injury in treated compared with control animals. Strikingly, the extent of necrotic and apoptotic cell death associated with inflammatory lesions in the spinal cord of anti-Ly6G–treated mice was much greater than that seen in brain tissue analyzed from the same mice. Thus, despite the decrease in the area populated by inflammatory cells in the spinal cords of neutrophil-depleted mice (Fig. 8C, left), there was only a 25% decrease in tissue injury (Fig. 8C, middle) compared with the 75% decrease in tissue injury seen in the brain (Fig. 7C). These results, together with the ability of neutrophil depletion to affect atypical but not classic EAE signs, indicate that preventing neutrophil infiltration during CNS autoimmunity has a much greater impact on tissue injury in the brain compared with the spinal cord.

**Discussion**

We show in the present study that the mechanism by which IL-17 and IFN-γ influence lesion formation in the brain arises from their ability to regulate, in an opposing fashion, CXCL2 induction and neutrophil recruitment to this microenvironment. Regulation of CXCL2 expression by IFN-γ is particularly striking, as IFN-γ inhibits CXCL2 production in the brain but promotes its induction in the spinal cord. Interestingly, we found that astrocytes are the predominant source of CXCL2 in both microenvironments, yet we...
found no differences by flow cytometry in IFN-γR expression by brain versus spinal cord astrocytes (data not shown). Our finding that IFN-γ signaling also induced IL-1β in the spinal cord but not the brain suggests that IFN-γ could induce CXCL2 indirectly in the spinal cord via induction of IL-1β, and it further highlights the profound differences in response to inflammatory stimuli within the brain versus spinal cord. Our results also establish a critical role for neutrophil recruitment to the brain by demonstrating that neutrophils are required to promote the disseminated leukocyte infiltration that leads to severe tissue damage in the brain. In the absence of neutrophils, inflammatory cells were largely retained in perivascular spaces, resulting in only mild gliosis in the surrounding brain tissue. This is consistent with other reports that neutrophils enhance blood/brain barrier permeability (19) and promote invasion of immune cells into tissue parenchyma (8, 20). Consistent with the critical role for neutrophils that we identified in mediating tissue injury in the brain, Stoolman et al. (see companion article, Ref. 21) found that CXCL2-mediated neutrophil recruitment to the brain was required for manifestation of atypical EAE even when disease was induced by Th1 cells that do not depend on IL-17 signaling. Our finding that neutrophils play a less obligatory role in promoting tissue damage in the spinal cord was therefore very surprising and has important therapeutic implications. Some tissue damage in the spinal cord was ameliorated by neutrophil depletion, but the protection afforded by this treatment was much less than that observed in the brain. It is possible that the small number of neutrophils that remain following treatment with anti-Ly6G is sufficient to induce injury in the spinal cord. However, whereas atypical EAE signs were abolished by treatment with a CXCR2 antagonist, 100% of these mice still developed classic EAE with comparable severity as that seen in control mice. This observation supports the notion that spinal cord tissue may be more sensitive than brain tissue to injury mediated by inflammatory cells other than neutrophils that are recruited via a CXCR2-independent mechanism. Consistent with our results, Stoolman et al. (21) suggest a more important role for monocytes rather than neutrophils in mediating spinal cord injury.

The mechanisms that regulate spinal cord inflammation in EAE have been controversial. IL-17 signaling was shown to be important in some (22–25), but not all (9, 10, 26), models of classic EAE. Likewise, IFN-γ was shown to promote spinal cord inflammation in some (2, 4, 27), but not all (9, 20), classic EAE models. Although these disparate results may reflect differences in EAE models, our data suggest that distinct inflammatory cell types may play more redundant roles in inducing spinal cord inflammation compared with the brain.

Previous studies in patients with MS, most of whom have lesions in the brain parenchyma, support a role for an IL-17–induced ELR+ chemokine pathway in the pathogenesis of the disease. IL-17 transcript–expressing mononuclear cells are enriched in blood and cerebrospinal fluid (CSF) of patients with MS compared with healthy controls, and they are enriched in the CSF of patients with MS during exacerbation compared with remission (28). Additionally, protein levels of CXCL8, the human ortholog for CXCL1 and CXCL2, are increased in the CSF of patients with MS (29). Importantly, early phase IIa clinical trials indicated that treatment with secukinumab, a mAb directed against IL-17A, resulted in a reduction in inflammatory lesions in the brain and a trend toward reduced clinical relapses in MS patients compared with placebo-treated controls (30). Nevertheless, a role for neutrophils in MS has been controversial because they are not often found in CNS tissue obtained either postmortem or by biopsy from patients with
MS. Notably, our studies suggest that neutrophils could be most relevant during the early stages of MS by facilitating the initial leukocyte trafficking from the perivascular space into the brain parenchyma, whereas tissue sections from MS patients are typically analyzed long after disease onset. The observations that the proportion of neutrophils in the CSF of pediatric MS patients decreases with increasing disease duration whereas the proportion of lymphocytes and monocytes increases (31) support the notion that neutrophils may be most important during the initial stages of disease. A role for neutrophils in chronic disease, however, is also suggested by the observation that neutrophils in the blood of MS patients exhibit a primed phenotype compared with healthy controls and that neutrophil numbers increase during disease relapse (32). Additionally, treatment with recombinant human G-CSF, which supports neutrophil activation, worsens the clinical status in patients with MS (33), and neutrophil depletion in a relapsing-remitting EAE model inhibited relapses (19). Taken together, these observations suggest that neutrophils may play a more important role in the pathogenesis of MS than previously appreciated. In conclusion, our results suggest that therapeutic strategies that decrease neutrophil recruitment may be most beneficial in patients with MS when lesions dominate in the brain compared with spinal cord. Thus, the predominant site of lesion activity in a patient may be an important parameter in designing effective therapies.

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Disclosures
The authors have no financial conflicts of interest.

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Supplementary figure 1. Gating strategy for analyzing specific immune cell subsets isolated from the CNS. Cells were isolated from the brain or spinal cord of mice with EAE and stained with antibodies specific for CD11b, Ly6G, Ly6C, TCR and CD4. Live cells were gated based on FSC and SSC. Neutrophils are within the CD11b$^+$Ly6G$^+$ gate, Monocytes are within the CD11b$^+$Ly6C$^{hi}$ gate, and CD4$^+$ T cells are within the TCR$^+$CD4$^+$ gate.