Inhibitory Role of CD19 in the Progression of Experimental Autoimmune Encephalomyelitis by Regulating Cytokine Response

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nerve system that is considered a T helper type 1 (Th1)-mediated autoimmune disease. EAE currently serves as an experimental animal model for multiple sclerosis in human. Cytokines, such as interferon-γ and interleukin-10, play a key role in the development and remission of EAE. Recent studies have also shown a role for B cells in the pathogenesis of EAE. Therefore, we examined the role of CD19, a B cell-specific surface molecule that defines signaling thresholds critical for B-cell responses and autoimmunity, on the development of EAE. Following immunization with myelin oligodendrocyte glycoprotein (MOG) peptide, CD19-deficient (CD19−/−) mice exhibited higher clinical and pathological severity scores of EAE than wild-type mice. The increased severity of EAE in CD19−/− mice was associated with polarized Th1 cytokines in the inflamed central nerve system but not with anti-MOG antibodies in the serum. MOG-primed CD19−/− B cells produced high levels of interferon-γ, and transfer of MOG-primed CD19−/− B cells to wild-type mice worsened the disease. Thus, CD19 modulates the Th1/Th2 cytokine balance in B cells and plays a critical role as a suppressive molecule in the development of EAE. (Am J Pathol 2006; 168:812–821; DOI: 10.2353/ajpath.2006.050923)
Inhibitory Role of CD19 on EAE

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experimentation of Kanazawa University Graduate School screened regularly for pathogens. All studies and procedures were approved by the Committee on Animal Experimentation of Kanazawa University Graduate School of Medical Science.

Materials and Methods

Mice

CD19−/− (C57BL/6 × 129) mice were generated as described26 and backcrossed 12 generations onto the C57BL/6 background before use in this study. Wild-type C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Lack of cell-surface CD19 expression was verified by two-color immunofluorescence staining with flow cytometric analysis. All mice were housed in a specific pathogen-free barrier facility and screened regularly for pathogens. All studies and procedures were approved by the Committee on Animal Experimentation of Kanazawa University Graduate School of Medical Science.

Peptide

Myelin oligodendrocyte glycoprotein (MOG) peptide (P35–55: MEVGWYRSPFSRVHLYRNGK) was synthesized by Multiple Peptide Systems (San Diego, CA).

Induction and Evaluation of EAE

Active EAE was induced in female mice (6 to 8 weeks old) by subcutaneous immunization with 100 µg of MOG peptide emulsified in complete Freund’s adjuvant (CFA) containing 1 mg/ml heat-killed Mycobacterium tuberculosis H37RA (Sigma-Aldrich, St. Louis, MO) on days 0 and 7. Additionally, mice received 200 ng of pertussis toxin (Sigma-Aldrich) intraperitoneally in 0.5 ml of phosphate-buffered saline (PBS) on days 0 and 2. Clinical signs of EAE were assessed daily with a 0 to 6 scoring system (0, no signs; 1, flaccid tail; 2, impaired righting reflex and/or gait; 3, partial hind limb paralysis; 4, total hind limb paralysis; 5, hind limb paralysis with partial fore limb paralysis; and 6, moribund or dead). Differences in total disease burdens between groups were analyzed with Mann-Whitney U-test.

Serological Evaluation of MOG Peptide-Specific Ig Production

Serum was obtained 14 and 28 days after immunization. Enzyme-linked immunosorbent assay (ELISA) was used for detection of MOG peptide-specific Ab. Briefly, 96-well microtiter plates (Costar, Cambridge, MA) were coated with 10 µg/ml MOG peptide. Plates were incubated with serum samples diluted 1:100. The bound Abs were detected with alkaline phosphatase-conjugated goat anti-mouse IgG or goat anti-mouse IgM Abs (Southern Biotechnology Associates, Inc., Birmingham, AL).

Histology

MOG-induced EAE mice were euthanized on day 28 and perfused by intracardiac injection of 4% paraformaldehyde and 1% glutaraldehyde in PBS. Transverse sections of the cervical, upper thoracic, lower thoracic, and lumbar region of the spinal cord were stained with hematoxylin and eosin (H&E) or Luxol Fast Blue-Periodic acid. Each spinal cord section was further subdivided into an anterior, posterior, and two lateral columns. Each subdivided area displaying either lymphocyte infiltration or demyelination was assigned a score of 1; thus, each animal had a potential maximum score of 16.

For immunohistochemistry, frozen tissue sections of the spinal cord were acetone-fixed and then incubated with 10% normal rabbit serum in PBS (10 minutes, 37°C) to block nonspecific staining. Sections were then incubated with mouse monoclonal antibodies (mAbs) specific for mouse macrophages (F4/80), CD4 (Clone RM4–5; BD Pharmingen, San Diego, CA), and CD8 (clone 53-6.7; BD Pharmingen). Rat IgG (Southern Biotechnology Associates, Inc.) was used as a control for nonspecific staining. Sections were then incubated sequentially (20 minutes, 37°C) with a biotinylated rabbit anti-rat IgG and then horseradish peroxidase-conjugated avidin-biotin complexes (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were developed with 3,3’-diaminobenzidine tetrahydrochloride and hydrogen peroxide and then counterstained with methyl green.

Isolation of Total RNA and Real-Time Reverse Transcription-PCR

After perfusion with PBS, spinal cord from each MOG-induced EAE mouse was collected. Total RNA from...
spinal cord was extracted using Qiagen RNeasy spin columns (Qiagen Ltd., Crawley, UK) and digested with DNase I (Qiagen Ltd.) to remove chromosomal DNA in accordance with the manufacturer’s protocol. Total RNA was reverse transcribed to cDNA using Reverse Transcription System with random hexamers (Promega, Madison, WI). Real-time quantitative reverse transcription-polymerase chain reaction (PCR) was performed using the TaqMan system (Applied Biosystems, Foster City, CA) on an ABI Prism 7000 Sequence Detector (Applied Biosystems) according to the manufacturer’s instructions. TaqMan probes and primers for IFN-γ, IL-12p40, TNF-α, IL-6, IL-10, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Applied Biosystems. Relative expression of real-time PCR products was determined using the ΔΔCt technique. Briefly, each set of samples was normalized using the difference in threshold cycle (Ct) between the target gene and housekeeping gene (GAPDH): ΔCt = (Ct target gene − Ct GAPDH). Relative mRNA levels were calculated by the expression 2−ΔΔCt, where ΔΔCt = ΔCt sample − ΔCt calibrator. Each reaction was performed in, at least, triplicate.

Isolation of Mononuclear Cells from the CNS and Analysis by Flow Cytometry

After perfusion with PBS, cells from the CNS were isolated as previously described. Briefly, tissues were dissociated by passing through a 100-μm cell strainer and then centrifuged at 400 × g for 10 minutes at 4°C. The pellet was resuspended in 70% isotonic Percoll (Amersham Biosciences, Piscataway, NJ), overlaid with equal volumes of 37% and 30% isotonic Percoll, and centrifuged at 500 × g for 20 minutes at room temperature. Cells were collected from the 37%-70% interface and washed.

For intracellular IFN-γ staining, collected cells were stimulated for 4 hours at 37°C with 25 ng/ml phorbol 12-myristate 13-acetate (Sigma-Aldrich) and 1 μg/ml ionomycin (Sigma-Aldrich) in 1 ml of complete medium containing the intracellular transport inhibitor brefeldin A (10 μg/ml; Sigma-Aldrich). The cells were stained with anti-CD4 (RM4–5; BD Pharmingen) and anti-CD8 (53-6.7; BD Pharmingen) Abs and then washed and treated with fluorescence-activated cell sorting (FACS)-permeabilizing solution (BD Biosciences, San Jose, CA) for 10 minutes at room temperature. These cells were incubated for 30 minutes in the dark with anti-IFN-γ Ab (XMG1.2; BD Biosciences). The cells were analyzed on a FACSScan flow cytometer (BD Biosciences).

Adoptive Transfer of MOG-Primed B Cells

CD19−/− and wild-type mice were immunized with MOG peptide to induce EAE. MOG-primed splenic B cells were collected from MOG-induced EAE on day 28 and purified (>95% B220+) by removing T cells with anti-Thy1.2 Ab-coated magnetic beads (Dynal Inc.). Then 10⁷ B cells from CD19−/− and wild-type mice were transferred intravenously into wild-type and CD19−/− mice, respectively. One day later, the recipient mice were immunized with MOG peptide to induce EAE.

Statistical Analysis

All data are expressed as the mean value ± SEM. Mann-Whitney U-test was used for determining the level of significance of differences between sample means.

Results

Increased Severity of EAE in CD19−/− Mice

To assess whether CD19 expression plays a role in the pathogenesis of EAE, we immunized CD19−/− mice and wild-type C57BL/6 mice with MOG peptide in CFA and then observed the course of the clinical disease. In both lines of mice, EAE was induced at days 12 to 14, with a peak at days 18 to 23, followed by plateau phases (Figure 1). However, compared with wild-type mice, CD19−/− mice showed significantly higher score at the peak and the plateau phase. Thus, CD19−/− mice were more susceptible to EAE. This was consistent with histological findings of the inflamed CNS tissue, which revealed that CD19−/− mice developed more severe inflammation (Fig-
ure 2A). To further evaluate the pathological severity, transverse sections of the cervical, upper thoracic, lower thoracic, and lumbar regions of the spinal cord were stained with H&E and Luxol Fast Blue-Periodic acid and scored for the degree of inflammation and demyelination. Areas of inflammation and demyelination were more widespread in CD19\(^{-/-}\) mice when compared with those in wild-type mice at day 28 (Figure 2E). The pathological scores in the CD19\(^{-/-}\) mice were significantly higher with 11.4 ± 1.1 for inflammation and 7.4 ± 1.8 for demyelination, whereas in the wild-type mice the scores for inflammation and demyelination were 5.2 ± 0.7 and 2.8 ± 0.5, respectively (\(P < 0.005\) and \(P < 0.05\), respectively; Figure 2E). Collectively, CD19\(^{-/-}\) mice developed more severe EAE, both clinically and pathologically, than wild-type mice.

CD8\(^{+}\) T Cells Were Selectively Increased in the Inflamed CNS from CD19\(^{-/-}\) Mice with EAE

To further evaluate the pathology of EAE in CD19\(^{-/-}\) mice, the infiltrating cells in the inflamed CNS were examined by immunostaining. As shown in Table 1, the frequency of infiltrating CD4\(^{+}\) T cells was not significantly different between CD19\(^{-/-}\) and wild-type mice, whereas the frequency of CD8\(^{+}\) T cells was significantly 1.9-fold higher in CD19\(^{-/-}\) mice than in wild-type mice. Representative photomicrographs of CNS-infiltrating CD4\(^{+}\) and CD8\(^{+}\) T cells are shown in Figure 2 (C and D). The ratio of CD4/CD8 T cells in CD19\(^{-/-}\) mice was 1.4 ± 0.5, significantly lower than that found in wild-type mice (3.3 ± 1.5). The CD4/CD8 T-cell ratio was not altered in the peripheral blood in CD19\(^{-/-}\) mice with or without EAE when compared with wild-type mice (data not shown). Additionally, the number and percentage of macrophages were not different between the two groups (Table 1), and B-cell infiltration was not found in either line of mice (data not shown). CD19 expression was not detected in infiltrating T lymphocytes or macrophages (data not shown). Thus, CD8\(^{+}\) T cells were selectively increased in the inflamed CNS from CD19\(^{-/-}\) mice with EAE.

CD19 Loss Attenuated Anti-MOG Ig Production in MOG-Induced EAE

To dissect the mechanism by which CD19-deficiency enhanced disease severity, serum IgM and IgG anti-MOG Ab levels were measured by ELISA. Sera were collected from CD19\(^{-/-}\) and wild-type mice with EAE on days 14 and 28. Serum IgM anti-MOG Abs levels in
wild-type mice with EAE were significantly increased compared to control mice immunized with CFA alone 28 days after immunization \((P < 0.005; \text{Figure 3A})\). Additionally, serum IgG anti-MOG Abs levels in wild-type mice with EAE were significantly elevated compared to normal controls 14 and 28 days after immunization \((P < 0.005\) and \(P < 0.005\), respectively; \text{Figure 3B})\). By contrast, the serum levels of IgM and IgG anti-MOG Abs remained significantly lower in CD19\(^{-/-}\) mice with EAE, which did not exhibit significantly higher levels than wild-type mice without MOG immunization (\text{Figure 3}). Thus, the loss of CD19 expression dramatically inhibited anti-MOG Ab production in EAE.

To test whether decreased anti-MOG Ab enhanced the disease severity in CD19\(^{-/-}\) mice, sera from wild-type mice with EAE were transferred to CD19\(^{-/-}\) mice with EAE. However, no alteration of disease course was observed in CD19\(^{-/-}\) mice receiving sera from wild-type mice with EAE (data not shown), suggesting that decreased anti-MOG Ab is not solely responsible for the increased severity of EAE in CD19\(^{-/-}\) mice.

### The Inflamed CNS Tissue in CD19\(^{-/-}\) Mice Displays a Th1 Bias

To assess the cytokine expression profile of CNS-infiltrating cells, spinal cords were harvested from wild-type mice and CD19\(^{-/-}\) mice 28 days after MOG immunization, and cytokine expression was analyzed by real-time PCR (\text{Figure 4A}). The mRNA expressions of Th1 cytokines, IFN-\(\gamma\) and IL-12p40, in CD19\(^{-/-}\) mice were increased compared with wild-type mice (2.5-fold; \(P < 0.05\) and 2.0-fold, respectively). The mRNA expression of TNF-\(\alpha\), a proinflammatory cytokine, in CD19\(^{-/-}\) mice was also slightly higher by 1.5-fold than in wild-type mice. IL-6 mRNA expression in CD19\(^{-/-}\) mice was comparable to wild-type mice. By contrast, spinal cords from CD19\(^{-/-}\) mice showed dramatically decreased mRNA expression of IL-10, a Th2 cytokine, compared with wild-type mice (77% decrease, \(P < 0.05\); \text{Figure 4A}). To address what cell types may be accounting for excess IFN-\(\gamma\) in the CNS of CD19\(^{-/-}\) mice when compared to wild-type mice, flow

### Table 1. Infiltrating Cell Profile in the Inflamed CNS with CD19\(^{-/-}\) and Wild-Type Mice

<table>
<thead>
<tr>
<th>Cell number/transverse section</th>
<th>Macrophage</th>
<th>CD4(^{+}) T cell</th>
<th>CD8(^{+}) T cell</th>
<th>Total infiltrating cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19(^{-/-})</td>
<td>51.2 ± 8.5</td>
<td>39.8 ± 10.7</td>
<td>28.0 ± 4.2</td>
<td>119.5 ± 20.8</td>
</tr>
<tr>
<td>Wild type</td>
<td>30.3 ± 8.4</td>
<td>29.8 ± 8.4</td>
<td>8.8 ± 2.4</td>
<td>69.5 ± 5.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage of cells</th>
<th>Macrophage</th>
<th>CD4(^{+}) T cell</th>
<th>CD8(^{+}) T cell</th>
<th>CD4/CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19(^{-/-})</td>
<td>44.6 ± 3.8</td>
<td>31.3 ± 2.8</td>
<td>24.3 ± 2.9</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Wild type</td>
<td>43.8 ± 13.0</td>
<td>43.3 ± 11.9</td>
<td>13.0 ± 3.6</td>
<td>3.3 ± 1.5</td>
</tr>
</tbody>
</table>

Values represent the numbers or percentages of infiltrating cells in the CNS with EAE or the CD4/8 ratio. Data are the mean ± SEM.

*The numbers or percentages of cells or the CD4/8 ratio was significantly different from that of wild-type mice, \(P < 0.05\), \(n = 5\)/group.

\(^{\dagger}\)\(P < 0.005\), \(n = 5\)/group.
cytometric intracellular cytokine staining was performed. IFN-γ production from CNS-infiltrating CD4+ T cells in CD19-/- mice was augmented compared to wild-type mice (Figure 4B). In addition, IFN-γ production from CD8+ T cells in CD19-/- mice was also augmented (Figure 4C). Thus, it was likely that excess IFN-γ in the CNS of CD19-/- mice was generated by CD4+ and CD8+ T-cell collaboration. These findings indicate that the inflamed CNS tissue of CD19-/- mice with EAE displays a Th1 bias and that this skewed cytokine balance may contribute to the augmented disease manifestation.

**CD19 Deficiency Suppressed IL-10 Production from B Cells**

Because a recent study has demonstrated that IL-10 from B cells is important for EAE recovery, we next investigated the difference between IL-10 production in B cells from CD19-/- mice and wild-type mice. Splenic B cells were purified from CD19-/- mice and wild-type mice at day 28, followed by in vitro stimulation. Incubation with MOG alone resulted in modest and comparable IL-10 production both in CD19-/- and wild-type B cells (Figure 5A). By contrast, an agonistic Ab to CD40 induced higher levels of IL-10 production in wild-type B cells than in CD19-/- B cells (Figure 5A). Concomitant stimulation with MOG and anti-CD40 Ab further increased IL-10 production in wild-type B cells, whereas it did not increase IL-10 production in CD19-/- B cells. In addition, IL-10 production from B cells was confirmed using intracellular cytokine staining. Wild-type B cells produced IL-10 with MOG and anti-CD40 Ab stimulation, whereas CD19-/- B cells did not (Figure 5B). Thus, anti-CD40-induced IL-10 production was impaired in CD19-/- B cells with or without antigen stimulation.

**CD19 Deficiency Increased IFN-γ Production from B Cells**

We also examined in vitro B-cell production of IFN-γ using purified splenic B cells from CD19-/- and wild-type mice 28 days after immunization. B cells from wild-type or CD19-/- mice incubated with MOG alone did not produce IFN-γ (Figure 5B). Anti-CD40 Ab induced only slight increase of IFN-γ production, if any, in wild-type B cells, whereas B cells from CD19-/- mice incubated with MOG alone did not produce IFN-γ (Figure 5B). Anti-CD40 Ab incubated with MOG alone and anti-CD40 Ab stimulation (Figure 5B). Furthermore, addition of MOG to anti-CD40 Ab greatly increased IFN-γ production in CD19-/- B cells, whereas wild-type B cells showed only modest IFN-γ production by MOG and anti-CD40 Ab.
Increased the Severity of EAE in Wild-Type Mice

We next assessed whether the altered B-cell functions, especially cytokine production, were responsible in vivo for increased severity of EAE in CD19−/− mice. First, MOG-primed B cells (10^7 cells, >95% B220^+), with T cells removed anti-Thy1.2 Ab, from wild-type mice with EAE were adoptively transferred to CD19−/− mice before EAE induction. However, CD19−/− mice that received wild-type B cells developed EAE with almost the same severity as CD19−/− mice receiving no B cells (Figure 6A). Therefore, the failure of IL-10 production from B cells alone did not explain the increased severity of EAE in CD19−/− mice. Next, MOG-primed B cells (10^7 cells, >95% B220^+) from CD19−/− mice with EAE were adoptively transferred to wild-type mice before EAE induction. Wild-type mice that received CD19−/− B cells developed EAE with significantly higher severity than wild-type mice receiving no B cells and exhibited as severe EAE as CD19−/− mice (Figure 6B). To exclude dendritic cell or natural killer cell contamination in adoptive transfer of B cells, we obtained high purity B cells (>99% B220^+) by removing T cells, natural killer cells, monocytes, macrophages, dendritic cells, granulocytes, and erythrocytes with antibodies for mouse CD43, CD4, and Ter-119. The results of adoptive transfer of B cells using high purity B cells (>99% B220^+) were comparable to using conventional purity B cells (>95% B220^+) by removing T cells with anti-Thy1.2 Ab (data not shown). Thus, exaggerated B-cell functions, especially excessive IFN-γ production

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(Figure 5A). In addition, IFN-γ production from B cells was confirmed using intracellular cytokine staining. IFN-γ production from CD19−/− B cells with MOG and anti-CD40 Ab stimulation was augmented compared to wildtype B cells (Figure 5C). Unlike IFN-γ, B cells from CD19−/− and wild-type mice with MOG and anti-CD40 Ab stimulation produced scant but comparable amounts of IL-12, another Th1 cytokine (data not shown). Therefore, anti-CD40-induced IFN-γ production was augmented in CD19−/− B cells and was further exaggerated by the presence of antigen. Collectively, CD19−/− B cells showed skewed cytokine response with increased IFN-γ production and decreased IL-10 production.

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from B cells, may increase severity of EAE in CD19<sup>−/−</sup> mice.

**Discussion**

In the current study, we assessed the role of CD19, a B-cell-specific cell-surface molecule, in the induction and recovery of EAE. CD19 loss resulted in both increased severity and prolonged recovery of the disease (Figure 1). This result was striking because CD19<sup>−/−</sup> mice generally exhibit an immunodeficient phenotype. CD19<sup>−/−</sup> mice with EAE showed increased CD8<sup>+</sup> T cell infiltration but not increased CD4<sup>+</sup> T cell infiltration in the inflamed CNS (Table 1 and Figure 2 (C and D)). Furthermore, cytokine expression profiles were shifted to Th1 in inflamed CNS (Table 1 and Figure 2 (C and D)). Thus, CD19 positively regulates IL-10 production and negatively regulates IFN-γ, with this shifted balance likely affecting the disease severity of EAE. The mechanism by which CD19 regulates cytokine production is still unclear. Because CD19 has been shown to regulate BCR-induced signal transducers and activators of transcription 1 (STAT1) phosphorylation, which has a critical role in IFN-induced response, alterations in STAT1 function by CD19 loss may affect the signaling pathway. CD19 may also regulate other cytokine signaling pathways such as Janus tyrosine kinase/STAT. Therefore, CD19 may directly regulate IL-10 and/or IFN-γ production pathway(s). Alternatively, CD19 regulation of the signal strength in response to stimuli may indirectly affect the cytokine balance. In either manner, CD19 can control Th1/Th2 cytokine balance in B cells by the reciprocal regulation between IFN-γ and IL-10.

The adoptive transfer experiments have demonstrated that wild-type B cells did not rescue EAE in CD19<sup>−/−</sup> mice but that CD19<sup>−/−</sup> B cells worsened EAE in wild-type mice. Therefore, B cells deficient for CD19 have a positive role for driving the disease. The most likely explanation for this phenomenon is excessive production of IFN-γ by CD19<sup>−/−</sup> B cells. Recent studies have emphasized B cells as an important source of IFN-γ. By CD19<sup>−/−</sup> B cells down-regulate the Th1 response via the release of IL-10. Additionally, autoreactive B cells function as highly efficient antigen-presenting cells, enhancing the selective action of MOG-specific T cells. CD19<sup>−/−</sup> B-cell-T-cell interactions with B-cell antigen presentation favors Th2, rather than Th1, differentiation. Therefore, excessive production of IFN-γ from CD19<sup>−/−</sup> B cells can negatively regulate B-cell antigen presentation and then can commit T cells to a Th1 phenotype. Intriguingly, the inflamed CNS of CD19<sup>−/−</sup> mice exhibited an increase in TNF-α and Th1 cytokines such as IFN-γ and IL-12p40 and a decrease in Th2 cytokine such as IL-10, although there was no B-cell infiltration. Thus, skewed cytokine production of B cells influenced the total cytokine profile in the tissue.

Another striking finding in the current study was a specific increase of CD8<sup>+</sup> T cells in spinal cord of CD19<sup>−/−</sup> mice. EAE is considered to be CD4<sup>+</sup> T cell-driven and associated with an inflammatory cytokine profile. However, recent studies have shown that CD8<sup>+</sup> T cells also have a disease-promoting role. Furthermore, it has been demonstrated that T cells infiltrating into active human multiple sclerosis lesions are dominated by CD8<sup>+</sup> T cells. Thus, the increase in CD8<sup>+</sup> T cells in
inflamed CNS of CD19−/− mice may be related to more severe EAE. Previous studies have shown that MOG-specific antibodies enhance demyelination and inflammation, resulting in increased severity of EAE.19,20 Furthermore, plasma exchange can reduce clinical disease activity in a subset of multiple sclerosis patients.46,47 Therefore, humoral factors play an important role in multiple sclerosis and EAE. However, MOG-specific antibodies had no direct effect on EAE induction in the current study. Although the reasons for this discrepancy are not clear, B-cell functions for cytokine-producing and antigen-presenting cells may be more important than MOG-specific antibody production in the current study. It is possible that CD19−/− B cells act as antigen-presenting cells and stimulate autoantigen-specific CD8+ T cells, contributing to exacerbation of EAE.

In conclusion, the current study has shown that CD19 plays an important role in the development of EAE, and CD19 is a critical factor for T cell commitment to the Th2 phenotype. Thus, modulating CD19 function or its pathway may be a potential therapeutic target of multiple sclerosis and other Th1 diseases.

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