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B Cells Promote Induction of Experimental Autoimmune Encephalomyelitis by Facilitating Reactivation of T Cells in the Central Nervous System

Emily R. Pierson, Ingunn M. Stromnes, and Joan M. Goverman

The efficacy of rituximab treatment in multiple sclerosis has renewed interest in the role of B cells in CNS autoimmunity. In this study, we show that B cells are the predominant MHC class II+ subset in the naive CNS in mice, and they constitutively express proinflammatory cytokines. Incidence of experimental autoimmune encephalomyelitis induced by adoptive transfer was significantly reduced in C3HeB/Fej μMT (B cell–deficient) mice, suggesting an important role for CNS B cells in initiating inflammatory responses. Initial T cell infiltration of the CNS occurred normally in μMT mice; however, lack of production of T cell cytokines and other immune mediators indicated impaired T cell reactivation. Subsequent recruitment of immune cells from the periphery driven by this initial T cell reactivation did not occur in μMT mice. B cells required exogenous IL-1β to reactivate Th17 but not Th1 cells in vitro. Similarly, reactivation of Th1 cells infiltrating the CNS was selectively impaired compared with Th17 cells in μMT mice, causing an increased Th17/Th1 ratio in the CNS at experimental autoimmune encephalomyelitis onset and enhanced brain inflammation. These studies reveal an important role for B cells within the CNS in reactivating T cells and influencing the clinical manifestation of disease. The Journal of Immunology, 2014, 192: 929–939.

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the CNS. Lesions comprised of immune cell infiltrates, plaques of demyelination, and axonal damage are hallmarks of MS, although substantial variation is seen among patients in both disease course and pathological features (1). Most patients with MS exhibit lesions primarily in the brain; only a small subset of patients exhibit lesions in the spinal cord without substantial involvement of the brain (2). The mechanisms that govern the pathological spectrum in MS, including localization of inflammatory lesions within the CNS, are poorly understood. Experimental autoimmune encephalomyelitis (EAE) is an animal model that has been widely used to study the pathogenesis of MS. EAE is induced by activation of T cells specific for myelin proteins such as myelin oligodendrocyte glycoprotein (MOG), either by immunization with myelin Ags in adjuvant (active induction) or by adoptive transfer of myelin-specific CD4+ T cells (passive induction) (3). Once the activated myelin-specific T cells cross the blood–brain barrier and infiltrate the CNS, they are reactivated upon encounter with local APCs presenting endogenous myelin Ag. T cell reactivation triggers an inflammatory cascade that ultimately results in demyelination and axonal loss (3).

In contrast to MS, parenchymal lesions are localized predominantly in the spinal cord in most EAE models. However, we developed a new EAE model in C3HeB/Fej mice in which significant inflammation and tissue damage occurs in the brain as well as the spinal cord (4). In contrast to the C57BL/6 EAE model, this model has the unique advantage that mechanisms responsible for regulating inflammation in the brain versus the spinal cord can be studied. We found that the ratio of myelin-specific T cells secreting IL-17 (Th17) to T cells secreting IFN-γ (Th1) infiltrating the CNS is a critical factor in determining whether inflammation is induced in the brain. T cells that are primed by immunization with MOG exhibit a higher Th17/Th1 ratio in C3HeB/Fej mice compared with other strains, and this higher ratio generates the conditions necessary to induce brain inflammation. In contrast, spinal cord inflammation occurred over a wide range of Th17/Th1 ratios, demonstrating that inflammatory responses triggered by T cell reactivation are regulated differently in the brain compared with the spinal cord.

Although much attention has focused on the role of T cells in EAE, the observation that treatment with a B cell–depleting mAb targeting CD20 (rituximab) can reduce the number of lesions and relapses in relapsing-remitting MS patients has renewed interest in the role of B cells in the pathogenesis of MS (5, 6). The mechanisms by which B cell depletion ameliorates the clinical signs of MS are not understood. Early studies in patients with MS demonstrated a role for B cells in the production of Abs that bind myelin components, promoting inflammation and demyelination (7). However, treatment with rituximab does not deplete Ab-secreting plasma cells, suggesting that other B cell functions such as Ag presentation or cytokine production may be responsible for their pathogenic effects in MS. In support of a pathogenic role for B cells in promoting T cell activity, B cell depletion in MS patients following rituximab treatment resulted in a decrease in absolute number of T cells in the cerebrospinal fluid (8), as well as decreased cytokine-producing and proliferating T cells among PBMCs (9).
Studies in EAE suggested that B cells exert distinct pathogenic or regulatory functions depending on the stage of disease pathogenesis. In some studies that actively induced EAE in B cell–deficient (μMT) mice, a regulatory role for B cells was observed that was associated with B cell–dependent production of IL-10 (10, 11). More recently, EAE has been studied using anti-CD20 mAb to deplete B cells. In one study, B cell depletion prior to active EAE induction with MOG35–55 peptide exacerbated disease, which was associated with loss of an IL-10–secreting regulatory B cell subset (12). In contrast, B cell depletion after EAE onset decreased disease severity and reduced the proliferation of naive T cells, suggesting that B cell–mediated activation of T cells may be more influential than regulatory activity later in disease (12).

Another study found that immunization with rMOG protein versus MOG peptide influenced the outcome of B cell depletion. Depletion of B cells either before or after active induction of EAE with MOG peptide resulted in more severe EAE (13). However, when EAE was induced by immunization with rMOG, B cell depletion before or after induction of EAE resulted in reduced severity of disease. This study suggested that B cells may promote EAE induction by acting as APCs that process and present MOG protein, as peripheral B cells exhibited evidence of activation upon immunization with MOG protein but not peptide (13).

Other studies that focused on T cell responses to myelin protein rather than synthetic peptide also implicated a pathogenic role for B cells as APCs. In a model that combined a MOG–specific transgenic TCR with a transgenic H chain from a MOG–specific BCR, the increased frequency of MOG–specific B cells promoted activation and cytokine production by the transgenic T cells in vivo, which increased the incidence of spontaneous EAE compared with mice expressing only the transgenic TCR (14, 15). In another study, mice expressing a different transgenic MOG–specific TCR developed spontaneous EAE that was accompanied by production of anti-MOG–specific Abs by endogenous B cells (16). Importantly, anti-CD20–mediated depletion of B cells in this model largely eliminated the incidence of spontaneous EAE, suggesting that T cell interactions with B cells played a crucial role in initiating the spontaneous disease. Cytokines secreted by B cells may play an important role in shaping the outcome of T cell/B cell interactions that occur during CNS autoimmune disease. Secretion of IL-10 by naive B cells and TNF-α, lymphotoxin, and IL-6 by memory B cells has been implicated in the regulation of MS and EAE (9, 11, 17, 18). Production of these and other cytokines during Ag presentation to T cells could influence the effector function of the activated T cells. However, it is not known whether B cell production of these cytokines is important in the periphery and/or the CNS.

The studies described above suggest that B cells can play different roles at different times during the pathogenesis of CNS autoimmunity. However, these studies did not determine whether the B cell activities that influence CNS autoimmunity occur in the periphery or within the CNS. In the present study we addressed the role of B cells within the CNS during the earliest phase of T cell entry when reactivation of T cells is required. Surprisingly, we found that B cells represent most MHC class II+ cells in the naive CNS, and that they constitutively express proinflammatory cytokines. In the absence of B cells, adoptively transferred T cells still infiltrate the CNS, but in contrast to wild-type (WT) mice, they do not trigger production of immune mediators necessary for recruitment of cells from the periphery. We also found that B cells preferentially reactivate IFN-γ–producing T cells. Similarly, the absence of B cells in μMT mice resulted in impaired reactivation of Th1 compared with Th17 cells in the CNS. This caused an increase in the Th17/Th1 ratio of T cells in the brain and altered the clinical manifestation of disease. Taken together, our data identify novel mechanisms by which B cells not only contribute to the initiation of inflammatory responses in the CNS but may also influence the localization of lesions within the brain and spinal cord.

### Materials and Methods

#### Mice

C3HeB/Fej, C3.SW-H-2b/Sn (C3H.SW), B6.129S2-Igh-6tm1Cgn/J, and B10.PL mice were purchased from The Jackson Laboratory and maintained in a specific pathogen-free facility at the University of Washington. To generate μMT mice, the B6.129S2-Igh-6tm1Cgn/J strain was backcrossed to C3HeB/Fej and C3H.SW mice for 12 generations. The Institutional Animal Care and Use Committee at the University of Washington approved all procedures.

**rMOG protein and peptides**

Rat rMOG protein (1–125) was produced in *Escherichia coli* and purified as previously described (19). MOG peptides 79–90 (GKVALRIQNVRF) and 97–114 (TCFRFDHYSQEAEEVLK) were synthesized by GenScript.

#### Active EAE Induction

Active EAE was induced by immunizing 8- to 12-wk-old mice s.c. with 100 μg rMOG emulsified in CFA containing 1 mg/ml heat-killed mycobacteria (Sigma-Aldrich), accompanied by two injections of 200 ng pertussis toxin (List Biological Laboratories), as previously described (20). Animals were observed daily for clinical signs. We scored the severity of EAE as follows: grade 1, paralyzed tail, hindlimb claspings, hyperactivity; grade 2, head tilt, hindlimb weakness; grade 3, one paralyzed leg, mild body leaning; grade 4, two paralyzed legs, moderate body leaning; grade 5, forelimb weakness, severe body leaning; grade 6, hunched, breathing difficulty, body rolling; grade 7, moribund. Atypical EAE was determined by the presence of one or more of the following symptoms: hyperactivity, head tilt, body leaning, and rolling.

#### Passive EAE induction/adoptive transfers

Cells were isolated from spleen and lymph nodes of WT mice 7 d after rMOG immunization and cultured at 1 × 10^7^ cells/ml for 3 d with MOG97–114 (10 μM). To generate cells for transfer with a Th1/Th1 ratio of 1:1, we included 10 ng/ml IL-23 (R&D Systems) in the culture. To skew cells toward a Th1 phenotype (Th1/Th1 ratio of ~1:8), we included 10 ng/ml IL-12 (eBioscience). To skew cells toward a Th17 phenotype (Th17/Th1 ratio of ~3:1), we included 10 ng/ml IL-23 (R&D Systems) and 10 μg/ml anti–IFN-γ (XMG1.2; eBioscience). Viable cells were isolated from a Lympholyte gradient (Cedarlane Laboratories) and i.p. injected (2 × 10^7^ cells/mouse) into mice that were sublethally irradiated (250 rads) on day −1. In some experiments, we purified the CD4^+^ T cells using a CD4^+^ T cell isolation kit and an autoMACS separator (Miltenyi Biotec) and injected 5 × 10^6^ CD4^+^ T cells i.p. into nonirradiated mice. The severity of EAE was scored as described above.

#### Passive EAE induction/adoptive transfers

Mononuclear cells were isolated from the CNS after cardiac perfusion with PBS as previously described (21). Briefly, brain and spinal cord were dissociated through sterile stainless steel mesh and centrifuged at 4°C for 10 min at 3000 rpm. Cell pellets were resuspended in 30% Percoll, overlaid onto 70% Percoll, and centrifuged without brake at 25°C for 20 min at 2600 rpm. Cells were collected from the 30–70% Percoll interface.

#### Flow cytometry

Cells were incubated with Fc block (clone 2.4G2; eBioscience) in 5% normal mouse serum for 15 min at room temperature, washed, and stained with mAbs for 30 min at 4°C. mAbs for CD45 (30-F11), CD19 (1D3), F4/80 (BM8), CD11b (M1/70), and CD11c (N418) were from eBioscience. mAbs for MHC class II (I-A^b^, 11-5.2), CD4 (RM4-5), Thy1.1 (OX7), CD79b (HM79b), CD138 (281-2), CD80 (16-10A1), CD86 (GL1), and CD43 (S7) were from BD Biosciences. Intracellular cytokine staining for IL-17 and IFN-γ was performed according to manufacturer’s directions using mAbs and staining kits from BD Biosciences. BrdU and annexin V staining kits were purchased from BD Biosciences.

#### In vivo T cell recruitment assay

Thy1.1^+^ T cells from MOG-immunized donors were activated in vitro for 3 d with MOG97–114 and transferred into WT Thy1.2 recipients. Either 3...
mg/kg FTY720 or vehicle (5% DMSO) was injected i.p. daily beginning on day 4 posttransfer. CNS mononuclear cells were isolated from mice on day 7 for analysis.

**ELISPOT assays**

Numbers of Ag-specific cytokine-producing cells were determined by culturing cells overnight with and without Ag in duplicate wells of 96-well ELISPOT plates (Millipore). ELISPOT assays were carried out according to BD Biosciences protocols and analyzed on an ImmunoSpot analyzer (Cellular Technology). IFN-γ-specific mAbs and IL-17–specific (TC11-18H10 and biotinylated IL-17–specific (TC11-8H4.1) mAbs were from BD Biosciences. For detection of IL-17– and IFN-γ–producing cells in the CNS of mice with EAE, total mononuclear cells isolated separately from the brains and spinal cords of perfused mice (typically 1–10 × 10⁶ cells/well) were plated with or without MOG₉₇₋₁₁₄ (10 μM).

**Th1 and Th17 reactivation assays**

Th1 and Th17 cells were generated by culturing cells isolated from spleen and lymph nodes of WT mice immunized 7 d earlier with rMOG at 1 × 10⁷ cells/ml for 3 d with MOG₉₇₋₁₁₄ (10 μM). The cultures included IL-12 (10 ng/ml; eBioscience) to generate Th1-skewed cells and IL-23 (10 ng/ml; R&D Systems) and anti–IFN-γ (10 μg/ml; XMGI.2; eBioscience) to generate Th17–skewed cells. Cells were split after 3 d and maintained in culture with 10 μM IL-2 without MOG₉₇₋₁₁₄ for an additional 4 d. CD₄⁺ T cells from these cultures as well as naive splenic B cells (CD19⁺CD43⁻) or non–B cells (CD43⁺, CD11c⁺, and CD11b⁺) were sorted on a FACSAria cell sorter (BD Biosciences) and the CD4⁺ T cells (5 × 10⁶ cells/well) were cocultured overnight with either B cells, non–B cells, or bulk splenocytes (each at 5 × 10⁵ cells/well) with or without 25 μg/ml rMOG. Where indicated as “activated,” the B or non–B cell APCs were stimulated with 10 μg/ml anti-CD40 (R&D Systems) and 20 μg/ml LPS for 4 h prior to culture. IL-23 (10 ng/ml; R&D Systems), IL-6 (20 ng/ml; eBioscience), and IL-1β (10 ng/ml; eBioscience) were included as indicated. The number of Ag-specific T cells producing IL-17 or IFN-γ was detected by ELISPOT as described. The percentage reactivation was calculated by normalizing the number of Ag-specific spots produced by either Th1 or Th17 cells in response to the indicated APCs to the number of Ag-specific IFN-γ or IL-17 spots obtained by coculture of either Th1 or Th17 cells with bulk naive splenocytes (100%). In ELISPOT experiments using CNS cells as APCs, bulk CNS cells were pooled from six to eight perfused, naive WT, or μMT mice and stained for MHC class II, CD19, CD11b, and CD11c to calculate the number of B cells and non–B cell APCs in the bulk population. The number of CNS cells plated in each ELISPOT well was normalized to the number of non–B cell APCs such that wells containing either WT or μMT CNS APCs had the same number of non–B cell APCs (≈7000/well), but wells with WT CNS APCs also contained ∼2 × 10⁶ B cells. Th1 and Th17 cells were generated and sorted as described above and cocultured overnight with the APCs and 25 μg/ml rMOG or media alone to determine numbers of Ag-specific IFN-γ or IL-17 spots.

**Real-time quantitative PCR of gene expression**

For analysis of gene expression in tissue, total RNA was isolated using the RNeasy Lipid Tissue Midi kit (Qiagen) from brains of recipients of MOG-specific T cells (Th17/Th1 ratio of ∼1:1) on day 5 posttransfer or after onset of EAE, or from healthy control mice on day 5 posttransfer of 5 × 10⁶ activated polyclonal T cells. The activated polyclonal T cells were generated by stimulating naive CD4⁺ T cells with anti-CD3/anti-CD28 Dynabeads according to Invitrogen protocols. For analysis of gene expression in spleen RNA, RNA was isolated from unstimulated cells sorted from the CNS of perfused naive WT mice or from mice of onset of EAE induced by adoptive transfer of CD4⁺ MOG-specific T cells (Th17/Th1 ratio of ∼1:1) into nonirradiated recipients. Mononuclear cells were pooled from the CNS of three to five mice, and ∼1 × 10⁵ B cells (CD45⁺CD19⁺CD11b⁻), plasmablasts (CD45⁺CD19⁺CD11b⁻), CD45⁺CD11b⁺CD11c⁺ cells, and microglia (CD45⁺CD11b⁻) were sorted using a FACSaria cell sorter (BD Biosciences). B cells were also sorted simultaneously from the spleen and blood of naive mice. Total RNA was isolated from cells directly ex vivo using the RNeasy Micro kit (Qiagen). cDNA was generated using the SuperScript III first-strand synthesis system (Invitrogen). Real-time quantitative PCR was performed in triplicate using SYBR Green PCR master mix and an AB7300 or ViiA7 (Applied Biosystems). All data were normalized to GAPDH. Data were analyzed using the comparative ΔΔCT method to obtain relative quantification values. Fold induction for day 5 brain tissue was calculated relative to healthy brain tissue from mice that had received anti-CD3/anti-CD28–activated T cells. Mouse primer sequences are listed in Supplemental Table I.

**Analysis of Th17 and Th1 cell priming in vivo**

WT or μMT mice were immunized with rMOG in CFA and injected with pertussis toxin. Splenocytes were isolated 7 d after immunization and plated at 1 × 10⁶ cells/well in anti-IL-17– or anti-IFN-γ–coated wells of an ELISPOT plate with or without rMOG (25 μg/ml), MOG₉₇₋₁₁₄ (10 μM), or MOG₉₇₋₁₁₄ (10 μM). After 16 h culture, IL-17– and IFN-γ–producing cells were detected as described above.

**Perfusion control**

Splenocytes isolated from naive WT mice were labeled with CFSE (1 μM; Molecular Probes) and 2 × 10⁶ cells were transferred i.v. into naive recipients. One hour later, half of the mice were perfused, whereas the other half were not perfused prior to isolation of CNS mononuclear cells. Cells were analyzed by flow cytometry to compare the percentage of CFSE⁺ cells within a CD45⁺CD19⁺ gate in CNS cells from perfused and unperfused mice.

**Statistical analysis**

Statistical analysis was performed using Prism software (GraphPad Software). Significance between groups was determined using either a Fisher exact test, χ² test, Student t test, or Mann–Whitney nonparametric t test, as indicated. A p value <0.05 was considered significant.

**Results**

**B cells influence both the priming and effector stages of EAE**

Our previous studies showed that C3HeB/Fej (H-2b) mice immunized with rMOG exhibited the spinal cord inflammation typically seen in EAE but differed from most mouse strains by also exhibiting extensive parenchymal brain inflammation. Inflammatory lesions were consistently observed in the cerebellar and periventricular white matter, brain stem, fimbria hippocampi, and cortex (4). Lesions in one or more of these areas of the brain correlate with clinical signs such as leaning, rolling, spasticity, and hyperreflexia, referred to as atypical EAE. In contrast, MHC congeneric C3H.SW (H-2b) mice developed classic EAE characterized by tail and hindlimb paralysis with parenchymal inflammation restricted predominantly to the spinal cord (4). The difference in inflammatory patterns was due to the preponderance of Th1 cells generated in C3HeB/Fej mice that respond to one of the epitopes in rMOG (MOG₉₇₋₁₁₄) presented by I-ₐ. The abundance of Th17 cells resulted in a Th17/Th1 ratio of ≥1 among T cells infiltrating the brain, which we found was a critical factor in promoting brain inflammation. In contrast, the MOG₃₅₋₅₅ epitope targeted in C3H.SW mice elicited a strong predominance of Th1 cells such that the Th17/Th1 ratio in the brain was <1 and brain inflammation was not induced. We also investigated whether B cells played a role in defining the clinical phenotype of EAE in C3HeB/Fej mice; however, our results showed that C3HeB/Fej WT and μMT (B cell–deficient) mice exhibited the same atypical EAE and neuroinflammatory patterns in actively induced EAE (4). Interestingly, the incidence of EAE in μMT mice was lower compared with WT mice, suggesting that B cells may influence the events that trigger EAE. To explore this finding further, we compared the incidence of EAE in both C3HeB/Fej and C3H.SW mice on the μMT background. The incidence of EAE induced by immunization with rodent rMOG protein was significantly reduced compared with WT mice for both strains (Table I). The μMT C3HeB/Fej and C3H.SW mice that did develop EAE exhibited a similar day of onset, clinical course, and severity compared with their WT controls. These data suggest that B cells may be important in EAE to trigger the initial inflammatory response, but may be less essential once the disease has been initiated in these strains.

Our results suggested that B cells play a pathogenic role in EAE by promoting initiation of disease, which is consistent with a function for B cells as APCs. We analyzed whether B cells influence priming
B cells are the predominant MHC class II+ population within T cell priming. In the effector stage of disease independent of their role during recipients (Table I), indicating that B cells play an important role to WT mice, significantly fewer T cells from immunized WT mice and restimulated in vitro with MOG97–114. Position of the MHC class II+ population such that CD11c+ cells inflammatory cells that occurs at onset of EAE changes the composition in the CNS during the initiation of EAE, which has not left. Our data on T cell priming are consistent with earlier studies that implicated a role for T cell/B cell interactions in the periphery in promoting EAE (14–16). However, the low incidence of EAE in µMT mice could also occur if B cells promote Ag presentation in the CNS during the initiation of EAE, which has not been addressed in previous studies. To investigate whether B cells play a role during the effector stage of disease when T cells are reactivated within the CNS, we induced EAE in C3HeB/Fej WT and µMT mice by adoptive transfer of T cells isolated from MOG- immunized WT mice and restimulated in vitro with MOG97–114. In these experiments, the ratio of Th17/Th1 cells in the trans­ferred population was ~1:1 (data not shown). The incidence of EAE was significantly reduced in µMT compared with WT recipients (Table I), indicating that B cells play an important role in the effector stage of disease independent of their role during T cell priming.

B cells are the predominant MHC class II+ population within the naive CNS

To determine whether B cells could influence the activity of CD4+ T cells that infiltrate the CNS, we first determined what percentage of MHC class II+ cells in the CNS of naive mice are B cells. Mononuclear cells were isolated from well-perfused, naive C3HeB/Fej mice and analyzed by flow cytometry. Surprisingly, we found that most MHC class II+ cells in the brain were CD19+ B cells (Fig. 1A, left), with the remaining MHC class II+ cells consisting primarily of CD11c+ dendritic cells. Control experiments that compared the B cell population in cells isolated from the CNS of perfused versus nonperfused mice following injection of labeled B cells confirmed that blood contamination did not account for the B cells that we observed in the naive CNS (Supplemental Fig. 2A). In the spinal cord, B cells represented an even greater fraction of the MHC class II+ population, with >90% of the MHC class II+ cells expressing CD19 (Fig. 1B). Thus, although the absolute number of B cells in the naive CNS is small, the abundance of B cells within the MHC class II+ population suggests that they could be the predominant APCs for the initial population of T cells infiltrating the noninflamed CNS. The large influx of inflammatory cells that occurs at onset of EAE changes the composition of the MHC class II+ population such that CD11c+ cells predominate and the percentage of CD19+ B cells is reduced (Fig. 1A, right). This suggests that B cells may be more influential as APCs during the initial reactivation of T cells required to trigger disease, whereas CD11c+ dendritic cells may be more influential at later stages of disease.

Because our data indicate that CD19+ cells comprise most MHC class II+ cells in the naive CNS, we examined their phenotype in greater detail. By analyzing CD19 expression among CD45+ cells, we detected two distinct populations of CD19+ cells in the brain and spinal cord that were differentiated by levels of CD45 and CD19 expression (Fig. 1C). The CD45hiCD19hi cells were MHC class II (I-Ak)+, CD79b+, CD138+, and predominantly IgM+, IgG1, IgG2a, and IgG3 (data not shown). This phenotype is typical of resting B cells found in secondary lymphoid tissues. In contrast, the CD45intCD19int cells expressed low–negative levels of I-Ak and were CD79b– and CD138– (Fig. 1C), a phenotype resembling short-lived, Ab-secreting plasmablasts. However, this plasmablast-like population observed in the CNS differed from plasmablasts in the spleen or blood of naive mice by their lack of CD79b expression and low expression of MHC class II and other activation markers. To confirm that these findings were not specific to the C3Heb/Fej strain, we analyzed CNS cells in B10.PL mice. The two CD19+ populations described above were also observed in this mouse strain, indicating that the predominance of B cells among MHC class II+ cells in the CNS is not unique to a particular mouse strain (Supplemental Fig. 2B).

Analyses of the absolute number of cells in the different cell subsets in the CNS of naive mice revealed that both the plasmablast-like cells and MHC class II+ B cells outnumbered the CD11c+ cells (Fig. 1D). In contrast, the number of CD11c+ dendritic cells in mice with EAE increased significantly accounting for their predominance in the MHC class II+ population. However, the absolute number of CD45hiCD19hi B cells also increased significantly in mice with EAE, suggesting that B cells may not only be the predominant APC at the initiation of disease, they could also play a substantial role after onset of EAE. Interestingly, the absolute number of CD45intCD19int plasmablast-like cells was dramatically reduced in mice with EAE, indicating that cells with this phenotype are not maintained in the inflammatory milieu in the CNS during EAE. However, a plasma cell population (CD45intCD19hiCD138−Syndecan-1−) was detected in mice with EAE that was comparable in number to the resting B cells (Fig. 1D).

To determine whether CNS B cells influence the local environment in the naive or inflamed CNS via cytokine production, we sorted B cells, plasmablasts, CD45hiCD11b+ cells, and microglia (CD45intCD11b+) from the CNS of naive and EAE mice and measured cytokine mRNA levels directly ex vivo (without stimulation). Interestingly, IL-12 p35 was expressed by CNS B cells from both naive and EAE mice at significantly higher levels than

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<th>Induction Method</th>
<th>Strain</th>
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<th>Mean Day of Onset</th>
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<td>Active C3H/Fej</td>
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*a Data shown (mean ± SEM) are from mice with EAE. The p values indicating significant differences between WT versus µMT mice are as follows: **p = 0.01, ***p = 0.03, ****p < 0.0001, χ² test.
by CD11b/c+ cells (Fig. 2). Naive CNS plasmablasts also expressed IL-12 p35. TNF-α mRNA expression was also detected in CNS B cells from both naive and EAE mice at higher levels than CD11b/c+ cells, although more TNF-α was produced by microglia than B cells during EAE. CNS B cells in naive mice constitutively expressed significantly higher levels of IL-12 p35 and TNF-α than B cells in the blood, indicating that CNS B cells have a functionally distinct phenotype compared with B cells in the periphery. IL-10, IL-1β, GM-CSF, IL-6, and IL-23 p19 transcripts were not detected in B cells sorted from the CNS of either naive or EAE mice (data not shown). Additionally, IL-12 p35 and TNF-α transcripts were not detected in CD45− cells from the CNS of naive mice, indicating that astrocytes do not produce these cytokines in healthy mice. Thus, CNS B cells and plasmablasts are the major source of IL-12 p35 and TNF-α within the noninflamed CNS during the initial T cell infiltration.

**CNS B cells promote T cell recruitment to the CNS**

Because most MHC class II+ cells in the naive CNS are B cells, we hypothesized that they may influence the earliest events in EAE induction that occur directly after T cells infiltrate the CNS. To test this, we transferred activated T cells isolated from rMOG-immunized mice into WT and μMT recipients and compared the numbers of donor T cells in the CNS on day 4 (preclinical) and day 7 (average day of EAE onset in WT mice). Comparable numbers of donor T cells were found in the CNS of both WT and μMT recipients on day 4 posttransfer (Fig. 3A). At this time point, the number of endogenous host T cells in the CNS of both types of recipients was not increased beyond that seen in the CNS of naive mice (data not shown). Donor T cells (5–10×10^5) were also found in the spleen on day 4 posttransfer in both WT and μMT recipients (data not shown). By day 7 posttransfer, the donor T cell number increased 100-fold relative to day 4 in the CNS of WT recipients (Fig. 3A), and the mice exhibited clinical signs of EAE. In contrast, donor T cell numbers decreased on day 7 in the CNS of μMT recipients relative to day 4 and the mice exhibited no clinical signs. Additionally, whereas the number of host T cells increased slightly in the WT CNS, there was no increase in the number of host T cells in the CNS of μMT recipients on day 7 (data not shown). These data indicate that the initial donor T cell infiltration to the CNS is not impaired in μMT mice. However, in the absence of B cells, infiltrating T cells fail to initiate events that lead to an increase in T cell number and ultimately to EAE.

The increase in T cell number in the CNS during the preclinical stage of EAE is usually attributed to a combination of proliferation of the initial infiltrating T cells and recruitment of a second
reactivated in vitro with MOG97–114 and transferred into Thy1.2 WT or μMT recipients. CNS mononuclear cells were isolated on days 4 and 7 posttransfer and analyzed for transcription directly ex vivo for the indicated cell types sorted from naive mice or mice with passive EAE. Each subset was analyzed in three to four independent samples generated by pooling cells from three to five mice prior to sorting. Relative transcript values were normalized to GAPDH.

* p < 0.05, Student t test.

Wave of T cells from the periphery. To determine whether proliferation of T cells that initially infiltrate the CNS is impaired in μMT mice, we analyzed BrdU incorporation in donor CD4+ T cells on day 4 but represented 75% on day 7 posttransfer in WT and μMT recipients on day 5 posttransfer. The percentage of annexin V+ donor T cells was similar in WT and μMT recipients, indicating that T cell survival in the CNS is not significantly enhanced by the presence of B cells (Supplemental Fig. 3B).

Because proliferation and apoptosis of donor T cells in the CNS were comparable in WT and μMT mice, we hypothesized that the increase in donor T cells in the CNS of WT mice on day 7 posttransfer resulted from recruitment of additional donor T cells from the periphery. This notion is supported by the observation that donor T cells represented ~35% of the total CD4+ CNS T cells on day 4 but represented 75% on day 7 posttransfer in WT recipients (Fig. 3B). To test this hypothesis, we administered FTY720, a sphingosine-1-phosphate receptor modulator that causes T cell retention in secondary lymphoid tissues (24), daily beginning on day 4 to WT recipients of MOG-specific T cells, as only these recipients exhibited an increase in T cell number between days 4 and 7. The donor CD4+ T cell number in the CNS was analyzed on day 4 and day 7. The donor T cells in the CNS of vehicle-treated WT mice increased significantly between day 4 and day 7 (Fig. 3C). In contrast, there were significantly fewer donor T cells on day 7 in mice treated with FTY720 compared with vehicle-treated mice (Fig. 3C). Thus, recruitment of additional donor T cells from the periphery, and not enhanced proliferation or survival of the original infiltrating T cells, accounts for the preclinical increase in CNS donor T cells. These data suggest that the recruitment of a second wave of T cells is impaired in the absence of CNS B cells.

B cells promote production of inflammatory mediators triggered by infiltrating T cells

We hypothesized that the failure to recruit peripheral T cells during preclinical EAE in B cell–deficient recipients is due to impaired reactivation of the initial T cells that infiltrated the CNS. Reactivation of T cells within the CNS is thought to induce production of multiple cytokines, including IL-17, IFN-γ, GM-CSF, and TNF-α (25). These cytokines act on various cell types within the CNS, including astrocytes and endothelial cells of the blood–brain barrier, to elicit expression of adhesion molecules, integrin ligands, and chemokines that are important for recruiting cells from the periphery (26). Our hypothesis predicts that expression of these gene products involved in facilitating T cell recruitment would be impaired in μMT mice. To test this prediction, we first confirmed that genes reported to be induced in other EAE models (25) were also induced in C3HeB/FeJ mice during EAE. Gene expression was compared by RT-PCR between brains of WT mice with EAE induced by adoptive transfer of MOG-specific T cells and brains of WT mice that received polyclonal CD4+ T cells
activated in vitro with anti-CD3 and anti-CD28 (healthy controls). Of the genes induced in the brains of mice with clinical EAE, we then determined which were induced in preclinical WT C3HeB/Fej mice (5 d posttransfer of MOG-specific CD4+ T cells) compared with healthy controls (Supplemental Table I). Using these genes as indicators of T cell reactivation, we compared their expression in the brains of WT and μMT mice 5 d after transfer of CD4+ MOG-specific T cells. To ensure that tissue was harvested from mice at the same preclinical stage, half of each brain was used for RT-PCR analyses, and cells were isolated from the other half of each brain to determine donor T cell numbers by flow cytometry. Only brains from WT and μMT recipients with comparable numbers of donor CD4+ T cells were used for gene expression analyses. Strikingly, the expression of all selected genes was significantly reduced in the brains of μMT recipients compared with WT recipients (Fig. 4, Table II). Impaired gene expression was observed for proinflammatory T cell– and non-T cell–derived cytokines, cell surface molecules involved in adhesion to and transmigration across the blood–brain barrier, and chemokines involved in peripheral T cell recruitment. Expression of a few genes was induced in μMT mice relative to healthy controls, but their level of expression was always significantly less than that seen in WT recipients (Table II). The greater variability in gene expression in WT mice likely reflects differences in the extent of T cell reactivation at this preclinical time point. These observations suggest that although equal numbers of donor T cells are initially present in the μMT CNS, their reactivation is significantly impaired in the CNS in the absence of B cells, resulting in an inability to recruit additional T cells from the periphery.

**Peripheral B cells reactivate Th1 but not Th17 cells in vitro**

B cells could influence reactivation of T cells infiltrating the noninflamed CNS by functioning as APCs. To test whether resting B cells can process and present MOG protein to CD4+ MOG-specific Th1 and Th17 cells, we sorted resting B cells (CD19+ CD25−) from spleens of naive C3HeB/Fej mice and cultured them with rMOG and MOG-specific CD4+ T cells skewed toward a Th1 or Th17 phenotype that had been sorted to remove contaminating APCs. The non–B cell fraction sorted from the splenocytes (containing dendritic cells and macrophages) as well as unsorted splenocytes were used as control APCs in separate cocultures with the MOG-specific Th1 and Th17 cells. MOG-specific stimulation of T cell secretion of IL-17 and IFN-γ by the different types of APCs was quantified by ELISPOT. The fraction of cells containing dendritic cells and macrophages efficiently reactivated T cells that had been skewed to either a Th1 or Th17 phenotype. In contrast, resting B cells reactivated the Th1-skewed but not the Th17-skewed cells (Fig. 5A). To determine whether activated B cells could reactivate Th17 cells, naive splenic B cells were stimulated with LPS and anti-CD40 prior to culturing with the T cells. A small increase in IL-17–producing T cells was observed when activated versus resting B cells were used as APCs; however, the extent of Th17 cell reactivation was still significantly reduced compared with the reactivation observed when non-B cells were used as APCs (Fig. 5A). The addition of IL-23 or IL-6 (Fig. 5B, 5C), reflecting the ability of IL-1β to reactivate MOG-specific Th17 cells, whereas reactivation of Th1 cells was unaffected by addition of IL-1β to promote IL-17 production in T cells (27), Thus, resting B cells do not appear competent to reactivate Th17 cells owing to a lack of production of IL-1β.

**CNS B cells promote reactivation of Th1 cells in vitro**

Owing to the small number of B cells in the naive CNS, purifying B cells from the CNS and utilizing them in vitro as APCs was not feasible. Therefore, we used an alternative approach to determine whether CNS B cells preferentially reactivated Th1 compared with Th17 cells, as we had observed for splenic B cells. Bulk CNS cells were isolated from perfused, naive WT, or μMT mice and used as APCs in ELISPOT assays to compare reactivation of Th1 and Th17 cells. The CNS cells were stained for MHC class II, CD19, CD11b, and CD11c and plated such that all wells contained the
same number of non–B cell APCs, but CNS cells from WT mice also contained B cells. The presence of B cells in the CNS APCs significantly enhanced reactivation of MOG-specific Th1 cells, but not Th17 cells, over levels achieved with µMT CNS APCs (Fig. 5D). These data suggest that naive CNS B cells are similar to peripheral B cells in their preferential ability to reactivate Th1 but not Th17 cells.

**B cells affect both the Th17/Th1 ratio in the CNS and the manifestation of EAE**

Upon observing the preferential reactivation of Th1 cells by CNS B cells in vitro, we hypothesized that the lack of B cells in µMT mice in vivo should differentially affect reactivation in the CNS of adoptively transferred Th1 compared with Th17 cells. If Th1 cells are reactivated less efficiently in the absence of B cells (whereas Th17 reactivation remains relatively unaffected), then the Th17/Th1 ratio of T cells within the CNS would increase, and this in turn should increase the susceptibility to brain inflammation. This hypothesis could not be tested in actively induced EAE in C3HeB/Fej mice because WT C3HeB/Fej mice already generate a high Th17/Th1 ratio that results in atypical disease. We therefore induced EAE by transfer of Th1-skewed cells that induces primarily classic EAE in C3HeB/Fej mice because WT C3HeB/Fej mice already generate a high Th17/Th1 ratio that results in atypical disease. Therefore, if Th17 reactivation remains relatively unaffected, then the Th17/Th1 ratio of T cells within the brain would increase, and this in turn should increase the susceptibility to brain inflammation. This hypothesis could not be tested in actively induced EAE in C3HeB/Fej mice because WT C3HeB/Fej mice already generate a high Th17/Th1 ratio that results in atypical disease. We therefore induced EAE by transfer of Th1-skewed cells that induces primarily classic EAE in C3HeB/Fej mice (4). MOG-specific T cells that were skewed to a Th17/Th1 ratio of either ~1:8 (Th1-skewed) or ~3:1 (Th17-skewed) were transferred into C3HeB/Fej WT and µMT mice. The incidence of disease was significantly reduced in µMT mice after transfer of either Th1- or Th17-skewed cells (reduction from 94 to 37% for Th1 cell transfers and from 97 to 36% for Th17 cell transfers; p < 0.001 for both Th1 and Th17, Fisher exact test). This reduced incidence in µMT mice is similar to that observed when the transferred T cells exhibited a Th17/Th1 ratio of 1:1 (Table I), and it likely reflects the loss of most MHC class II* APCs in the CNS. The day of onset (days 6–7) and severity (score of 4–5) were not significantly different between WT and µMT recipients of either Th1- or Th17-skewed cells, and they were also similar to the results shown in Table I. Importantly, there were differences in the clinical manifestation of EAE in the WT and µMT recipients of Th1-skewed cells. As we previously observed, Th1-skewed cells induced predominantly classic EAE, and Th17-skewed cells induced predominantly atypical EAE in WT recipients (4). In contrast, in the µMT recipients that did develop EAE, both Th1- and Th17-skewed cells induced predominantly atypical EAE, with most mice exhibiting ataxia, leaning, and rolling in addition to spinal cord signs (Fig. 6). As predicted, this shift from classic to atypical EAE when Th1-skewed cells were transferred into µMT instead of WT mice correlated with a significant increase in the Th17/Th1 ratio of cells in the brains of µMT compared with WT recipients at EAE onset (Fig. 7A). Consistent with our in vitro observation that B cells preferentially reactivate Th1 compared with Th17 cells, there were significantly fewer IFN-γ- and IL-17-producing T cells detected in the brains of µMT compared with WT mice that received Th1-skewed cells (Fig. 7B). The number of IL-17–producing T cells was also decreased in µMT compared with WT mice, but the difference was not significant. These data suggest that B cells preferentially promote reactivation of Th1 cells in the CNS. In mice that received Th17-skewed cells, the Th17/Th1 ratio in the brains of µMT recipients was not significantly different compared with WT recipients (Fig. 7C). The absolute numbers of both IFN-γ- and IL-17–producing T cells were significantly
decreased in the brains of these μMT recipients compared with WT (Fig. 7D), indicating that the absence of B cells has a global effect on Ag presentation in the CNS. However, the fold decrease in IFN-γ–producing cells was significantly greater than the fold decrease in IL-17–producing cells in the brains of μMT relative to WT recipients (7.6 ± 1.6 versus 3.7 ± 0.7; p = 0.04, Student t test). Taken together, these data suggest that B cells promote reactivation of T cells producing IFN-γ more strongly than T cells producing IL-17 within the CNS.

Discussion
Defining the mechanisms by which B cells influence CNS autoimmunity is of great interest in light of the efficacy of rituximab-mediated B cell depletion in MS patients (5, 6). Because rituximab does not deplete plasma cells (5), its therapeutic benefit suggests that B cells play a pathogenic role independent of Ab production in MS. Rituximab depletes B cells from both the periphery and the cerebrospinal fluid (8), and it is not clear whether B cells exert a pathogenic effect in one or both compartments. Our studies demonstrate that B cells play an important role in the pathogenesis of EAE by promoting the reactivation of T cells infiltrating the CNS that is required to trigger inflammation. The presence of B cells in the CNS also results in preferential reactivation of Th1 cells, and this differential reactivation of Th1 versus Th17 cells can influence the regional localization of inflammation. Taken together, these findings reveal an important role for CNS B cells in promoting initial inflammatory responses and shaping neuroinflammatory patterns.

B cells appear to contribute to multiple steps in the pathogenesis of CNS autoimmunity, accounting for reports of both pathogenic and regulatory B cell activities in EAE. Most studies have analyzed the effects of eliminating B cells in models of active EAE induction using μMT mice. Therefore, results from these studies reflect the cumulative effects of peripheral B cells on T cell priming, the contribution of regulatory B cells and the activity of B cells in the CNS. The impact of these different B cell functions may vary in different systems, depending on the strength and nature of the effector T cell response and the ability of regulatory B cells to modulate the response. B cells appeared to enhance pathogenicity in EAE models employing certain strain and Ag combinations (28–30), but they exerted a regulatory role or had no impact in other models (10, 30, 31). Both pathogenic and regulatory effects in EAE have also been observed when anti-CD20 was used to deplete B cells (12, 13). Anti-CD20 depletion can be used to determine the effects of depleting B cells either before or after disease onset; however, treatment before initiation of disease is unlikely to deplete B cells within the CNS, as Abs do not efficiently cross the intact blood–brain barrier. Thus, this strategy

FIGURE 6. EAE shifts from classic to atypical disease in B cell–deficient recipients of Th1 cells. EAE was induced by adoptive transfer of Th1- or Th17-skewed cells into C3HeB/Fej WT or μMT recipients. The percentages of classic and atypical EAE observed in recipient mice that developed EAE are shown (n = 7–24/group). A significant difference between WT and μMT recipients of Th1 cells was observed. ****p < 0.0001, Fisher exact test.

FIGURE 7. B cell–deficient recipients of Th1-skewed cells that developed EAE exhibit higher Th17/Th1 ratios in the brain compared with WT recipients. EAE was induced by adoptive transfer of Th1- or Th17-skewed cells into C3HeB/Fej WT or μMT recipients. At EAE onset, CNS cells were isolated from the brain and spinal cord and plated in ELISPOT assays to detect IL-17– or IFN-γ–producing cells. Numbers of Ag-specific spots were determined by comparing spots in wells with and without MOG97–114. (A and B) Mice received Th1-skewed cells. (C and D) Mice received Th17-skewed cells. (A and C) The Th17/Th1 ratio is shown, calculated from the number of IL-17/IFN-γ–producing cells in each culture. Each datum point represents a single mouse. Data are pooled from at least three independent experiments. *p < 0.05, ***p < 0.001, Student t test.
does not permit assessment of the role of CNS B cells during initiation of disease.

In our studies, we used an adoptive transfer model in C3HeB/Fej μMT mice to analyze the contribution of B cells residing within the CNS to the pathogenesis of EAE. Similar to our results in active EAE induction, a significant decrease in incidence rather than severity was observed in C3HeB/Fej μMT mice following adoptive transfer of MOG-specific T cells. This decrease in incidence of EAE demonstrated that B cells can increase susceptibility to disease by acting at a point subsequent to T cell priming but prior to onset of clinical signs. Importantly, the initial T cell infiltration of the CNS was not impaired in μMT mice. Thus, regardless of whether B cells in peripheral lymph nodes play a role in either the priming of myelin-specific T cells or in promoting expression of tissue-homing cell-surface molecules on activated T cells that enter peripheral lymph nodes (32), our adoptive transfer model allowed us to avoid effects on T cell activation in the periphery due to the altered lymphoid architecture seen in μMT mice (33), and instead investigate how B cells influence the activity of T cells once they enter the CNS.

Our results indicate that the inflammatory cascade triggered by early T cell reactivation in the CNS that generates immune mediators necessary to recruit additional T cells from the periphery was severely impaired in the absence of B cells, thus preventing initiation of EAE. The impaired reactivation of the myelin-specific T cells suggests that B cells play a critical role as APCs within the CNS during the early stages of EAE induction. Although other studies have suggested that B cells may act as APCs in the CNS at a later stage after disease onset (12, 13), the role of B cells in the CNS during initial T cell reactivation has not been previously studied. The fact that B cells comprise the predominant MHC class II+ population in the naive CNS supports this early role for CNS B cells as APCs. It is not yet known whether BCR specificity for myelin Ag is required for their APC function, or whether the CNS B cells acquire myelin Ag via a non-BCR–dependent mechanism as previously described for peripheral B cells (34).

Recent work has shown that C57BL/6 mice with MHC class II expression restricted to CD11b+ B cells do not develop EAE, suggesting that B cells are not sufficient as the sole APCs in initiating disease (35). Early studies indicated that dendritic cells were sufficient as APCs to initiate T cell responses (36), but more recent studies indicate that a requirement for dendritic cells in EAE is controversial (37–39). Our studies demonstrate that B cells are not essential to initiate EAE, as some μMT mice still succumb to EAE. However, our data show that B cells play an important role in promoting the reactivation of CNS-infiltrating, myelin-specific T cells. The population of B cells in the naive CNS significantly expands the pool of APCs that infiltrating T cells would encounter and thus would increase the likelihood of reactivation events during the initiation of EAE.

B cells in the naive CNS differed functionally from B cells in the spleen and blood with respect to constitutive cytokine production, potentially reflecting the unique microenvironment of the CNS. Similarly, the plasmablasts that we identified in the naive CNS also differed from plasmablasts in the periphery in that CNS plasmablasts did not express activation markers or MHC class II. The origin of these plasmablasts is unknown; they may either differentiate within the CNS from the B cell population into phenotypically distinct plasmablasts, or infiltrate the CNS as plasmablasts and undergo a phenotypic change within the microenvironment of the CNS. It is not clear whether these plasmablasts significantly influence immune responses in the CNS, as their cytokine production is much lower than that of CNS resting B cells and their lack of MHC class II expression indicates that they do not participate in T cell reactivation.

In the naive CNS, B cells expressed significantly higher levels of IL-12 p35 and TNF-α compared with CD11b/c+ cells, and both of these cytokines could influence the responses of T cells that initially infiltrate the CNS. T cell proliferation and IFN-γ production are enhanced by B cell–derived TNF-α in vitro (9). In addition to promoting Th1 cell differentiation, IL-12 produced by B cells in vitro has been shown to stimulate IFN-γ production from effector Th1 cells (40, 41). Although p35 is most widely recognized as a subunit of IL-12, and increased expression of IL-12 by B cells in WT mice is consistent with preferential reactivation of Th1 cells in the CNS of WT compared with B cell–deficient mice, p35 is also a subunit of IL-35. Thus, it is possible that the increased expression of p35 could enhance production of IL-35, a cytokine that may have regulatory functions (42). We did not detect IL-6, IL-1β, IL-23 p19, or IL-10 mRNA in B cells analyzed directly ex vivo from the CNS. The lack of IL-10 expression suggests that CNS B cells may not play a strong regulatory role during disease initiation in our model. IL-6 production by B cells was previously shown to influence the severity, but not the incidence, of EAE, suggesting that B cells expressing this cytokine may play a role later in disease (18).

Constitutive production of IL-12 p35 and TNF-α by CNS B cells suggested that they may promote reactivation of Th1 cells more efficiently than Th17 cells. Our observations that splenic B cells required exogenous IL-1β to reactivate Th17 but not Th1 cells, and that IL-1β is not expressed in the naive CNS and is only marginally increased in the preclinical stage of EAE, also raised the possibility that B cells in the CNS may preferentially reactivate Th1 cells. Our in vitro data using CNS cells as APCs confirmed a deficiency in the ability of CNS cells from μMT mice to reactivate Th1 cells compared with CNS cells from WT mice. Consistent with these in vitro data, ex vivo analysis showed that the Th17/Th1 ratio in the brain increased following transfer of Th1-skewed cells into μMT C3HeB/Fej mice due to a greater loss of T cells producing IFN-γ compared with IL-17. In this model, the increase in the Th17/Th1 ratio in μMT mice caused a shift from the classic EAE seen in WT mice to atypical EAE, confirming that CNS B cells can play a critical role in determining the localization of inflammation in the CNS by preferentially reactivating Th1 cells during the initial infiltration. However, the clinical impact of this preferential reactivation will depend on the relative abundance of Th1 and Th17 cells that initially infiltrate the CNS. If the percentage of Th17 cells in the population is very small, as is the case in C3H.SW mice, even eliminating the preferential reactivation of Th1 cells by B cells in the CNS may not be sufficient to increase the Th17/Th1 ratio enough to permit brain inflammation. Additionally, the influence of B cells on the localization of CNS lesions may be greatest at the initiation of disease or of a relapse when the CNS is relatively noninflamed, as the increase in IL-1β during peak inflammatory conditions should allow B cells to become proficient in activating Th17 as well as Th1 cells.

In conclusion, our studies indicate an important role for B cells as APCs in the CNS during the initial reactivation of myelin-specific T cells in EAE. Their differential ability to reactivate Th1 versus Th17 cells in a noninflammatory milieu may influence the initial localization of lesions in the CNS. A similar role for human CNS B cells as APCs could be clinically relevant during newly occurring MS relapses in which the CNS milieu may be similar to a healthy CNS. Our studies suggest that the effectiveness of rituximab treatment may in part reflect an overall decrease of APCs in the CNS by eliminating B cells, and that this therapy may be especially effective in patients in which Th1 cells are more prominent in the infiltrating population.
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Disclosures

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References


