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B cells limit epitope spreading and reduce severity of EAE induced with PLP peptide in BALB/c mice

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Abstract

The role of B cells and antibody in Experimental Autoimmune Encephalomyelitis (EAE) appears to differ based on the identity and state (protein vs. encephalitogenic peptide) of the inducing antigen and the strain of mouse utilized. The involvement of B cells in the induction of EAE by peptides of proteolipid protein (PLP) in BALB/c mice was investigated. Wild-type and B cell-deficient (B cell $-/-$) mice on the BALB/c background were immunized with overlapping PLP peptides, and the disease course was followed. Although incidence and onset of PLP_{180–199}-induced EAE was similar in WT and B cell $-/-$ mice, the clinical course was more severe in B cell $-/-$ mice. During acute disease, proliferation and interferon- γ production by lymphoid cells from both strains were similar and were elicited predominantly in response to the immunizing antigen. However, during chronic disease lymphoid cells isolated from B cell $-/-$ mice proliferated to a greater extent and produced more interferon- γ in response to the overlapping peptide PLP_{185–206} and to the smaller internal peptide PLP_{185–199} than did WT mice. These data suggest that B cells regulate PLP-induced EAE in BALB/c mice through control of epitope spreading.

Keywords

Experimental Autoimmune Encephalomyelitis; B cells; Antibody; Proteolipid Protein; Epitope spreading

1. Introduction

The role of B cells and antibody (Ab) in multiple sclerosis (MS) and its primary animal model, experimental autoimmune encephalomyelitis (EAE), remains controversial. Several factors suggest a role for B cells/Ab in MS pathogenesis. Plasma cells are numerous in active [1] and chronic [2] lesions. Oligoclonal immunoglobulin (Ig) in the cerebrospinal fluid is a hallmark of MS, the presence of which correlates with worse prognosis [3–5]. Furthermore, Ab specific for myelin proteins has been observed bound to demyelinating axons in active MS [6] and EAE [7] lesions.

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Early studies of myelin protein-induced EAE with rats and mice depleted of B cells from birth supported a role for B cells/Ab in disease pathogenesis [8–11], while subsequent studies of peptide-induced EAE using genetically modified B cell deficient mice failed to support a role for B cells and their products in EAE [12–14]. More recent studies from this group have supported a role for B cells/Ab in protein-induced, but not peptide-induced EAE, in C57BL/6 mice [15,16].

BALB/c (H-2^d) mice have traditionally been considered resistant to EAE [17,18]. However, we recently demonstrated EAE induction in BALB/c mice upon immunization with proteolipid protein (PLP) and PLP peptides, aa180–199 and aa185–206 [19]. It is probable that the role of B cells in EAE pathogenesis varies depending on the genetic background of the animal strain and the myelin protein used for disease induction. Thus, we sought to characterize the role of B cells in the pathogenesis of PLP peptide-induced EAE in wild-type (WT) and B cell-deficient (B cell^{−/−}) BALB/c mice.

2. Materials and Methods

Mice

Pathogen-free female WT and B cell^{−/−} BALB/c mice were maintained in microisolator cages in accordance with University and NIH guidelines. WT mice were purchased from Taconic (Germantown, NY). B cell^{−/−} mice on the BALB/c background were deficient in B cells due to a targeted disruption of the *Jh* locus of the Ig heavy chain *JhD*; [20], and were either purchased from Taconic or bred in-house from breeding pairs generously provided by M. Shlomchik (Yale University). There were no differences in EAE induction in B cell^{−/−} mice purchased from Taconic and those bred in-house. All mice were used in experiments at 6–8 weeks of age. Sentinel mice showed no infection.

Antigens

PLP aa185–206 was synthesized (S-I-A-F-P-S-K-T-S-A-S-I-G-S-L-C-A-D-A-R-M-Y) by the Peptide Synthesis Facility, Washington University, St. Louis, MO. PLPaa180–199 (W-T-T-C-Q-S-I-A-F-P-S-K-T-S-A-S-I-G-S-L) and PLP185–199 (S-I-A-F-P-S-K-T-S-A-S-I-G-S-L) were synthesized by Sigma-Genosys (The Woodlands, TX). Purity of all peptides was confirmed by HPLC.

EAE Induction and Clinical Scoring

Mice were immunized (s.c.) with PLP_{180–199} (200μg/mouse) or PLP_{185–206} (400μg/mouse) or PLP_{185–199} (200μg or 500μg/mouse) emulsified (1:1) in IFA containing 0.5mg/mouse *Mycobacterium tuberculosis*, strain H37RA, 0.1ml emulsion/mouse. Emulsions were prepared using an Omni-Mixer (Omni International, Warrenton, VA). Mice also received 300ng pertussis toxin (List Biological Laboratories, Campbell, CA) intravenously at the time of immunization and 72h later. The development of EAE was followed and graded on a scale of 0–5 by a blinded observer as previously described [21].

Histology

Mice were perfused with buffered 4% paraformaldehyde, the CNS dissected, and 1mm CNS slices were prepared. The CNS tissue was then embedded in paraffin, and four micron thick CNS tissue sections were placed on glass slides. Slides were stained with H&E stain to assess inflammation and axonal loss, and Luxol Fast Blue to assess degree of demyelination. Scoring was done by a blinded observer (AHC) on a scale of 0–5 as published [21].

Proliferation Assays

Spleen and lymph node cells were isolated from immunized mice. Cells from 3–5 mice were pooled from each respective organ and cultured in quadruplicate at 2.5×10^6 cells/ml with antigen [PLP_{180–199} or PLP_{185–206} or PLP_{185–199}] or mitogen (ConA, 1 µg/ml) and 5% FBS in complete RPMI-1640. ^3H -thymidine (0.5 µCi/well) was added during the final 18h, and its incorporation counted (Betaplate 1205; Wallac, Gaithersburg, MD).

Cytokine ELISA

Spleen and lymph node cells were isolated from immunized mice and cultured at 2.5×10^6 cells/ml with the indicated peptide antigen at 10 µg/ml and 5% FBS in complete RPMI-1640. Cell culture supernatants were collected at the indicated times and frozen at -80°C until assayed. Supernatant fluids were analyzed in duplicate for IFN γ (detection limit: 2pg/ml), TNF α (detection limit: 5.1pg/ml), IL-17 (detection limit: 5pg/ml) and IL-10 (detection limit: 4pg/ml) by QuantikineM ELISA (R&D Systems, Minneapolis, MN) according to manufacturer's instructions.

Serum antibody ELISA

Microtiter plates (Nunc, Rochester, NY) were coated with 10 µg/ml PLP_{180–199} or PLP_{185–206} in bicarbonate coating buffer (pH 9.0) overnight at 4°C . Plate were washed in PBS-0.05% Tween-20 (PBST) and blocked with 3% BSA at room temperature (RT) for 2h. Serum samples isolated from WT or B cell $^{-/-}$ BALB/c mice were diluted 1:100 and 1:400 in dilution buffer (PBS/0.5% BSA/0.05% Tween 20) and added to wells (100 µl) and incubated 1h at RT. Plates were washed with PBST and horseradish peroxidase-coupled secondary antibody diluted in dilution buffer (100 µl; anti-IgM [1:500]; anti-IgG [1:40 000]; both from Jackson ImmunoResearch, West Grove, PA) was added to the appropriate wells for 1h at RT. Plates were washed and 100 µl of substrate (OptEIA; BD Biosciences) was added to each well. Reactions were stopped by the addition of 100 µl of 2.5N sulfuric acid (LabChem, Pittsburgh, PA). The absorbance at 450nm was read on a ChemWell microplate analyzer (Awareness Technology, Palm City, FL). Results are reported as the Abs₄₅₀ of diluted serum samples incubated with peptide minus the Abs₄₅₀ of diluted serum samples incubated in the absence of peptide. Background values were typically Abs₄₅₀ <0.07. Background-subtracted results of Abs₄₅₀ >0.1 were considered positive.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 3.0 (San Diego, CA). Fisher's Exact Test, unpaired t-test, Mann-Whitney test and 2-way ANOVAs were applied, as indicated. $P < 0.05$ was considered significant.

3. Results

B cell-deficient BALB/c mice are more susceptible to EAE induced by PLP peptides

To investigate the role of B cells in PLP-induced EAE in BALB/c mice, WT and B cell $^{-/-}$ mice were immunized with PLP_{180–199} emulsified in CFA. Both groups of mice presented with clinical signs at similar times post-immunization (Table 1). However, EAE was worse in B cell $^{-/-}$ mice, reflected in a more severe and prolonged disease course (Figure 1A; $P < 0.0001$ by two way ANOVA) and a higher median maximal score (Table 1; $P = 0.021$). There was a trend for a higher disease incidence in B cell $^{-/-}$ mice compared to WT mice ($P = 0.06$). Similar results were obtained when animals were immunized with PLP_{185–206}, but disease incidence was lower in both WT and B cell $^{-/-}$ mice, as observed previously (Figure 1B, Table 1; [19]).

The above data indicate that B cell^{-/-} mice on the BALB/c background develop worse clinical signs with PLP peptide-induced EAE than their WT counterparts. Previous data from this laboratory demonstrated that the core PLP₁₈₅₋₁₉₉, common to both PLP₁₈₀₋₁₉₉ and PLP₁₈₅₋₂₀₆, was not encephalitogenic in WT mice when mice were followed for 35d post immunization [19]. Thus, WT and B cell^{-/-} BALB/c mice were immunized with the core PLP₁₈₅₋₁₉₉ peptide and followed for a longer period of time to determine if the B cell^{-/-} would be susceptible to EAE induction with this peptide. This peptide was found to be weakly encephalitogenic in both strains with onset in one of four B cell^{-/-} mice at 40dpi, and in one of four WT mice at 47dpi (Table 1). Disease severity was similar in both strains. After development of EAE, the WT mouse remained at a grade 2.0 for the duration of the experiment (62d). The B cell^{-/-} mouse recovered fully from the first episode by 47dpi, relapsed at 50dpi and subsequently succumbed to EAE at 58dpi. To determine if a higher dose of PLP₁₈₅₋₁₉₉ would be more effective at disease induction, a second group of mice was immunized with 500µg peptide emulsified in CFA (Figure 1C). This higher peptide dose resulted in greater disease incidence with similar severity in WT and B cell^{-/-} mice (Table 1).

Histologic characterization of CNS pathology

Antibody is generally considered important to the process of demyelination. To determine whether B cell^{-/-} mice would display less demyelination or other differences in histopathology compared to WT mice, CNS sections were prepared from immunized mice and observed by light microscopy. The amount of inflammation and demyelination were consistent with clinical severity in both WT and B cell-deficient mice (Table 2).

Detection of serum antibody specific for PLP peptides

To demonstrate the activation of a PLP peptide-specific B cell response in WT mice with EAE, ELISAs were performed on serum samples isolated from WT BALB/c mice 14d post immunization with PLP₁₈₀₋₁₉₉ or PLP₁₈₅₋₂₀₆ (Figure 2). In each case, low titers of IgM and higher titers of IgG were detected to the immunizing antigen, but not to the overlapping peptide. As would be expected, no serum antibody was detected in B cell^{-/-} mice (not shown).

Characterization of the immune response to overlapping PLP peptides in WT and B cell^{-/-} mice

We sought to characterize differences in the T cell response that may explain the increased disease severity in B cell^{-/-} mice. WT and B cell^{-/-} BALB/c mice were immunized with PLP₁₈₀₋₁₉₉, and lymph nodes and spleens were removed 10–14 days later, prior to disease onset. Single cell suspensions were prepared, and proliferation in response to PLP₁₈₀₋₁₉₉, PLP₁₈₅₋₂₀₆, and PLP₁₈₅₋₁₉₉ was assessed by ³H-thymidine incorporation. Data represents cells isolated from a total 6 six mice in two separate experiments. Lymph node and spleen cell populations from WT and B cell^{-/-} strains responded similarly to the immunizing PLP₁₈₀₋₁₉₉ and was the predominant response detected in each case (Figure 3A and data not show). Lesser responses to the overlapping peptides were also detected, with a significantly greater response to the core PLP₁₈₅₋₁₉₉ peptide detected in B cell^{-/-} mice.

Cytokines produced in response to the overlapping PLP peptides were characterized by ELISA of cell culture supernatants. Lymph nodes and spleens were isolated from WT and B cell^{-/-} mice immunized 10–14d prior with PLP₁₈₀₋₁₉₉. Cells were cultured with PLP₁₈₀₋₁₉₉, PLP₁₈₅₋₂₀₆, or PLP₁₈₅₋₁₉₉, and cell culture supernatants harvested. Similar levels of IFNγ in response the immunizing PLP₁₈₀₋₁₉₉ peptide by spleen cells (Figure 4A) and lymph node cells (not shown). Lower, similar, amounts of IFNγ in response to the overlapping peptides was also noted (Figure 4A). TNFα was detected in response to all three peptides in supernatants from spleen (Figure 4B) and lymph node cultures (not shown), with significantly higher levels

detected in WT mice compared to B cell^{-/-} mice. No significant differences in IL-17 expression were noted between WT and B cell^{-/-} mice (not shown).⁷

Analysis of the immune response early in the disease process did not provide an explanation for more severe EAE in B cell^{-/-} mice compared to WT BALB/c mice. Thus, cell proliferation and cytokine production were analyzed during the chronic stage of disease of PLP₁₈₀₋₁₉₉-immunized mice. Spleen cells from WT and B cell^{-/-} mice responded similarly to the immunizing PLP₁₈₀₋₁₉₉ at this late time-point with proliferation (Figure 3B) and cytokine production (Figure 5). The difference in proliferation to PLP₁₈₅₋₁₉₉ by B cell^{-/-} vs. WT splenocytes that was noted at 14dpi was more pronounced during chronic disease. In addition, spleen cells from B cell^{-/-} mice showed significantly higher proliferation to PLP₁₈₆₋₂₀₆ than did WT mice (Figure 3B). Furthermore, B cell^{-/-} splenocytes produced significantly more IFN γ in response to the overlapping PLP₁₈₅₋₂₀₆ (2–3 fold more) and PLP₁₈₅₋₁₉₉ (3–4 fold more) than did WT mice (Figure 5A). IFN γ in response to the immunizing PLP₁₈₀₋₁₉₉ was similar in the two strains (Figure 5A). The significantly increased production of TNF α by WT mice in response to all 3 peptides that was noted at 14dpi was no longer evident, with similar production noted in both strains (Figure 5B). On the other hand, characterization of interleukin-10 (IL-10) demonstrated higher levels of this protective cytokine produced by primed WT than primed B cell^{-/-} splenocytes in response to the immunizing peptide PLP₁₈₀₋₁₉₉ (Figure 5C). Neither strain produced significant amounts of IL-10 in response to PLP₁₈₅₋₂₀₆ or PLP₁₈₅₋₁₉₉ (data not shown).

4. Discussion

Clinical findings from MS patients support a role for B cells and/or antibody in disease pathogenesis [1,3,6,22–24]. However, evidence supporting a role of B cells in EAE, the primary animal model for MS, has not been universally observed. Results differ depending on the mouse strain and antigen used for disease induction [12,14–16,25].

Historically, BALB/c mice were considered resistant to EAE induction [17,18]. We recently characterized the encephalitogenicity of myelin proteolipid protein (PLP) in the BALB/cJ and BALB/cBy substrains, identifying PLP₁₈₀₋₁₉₉ and PLP₁₈₅₋₂₀₆ as encephalitogenic epitopes [19]. This allowed us to investigate the role of B cells in the BALB/c model using the JhD B cell-deficient strain. Immunization of WT and B cell^{-/-} mice on the BALB/c background with either PLP₁₈₀₋₁₉₉ or PLP₁₈₅₋₂₀₆ demonstrated a more severe disease course in the absence of B cells. This correlated with evidence of epitope spreading in B cell^{-/-} mice.

Our previous work suggested that the core PLP₁₈₅₋₁₉₉ common to the encephalitogenic PLP₁₈₀₋₁₉₉ and PLP₁₈₅₋₂₀₆ peptides was not encephalitogenic [19]. We show here that this peptide can be encephalitogenic in WT and B cell^{-/-} mice, but disease incidence is low, of moderate severity, and much delayed in onset in both strains when mice are immunized with a dose similar to that used for the longer overlapping peptides. The encephalitogenicity of the PLP₁₈₅₋₁₉₉ peptide was increased by immunization with a larger dose of peptide. Thus, the PLP₁₈₅₋₁₉₉ core epitope shared by the encephalitogenic PLP₁₈₀₋₁₉₉ and PLP₁₈₅₋₂₀₆ peptides is a weaker epitope for EAE induction than the overlapping peptides, and may be the minimal encephalitogenic epitope in BALB/c mice.

Characterization of cell proliferation and cytokine production by lymphoid cells during acute EAE revealed greater proliferation to the core PLP₁₈₅₋₁₉₉ peptide by cells isolated from B cell^{-/-} mice compared to WT mice. This difference in the epitope response broadened to include the PLP₁₈₅₋₂₀₆ epitope during chronic EAE. Analysis of cytokine production showed a similar pattern. Early in the disease process, both strains produced similar levels of IFN γ in response to all 3 peptides. Paradoxically, lymphoid cells from WT mice produced significantly more

TNF α during acute disease, with very little of this proinflammatory cytokine detected in B cell $-/-$ cultures. However, during chronic disease significantly higher production of IFN γ in response to PLP_{185–199} and PLP_{185–206} was evident in PLP_{180–199}-immunized B cell $-/-$ mice compared to WT mice. The *in vivo* processing of PLP_{180–199} to additional epitopes shared with PLP_{185–206} or PLP_{185–199} in the absence of B cells could influence the induction of the pathogenic T cell population and the breadth of autoimmune responses which contribute to EAE induction.

IL-10 is considered a protective cytokine in the EAE model and multiple sclerosis [26]. WT mice produced significantly more IL-10 in response to PLP_{180–199} than did B cell $-/-$ mice. A variety of cells produce IL-10, including T cells, B cells, and macrophages. The source of IL-10 in WT mice was not elucidated, but it is tempting to speculate that B cells may have contributed, as been suggested in EAE induced by myelin oligodendrocyte glycoprotein [27,28]. The observed difference in the cytokine profile in WT and B cell $-/-$ mice in response to the overlapping epitopes could be responsible for the differences in disease severity noted between these two strains: lack of IL-10 production to temper disease severity coupled with a broader epitope response to PLP in B cell $-/-$ mice could result in the more severe disease noted in the B cell $-/-$ mice.

Activated B cells are very efficient at presenting antigen (Ag) to T cells and focusing the T cell response when both B cell and T cell are specific for the same Ag [29]. Increased IFN γ production to overlapping peptides by the B cell $-/-$ splenocytes suggested that epitope spreading had occurred to a larger extent in B cell $-/-$ mice. In some systems, targeting Ag to B cells has been shown to induce a Th2-like response [30–32], while in others a Th1-like response is induced [33,34]. Factors governing the role of B cell-mediated antigen presentation in Th1 versus Th2 differentiation include costimulatory molecule expression [35], kinetics of B cell receptor recognition of antigen [36], and adjuvants [32,33]. Thus, the lower levels of Th1 cytokines and higher levels of IL-10 in WT mice as compared to B cell $-/-$ mice may be due in part to B cell-mediated antigen presentation of the PLP peptides.

The function of antibody in PLP-induced disease in BALB/c mice is unknown. Although it is accepted that antibody plays a role in demyelination [3,37–40], this does not appear to be a major role in our model. Histopathologic characterization of lesions in WT and B cell $-/-$ mice demonstrated inflammation and demyelination characteristic of EAE in both strains. This is in agreement with MOG-induced disease in C57BL/6 mice with and without B cells [13,16].

A role for antibody in directing antigen processing and presentation in model systems [41–46], viral infection [47], and autoimmunity [48–50] is well-recognized. Antibodies can potentiate the T cell response to some epitopes while suppressing the response to others [41, 45]. Antibodies specific for glutamic acid decarboxylase 65 (GAD65), considered to be an autoantigen important in Type I diabetes, were found to suppress the generation of GAD65-specific T cell epitopes when both resided within the same peptide, yet enhance T cell epitope generation when the T cell epitope was located distant from the B cell epitope [48]. Similarly, thyroxine-specific autoantibodies were found to inhibit the generation of pathogenic T cell epitopes in a mouse model of thyroiditis [50]. Most commonly, suppression of T cell epitope generation was mediated by Ab of the IgG subclass via Fc γ receptor [41,48]. A similar mechanism could be responsible for the observations presented here. Initial studies indicate that serum IgG may be responsible for mediating EAE severity in our model (unpublished observation). Indeed, a robust IgG response to the immunizing peptide was detected in WT mice. Thus, in WT mice IgG specific for the immunizing peptide could block the presentation of the overlapping peptides, preventing the spread of the T cell response to these other epitopes. In the absence of Ab in the B cell $-/-$ mice, these epitopes would not be protected, allowing epitope spreading to occur. This model, as well as a role for Fc γ R, warrants further study.

We previously characterized a pathogenic role for B cells/Ab in rMOG-induced EAE, but not MOG_{35–55}-induced EAE, in C57BL/6 mice [15,16]. Studies of peptide-induced EAE in other strains of mice were inconsistent in supporting a role for B cells in disease [12,14,25]. Studies in B10.PL mice indicate that B cells are involved in the recovery phase of disease [12,14]. Data presented here are the first to support a role for B cells in PLP peptide-induced EAE. In this case, B cells temper the disease. The differences in the requirement and roles for B cells in PLP peptide-induced EAE vs. MOG peptide-induced EAE are likely due to differences in the myelin antigen used for immunization as well as genetic differences between mouse strains. Whether B cells will be necessary for EAE induction by whole PLP in BALB/c mice has yet to be investigated. However, whole PLP is difficult to purify in large quantities and these investigations will likely require development of a recombinant form (personal communication, John L. Trotter, M.D.).

We present data here supporting a role for B cells in moderating PLP-induced disease in a BALB/c model of EAE. The mechanism of disease amelioration warrants further investigation, as T cell responses specific for the same PLP peptides used for disease induction in BALB/c mice have also been noted in MS patients [51–53]. Thus, these findings may be applicable to the human disease state and could contribute to the development of effective therapies.

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Figure 1. PLP peptide-induced EAE in WT and B cell^{-/-} BALB/c mice

WT (■) and B cell^{-/-} (□) BALB/c mice were immunized with (A) PLP₁₈₀₋₁₉₉ [WT N=14 through d44; N=5 d45-80; B cell^{-/-} N=15 through d44; N=5 d45-80], (B) PLP₁₈₅₋₂₀₆ [WT N=10; B cell^{-/-} N=10], or (C) PLP₁₈₅₋₁₉₉ [WT N=4; B cell^{-/-} N=4] in CFA and administered pertussis toxin. All mice were graded daily on a scale of 0-5 for signs of EAE. Error bars represent S.E.M. Results were analyzed by two-way ANOVA: $p < 0.0001$ WT vs. B cell^{-/-} (A&B); $p = \text{not significant}$ (C).

Figure 2. Serum antibody specific for PLP peptides in EAE mice

WT and B cell^{-/-} mice were immunized (imm.) with PLP₁₈₀₋₁₉₉ or PLP₁₈₅₋₂₀₆ and 14d later serum was collected. Serum IgM and IgG specific for (A) PLP₁₈₀₋₁₉₉ and (B) PLP₁₈₅₋₂₀₆ was determined by ELISA using 1:100 and 1:400 dilutions of serum.

Figure 3. Proliferation of spleen cells isolated from PLP₁₈₀₋₁₉₉ immunized mice

WT and B cell^{-/-} BALB/c mice were immunized with PLP₁₈₀₋₁₉₉. Spleen cells were isolated (A) 10–14d post immunization (dpi) and (B) 60–70dpi. Proliferation in response to PLP₁₈₀₋₁₉₉, PLP₁₈₅₋₂₀₆, and PLP₁₈₅₋₁₉₉ was assessed by ³H-thymidine incorporation. Results expressed as Stimulation Index (S.I.; [cpm in presence of Ag]/[cpm in absence of Ag]) and are representative of 6 mice in each group analyzed in 2 separate experiments. Peak proliferation by each group in response to each peptide each group was compared by unpaired t-test. Only statistically significant results are noted.

Figure 4. Cytokine production by spleen cells isolated from PLP₁₈₀₋₁₉₉ immunized WT and B cell^{-/-} mice during acute EAE

WT and B cell^{-/-} BALB/c mice were immunized with PLP₁₈₀₋₁₉₉. Production of (A) IFN γ and (B) TNF α by spleen cells in response to the indicated PLP peptides was characterized by ELISA at 10–14dpi. Results expressed as cytokine produced in the presence of peptide minus cytokine produced in presence of media alone from 6 mice in each group analyzed in 2 separate experiments. Peak cytokine production by each group in response to each peptide was compared by unpaired t-test, and significant results are noted.

Figure 5. Cytokine production by spleen cells isolated from PLP₁₈₀₋₁₉₉ immunized WT and B cell^{-/-} mice during chronic EAE

WT and B cell^{-/-} BALB/c mice were immunized with PLP₁₈₀₋₁₉₉ and spleens were removed during chronic disease. (A) IFN γ , (B) TNF α and (C) IL-10 production was measured by ELISA. Results expressed as cytokine produced in the presence of peptide minus cytokine produced in presence of media alone. Data are representative of 6 mice analyzed in 2 separate experiments. Peak cytokine production by each group in response to each peptide was compared by unpaired t-test, and significant results are noted. N.D.: Not detected.

Table 1
PLP peptide-induced EAE in WT vs. B cell^{-/-} BALB/c mice

Antigen	Incidence	Day of Onset ^a (dpi) Mean ± S.D.	Max. Score ^b Median (range)
PLP ₁₈₀₋₁₉₉			
WT	6/14 ^d	16 ± 9.1 ^e	0 (0-4.0) ^f
B cell ^{-/-}	12/15 ^d	11.2 ± 3.1 ^e	4.0 (0-5.0) ^f
PLP ₁₈₅₋₂₀₆			
WT	2/10 ^c	17.5 ± 2.1 ^e	0 (0-4.0) ^g
B cell ^{-/-}	7/10 ^c	15.0 ± 2.3 ^e	3.5 (0-4.0) ^g
PLP ₁₈₅₋₁₉₉ 200μg/mouse			
WT	1/4	47	0.6
B cell ^{-/-}	1/4	40	0.7
500μg/mouse			
WT	4/4	20.2 ± 9.8 ^e	2.5 (2.0-4.0) ^e
B cell ^{-/-}	3/4	15 ± 1.2 ^e	3.75 (0-4.0) ^e

Results expressed as median (range).

^a of mice presenting with clinical signs

^b of all mice

^c P=0.07 (Fisher's Exact Test)

^d P=0.06 (Fisher's Exact Test)

^e not significant (unpaired t-test [day of onset] or Mann-Whitney test [median max score])

^f P=0.021 (Mann-Whitney test)

^g P=0.043 (Mann-Whitney test); dpi: day post immunization

Table 2
Histologic characterization of CNS tissue from PLP₁₈₀₋₁₉₉ immunized mice during acute EAE

Strain	Clinical Score	Clinical Stage	Inflammation	Demyelination	Remyelination	Axonal loss
WT	1	Onset	2	1	1	0
WT	4	Peak Acute	3	3	1	1
B cell ^{-/-}	1.5	Onset	0.5	0	0	0
B cell ^{-/-}	4	Peak Acute	3	2.5	0	2.5