Cutting Edge: B Cell-Deficient Mice Develop Experimental Allergic Encephalomyelitis with Demyelination After Myelin Oligodendrocyte Glycoprotein Sensitization[1]

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Myelin oligodendrocyte glycoprotein (MOG) induced experimental allergic encephalomyelitis (EAE) is an animal model for the central nervous system disease multiple sclerosis (MS). The roles of individual components of the immune system have not been completely defined in the mouse model, and to determine the role of B cells and Abs in the induction of EAE and demyelination, B cell-deficient μMT (H-2^b) mice were immunized with MOG peptide 35–55. The μMT mice were susceptible to MOG-induced EAE and developed a chronic sustained disease, with inflammatory lesions and primary demyelination in the spinal cord, brain, and optic nerves, similar to that seen in wild-type C57BL/6 mice. The inflammatory cells in the central nervous system of μMT mice included both activated and memory T cells and macrophages. The data suggest that B cells and Abs are not necessary for primary demyelination in MOG-induced EAE in mice. ***The Journal of Immunology*****, 1998, 161: 4480–4483.**

Experimental allergic encephalomyelitis (EAE)[3] is a paralytic, inflammatory disease of the central nervous system (CNS) that has been extensively investigated as a model of multiple sclerosis (MS). EAE can be induced by active immunization with myelin components emulsified in appropriate adjuvants or by passive transfer of activated CD4$^+$ T cells. In contrast to MS, not all models of EAE are characterized by primary demyelination in the CNS, and it has been proposed that autoantibodies might be involved in the destruction of myelin in some animal models (1).

A candidate autoantigen for Ab-mediated myelin destruction is myelin oligodendrocyte glycoprotein (MOG), a membrane protein found primarily on the extracellular surface of oligodendrocytes in the outermost lamellae of the myelin sheath (2, 3). In contrast to most other Ags used to induce EAE, MOG is directly accessible to a humoral immune response within the CNS, and numerous studies both in vitro (4–6) and in vivo (7–13) have shown that Abs to MOG can mediate demyelination. Rats with classical nondemyelinating EAE, transferred by myelin basic protein (MBP)-specific T cells, develop demyelination after injection of a mAb specific for MOG (7, 9–11). Severe disease and demyelination in Lewis rats immunized with MOG 35–55 peptide does not appear until specific IgG anti-MOG Abs are present, suggesting that Abs are necessary for full development of MOG-induced EAE (14). Abs to MOG (15) and anti-MOG Ab-secreting B cells (16) are found in cerebrospinal fluid of MS patients. Recently, a study reported that transgenic "knock-in" mice with the germline J_H locus replaced with the rearranged Ig H-chain variable (V) gene of a pathogenic MOG-specific mAb developed a more severe disease, with increased incidence and accelerated disease onset, after challenge with encephalitogenic Ags or T cells (17). However, no previous study has analyzed whether Abs are required for demyelination in MOG-induced EAE.

In this study, we analyzed the induction of EAE and demyelination in C57BL/6 H-2^b mice rendered deficient for B cells by genetic disruption of the μ heavy chain transmembrane exon (μMT mice) (18). We show that B cell-deficient μMT mice are susceptible to EAE induced by MOG peptide 35–55 and that the disease is characterized by both inflammatory lesions and primary demyelination.

Materials and Methods

Mice

Female C57BL/6 wild-type (WT) and two separate groups of C57BL/6 μMT H-2^b mice (18) were purchased from The Jackson Laboratory (Bar Harbor, ME) (with permission from Drs. Klaus Rajewsky and Werner Müller). All mice were 8 to 10 wk of age at use.

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[3] Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis; CNS, central nervous system; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; MS, multiple sclerosis; PE, phycoerythrin; SC, spinal cord; WT, wild-type.

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Table I. *B cell-deficient μMT mice are susceptible to MOG 35-55-induced EAE*[a]

	C57BL/6	μMT[b]	p[c]
Incidence (sick/total)	5/5	10/10	NS
Mortality (death/total)	0/5	1/10	NS
Average day of disease onset	12.8 ± 1.1 (10–16)[d]	11.3 ± 0.7 (8–14)	NS
Average maximum disease severity	2.8 ± 0.2 (2–3)	3.2 ± 0.2 (3–5)	NS

[a] EAE was induced and the mice were scored for clinical signs as described in *Materials and Methods*.
[b] Average of two separate experiments.
[c] The significance of the differences was analyzed by Fisher's exact test or Student's *t* test.
[d] Mean ± SEM (range).

MOG peptide

MOG peptide 35–55 (MEVGWYRSPFSRVVHLYRNGK), of murine origin, was synthesized by the W. M. Keck Biotechnology Resource Center at Yale University. The peptide was purified using a reverse phase (C_{18}) column HPLC and a trifluoroacetic acid/acetonitrile gradient.

Active induction of EAE

EAE was induced by s.c. flank injections of 300 μg of MOG 35–55 peptide in CFA (Difco, Detroit, MI) with 500 μg of *Mycobacterium tuberculosis* on days 0 and 7, supplemented by i.v. injections of 500 ng of pertussis toxin (List Biologic, Campbell, CA), as described previously (19). The mice were observed daily for clinical signs and scored on a scale of 0 to 5 with graduations of 0.5 for intermediate scores: 0, no clinical signs; 1, flaccid tail; 2, hind limb weakness or abnormal gait; 3, complete hind limb paralysis; 4, complete hind limb paralysis with forelimb weakness or paralysis; 5, moribund or deceased. Supplementary food and water were provided on the cage floor for disabled animals.

Histopathologic examination

Mice were deeply anesthetized and perfused intracardially with 4% formaldehyde and 2% glutaraldehyde in 0.14 M Sorenson's phosphate buffer (pH 7.2). The brain, spinal cord (SC), and optic nerves were removed. Tissues were embedded in Epon, sectioned at 1 μm, and stained with toluidine blue.

Flow cytometric analysis

Mice were deeply anesthetized and perfused intracardially with RPMI 1640 medium (Life Technologies, Gaithersburg, MD). Brain and SC cell suspensions were incubated with collagenase II (Sigma, St. Louis, MO), 1 mg/ml, at 37°C for 2 h, and mononuclear cells were isolated by discontinuous Percoll (Pharmacia, Piscataway, NY) gradient. After blocking with purified rat, hamster, and goat IgG (Pierce, Rockford, IL), cells were incubated with directly conjugated Abs in FACS buffer (1% BSA, 0.1% sodium azide in PBS). The CD4-FITC, B220-phycoerythrin (PE), IgM-FITC, CD69-PE, Mac-1-PE, L-selectin-PE, and CD44-Cychrome Abs were all from PharMingen (San Diego, CA). Samples were analyzed with a FACScan flow cytometer (Becton Dickinson) with Cellquest software. Each experiment was done twice, at 20 and 40 days after immunization, with a minimum of two animals examined in each group.

ELISA

Serum samples were prepared from peripheral blood obtained by retroorbital puncture 20 and 35 days after immunization. ELISA was performed as previously described, using purified rat MOG 35–55 peptide as Ag to detect specific anti-MOG Abs and a capturing goat anti-mouse Ig (G + A + M) Ab (Pierce) to detect total Ab levels in serum (19).

Results

B cell-deficient μMT mice were susceptible to MOG-induced EAE

To examine the role of B cells and Abs in MOG-induced EAE, B cell-deficient μMT mice ($n = 10$) and WT mice were immunized with MOG 35–55 and observed daily for the presence of neurologic signs. The μMT mice were susceptible to MOG-induced EAE and developed a chronic sustained disease similar to that seen in WT mice. The incidence of disease (10 of 10), mortality (1 of 10), average day of onset of disease (11.3 ± 0.7), and average maximum disease severity (3.2 ± 0.2) of the μMT mice were all similar to those in WT mice (Table I).

μMT mice exhibited extensive CNS inflammation and demyelination after MOG immunization

The brain, SC, and optic nerves of two MOG-immunized μMT mice, one control μMT mouse immunized with adjuvant alone, and one MOG-immunized WT C57BL/6 mouse were evaluated histologically to determine the extent of inflammation and demyelination in the CNS. Both the WT and μMT mice immunized with MOG exhibited extensive inflammatory infiltration, which often extended into the parenchyma, in several areas of the CNS, while the control mouse immunized with adjuvant alone did not show any signs of inflammation (Fig. 1). Primary demyelination was apparent in the SC and occasional demyelinated nerve fibers were also found in the optic nerve of the MOG immunized μMT (Fig. 1) and WT mice.

No B cells, Abs, or anti-MOG Abs could be found in μMT mice

There were IgM^+ cells (~6%) and $B220^+$ B cells (~13%) in the CNS infiltrate of MOG-immunized WT mice (Fig. 2, *A* and *C*). However, no IgM^+ cells or $B220^+$ B cells were detectable in the CNS (Fig. 2, *B* and *D*) or in the spleen (data not shown) of the MOG-immunized μMT mice. The serum from the μMT mice did not contain total Abs or anti-MOG 35–55-specific IgM or IgG Abs, while the WT C57BL/6 mice had normal concentrations of Abs in the serum and developed both IgM and IgG Ab titers against the immunizing peptide (data not shown).

The inflammatory infiltrate of μMT mice contained activated T cells and macrophages

To determine the nature and activation state of the cells in the inflammatory lesions in the CNS of MOG immunized μMT and WT mice, FACS analysis was conducted with cell phenotype and activation markers. The numbers of $CD4^+$ T cells (~31%) and Mac-1^+ monocytes (~60%) in the CNS infiltrate of μMT mice (Fig. 2, *D* and *F*) were comparable with the numbers of $CD4^+$ T cells (~41%) and Mac-1^+ monocytes (~64%) found in the CNS infiltrate of the WT mice (Fig. 2, *C* and *E*). In both μMT and WT mice, the $CD4^+$ T cells included almost no naive L-selectin high, CD44 low cells, but many activated and memory (L-selectin low, CD44 high) cells (Fig. 2, *G* and *H*). There were no differences in the ratio of memory:naive T cells between the infiltrates in WT and μMT mice 20 and 40 days after immunization (data not shown). The activation state of the cells was further determined by examining CD69 expression. As shown in Figure 2, *I* and *J*, ~27% of the infiltrating $CD4^+$ T cells in the WT and ~42% of the infiltrating $CD4^+$ T cells in the μMT mice expressed CD69.

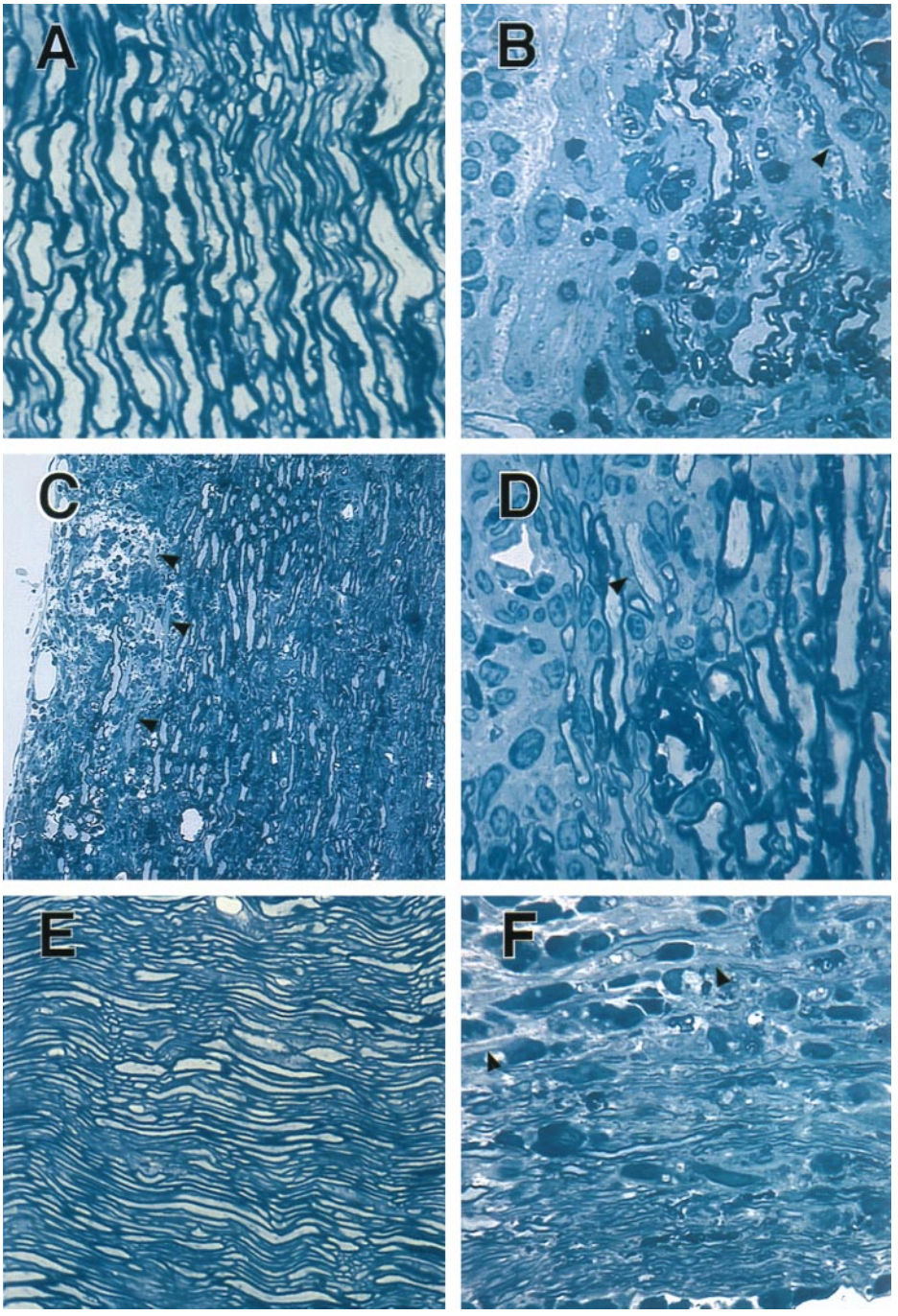

FIGURE 1. B cell-deficient μMT mice show CNS inflammation and demyelination after a 20-day immunization with MOG peptide. Representative light micrographs of 1-μm epoxy sections from the SC and optic nerves stained with toluidine blue. The original magnification is $\times$800 in all panels except *C*, where the original magnification is $\times$200. Demyelinated nerve fibers are indicated with arrowheads. *A*, Normal SC of a μMT mouse after immunization with adjuvant alone; *B*, Inflammation and demyelination in the SC of a MOG-immunized WT C57BL/6 mouse; *C*, A lower magnification micrograph showing the extent of an inflammatory lesion in the SC of a MOG-immunized μMT mouse. Several demyelinated nerve fibers are present; *D*, Inflammation and demyelination in the SC of a MOG-immunized μMT mouse; *E*, Normal optic nerve of a μMT mouse immunized with adjuvant alone; *F*, Inflammation and demyelination in the optic nerve of a MOG-immunized μMT mouse.

Discussion

It has been proposed that Abs are mediators of demyelination in MOG-induced EAE, but no study thus far has analyzed whether Abs are necessary for demyelination in MOG-induced EAE. We show here that genetically B cell-deficient μMT mice are susceptible to MOG-induced EAE and develop inflammatory lesions with primary demyelination in the CNS. Abs against MOG, or any other myelin Ag, are thus not necessary for demyelination in MOG-induced EAE, even though it cannot be excluded that Abs might influence the disease course and/or severity of the lesions. However, the B cell-deficient μMT mice had a chronic-sustained disease indistinguishable from that seen in C57BL/6 WT mice after immunization with rat MOG 35–55 peptide, and both the inflammation and demyelination were similar to those seen in WT mice in this and our previous experiments (19). Our results in MOG-immunized H-2^b μMT mice are in agreement with data from Wolf et al. (20) showing that MBP-immunized genetically B cell-deficient B10.PLμMT (H-2^u) mice lacking Abs develop EAE.

Our results are surprising considering the amount of evidence indicating that anti-MOG Abs mediate demyelination both in vitro (4–6) and in vivo (7–13, 17) in different animal models. Most studies on the role of Abs in demyelination in MOG-induced EAE have been conducted in species other than the mouse, which could explain some of the differences between our studies. Recently, however, a study was published using transgenic knock-in mice with the germline J_H locus replaced with the rearranged Ig H-chain variable (V) gene of a pathogenic MOG-specific mAb. These transgenic mice developed a more severe disease, with increased incidence and accelerated disease onset, after challenge with encephalitogenic Ags or T cells (17), thus implicating a role for

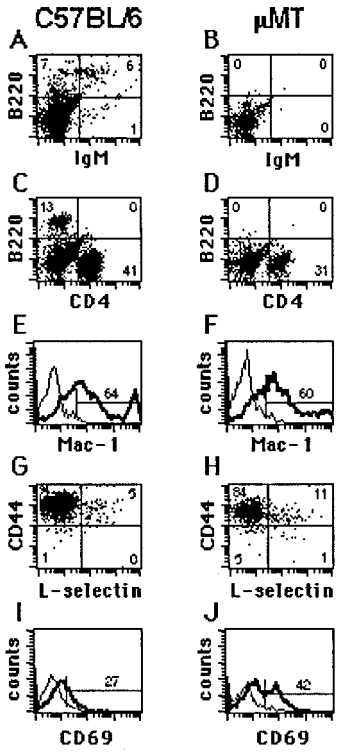

FIGURE 2. The autoimmune infiltrate in the CNS of B cell-deficient μMT mice contains T cells and macrophages, but no B cells or IgM-positive cells. Cells were isolated from the CNS of MOG-immunized WT (*left*) and μMT (*right*) mice and stained for the expression of B220 and IgM (*A* and *B*), B220 and CD4 (*C* and *D*), Mac-1 (*E* and *F*), CD44 and L-selectin gated on $CD4^+$ cells (*G* and *H*), and CD69 gated on $CD4^+$ cells (*I* and *J*). Representative data from two independent experiments using cells pooled from several mice are shown with the percentage of positive cells in relevant quadrants.

anti-MOG Abs also in mouse models of EAE. Nevertheless, our studies in a genetically B cell-deficient mouse model show that Abs are not necessary for demyelination but that there are other factors that can fully mediate the effector phase of the disease. Studies of the autoimmune infiltrate in MOG-immunized μMT mice indicate that both activated and memory T cells and macrophages are present in the CNS lesions; cells that could mediate myelin damage through either direct or indirect mechanisms. We are currently analyzing the composition of the CNS lesions in μMT and WT MOG-immunized mice in more detail to determine the role of individual components in the inflammatory process.

While this article was under review, a communication was published showing that B cell- and Ab-deficient RAG-$1^{-/-}$ mice are susceptible to EAE adoptively transferred by MOG 35–55-specific T cells (21). Even though that study did not include an analysis of inflammation and demyelination in the RAG-$1^{-/-}$ mice, it supports our finding that B cells and Abs are not necessary for full clinical disease in MOG-induced EAE. It is still possible, however, that in fully immunocompetent mice, both T and B cells could play a role in the pathogenesis, and we cannot rule out the role of B cells and Ab in MOG-induced EAE in WT mice. B cell-deficient RAG-$1^{-/-}$ mice after adoptive transfer of MOG 35–55-specific T cells (21) and B cell-deficient B10.PLμMT mice after active MBP immunization (20) fail to recover spontaneously, in contrast to their WT littermates, but have sustained clinical signs. These data suggest that B cells or their products may play a role in immune regulation in EAE. They could influence cytokine production and immune deviation or regulation and selection of functional T cell repertoires. The elucidation of the role of B cells and their products in the regulation of inflammatory processes and clinical disease in MS and EAE will be the subject of further studies.

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