Published in final edited form as:

Exp Mol Pathol. 2008 August ; 85(1): 28–39. doi:10.1016/j.yexmp.2008.03.005.

ROLE OF B:T CELL RATIO IN SUPPRESSION OF CLINICAL SIGNS: A MODEL FOR SILENT MS

Lisa K. Peterson, PhD, Ikuo Tsunoda, Md, PhD, Jane E. Libbey, MS, and Robert S. Fujinami, PhD

Department of Pathology, University of Utah School of Medicine, 30 North 1900 East, 3R330 SOM, Salt Lake City, Utah 84132

Abstract

B10.S mice have been considered resistant to experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. However, sensitization with a myelin oligodendrocyte glycoprotein (MOG) peptide, MOG_{92–106}, induced clinical signs in 30% of mice and central nervous system (CNS) pathology in 93% of mice. Symptomatic mice had more demyelination, inflammation, perivascular cuffing and axonal damage in the CNS compared to asymptomatic mice, but no strong correlations between CNS pathology and clinical score were found. Interestingly, the ratio of B cells to T cells in cellular infiltrates correlated with clinical score. This suggests that the balance between B and T cells contributes to expression of clinical signs.

Keywords

Axonal damage; CNS-demyelinating autoimmune diseases; CNS disease pathology; Experimental autoimmune encephalomyelitis; Myelin oligodendrocyte glycoprotein; Regulatory T cells

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), which encompasses a diverse spectrum of disease presentation (Willenborg and Staykova, 2003). The clinical course of MS can be categorized into four forms based on the presence or absence of relapses and remission and/or progression of neurological deficits: relapsing-remitting (RR), primary-progressive (PP), secondary progressive (SP) and progressive-relapsing (PR) (Lublin and Reingold, 1996). The MS clinical spectrum has also been suggested to include Devic's neuromyelitis optica, Balo's disease, Marburg's disease, clinically isolated syndromes (CIS) and clinically silent MS.

Clinically silent (also referred to as asymptomatic or subclinical) MS describes lesions suggestive of MS that are detected by chance through imaging or at the time of autopsy (Siva, 2006). Lesions suggestive of MS have been identified by radiological assessment in twins or relatives of patients with MS, and during autopsies of patients with no history of neurological

*Correspondence and reprint requests to: Robert S. Fujinami, PhD, Department of Pathology, University of Utah School of Medicine, 30 North 1900 East, 3R330 SOM, Salt Lake City, Utah 84132, Phone: 801-585-3305, FAX: 801-585-3311, E-mail: Robert.Fujinami@hsc.utah.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

symptoms typical of MS, indicating that the presence of CNS lesions does not directly correlate with clinical symptoms. Studies have suggested that, for every four people diagnosed with MS, there is one person with clinically silent disease (Engell, 1989). Other studies have indicated a higher frequency of clinically silent patients, and suggested that, for every patient diagnosed with MS, there are one or two people with clinically silent disease (Gilbert and Sadler, 1983). Mechanisms of transition and differences between asymptomatic and symptomatic MS are not well understood, due in part to a lack of appropriate animal models (Thompson et al., 1997).

Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS. RR- and PP-EAE can be induced in two different strains of *H-2^s* mice using the same encephalitogenic peptide myelin oligodendrocyte glycoprotein (MOG)_{92–106} (Tsunoda et al., 2000). SJL/J mice immunized with MOG_{92–106} develop RR-EAE characterized by a mild demyelinating disease with T cell infiltration. In contrast, A.SW mice sensitized with MOG_{92–106} develop PP-EAE characterized by large areas of plaque-like demyelination and have high titers of serum MOG specific antibody and immunoglobulin (Ig) deposition in the CNS (Tsunoda et al., 2000). Both SJL/J mice and A.SW mice are of the major histocompatibility complex (MHC) H-2 haplotype “s”. However, the s haplotype can be further subdivided to clarify differences in the genotype in some of the distal portions of the MHC locus, particularly class Ib (Fischer Lindahl, 1997) (Fig. 1). According to this nomenclature, the haplotype of SJL/J mice is *s2*, while that of A.SW is *s*. Although class I and class II genes are identical between the two strains, there are differences in the distal MHC locus, where class Ib, including Qa1 and Tla antigens, and MOG are encoded (Stroynowski, 1990) (Fig. 1).

B10.S mice, whose MHC haplotype is *H-2^s* (Fischer Lindahl, 1997; Stroynowski, 1990), have typically been considered to be resistant to EAE, and have been used as the prototypical resistant strain in genetic studies of genes responsible for EAE development. However, resistance to EAE is not conferred by the B10 background in combination with all MHC haplotypes. B10.RIII (*H-2^r*) and B10.PL (*H-2^u*) were shown to be highly susceptible to EAE induced with myelin basic protein (MBP)_{89–101} and MBPAc1-9, respectively (Jansson et al., 1991; Urban et al., 1988).

A number of different reasons have been proposed to explain the resistance of B10.S mice to EAE. Radiation bone marrow chimeras and adoptive transfers between B10.S and SJL/J, which both bear the MHC *H-2^s* haplotype, have revealed that non-bone marrow derived cells and the milieu in which encephalitogenic T cells are primed determine susceptibility or resistance to EAE (Chen et al., 2006; Korngold et al., 1986; Lublin et al., 1986). Differences in the permeability of the blood-brain barrier have been reported between B10.S and SJL/J mice, but the precise impact on susceptibility has not been determined (Jemison et al., 1991). Resistance of B10.S mice to MBP induced EAE correlated with a deficiency and/or a functional defect in MBP-specific T cells, but was not associated with defects in antigen presentation (Binder et al., 1991). The functional defect in MBP-specific T cells was suggested to be their inability to produce interferon (IFN)- γ upon stimulation with MBP as demonstrated by *in vitro* experiments.

Exposure of MBP-specific T cells to interleukin (IL)-12 *in vitro* prior to transfer into naïve B10.S mice resulted in the development of EAE (Segal and Shevach, 1996). Irradiation, immunosuppression induced with cyclophosphamide and treatment with antibodies against IFN- γ , IL-12, CD3 and CD25 have been shown to convert an EAE resistant phenotype into a susceptible one (Arnon, 1981; Billiau et al., 1988; Reddy et al., 2004; Zhao et al., 1992). Naïve B10.S mice were shown to have more myelin proteolipid protein (PLP)_{139–151} reactive CD4⁺CD25⁺ T cells in spleen and lymph nodes than SJL/J mice, suggesting a role for regulatory T cells in resistance to EAE. Depletion of these cells prior to sensitization with PLP_{139–151} resulted in the development of clinical signs in approximately a third of the mice,

with increased antigen-specific proliferation and IFN- γ production. These results indicate that regulatory T cells are necessary, but not sufficient, for resistance to EAE induction (Reddy et al., 2004). Interestingly, sensitization of B10.S mice with spinal cord homogenate prepared from SJL mice resulted in clinical signs in 16% of the mice and CNS pathology in 68% of the mice. In contrast, sensitization with spinal cord homogenate prepared from B10.S mice did not result in clinical signs or CNS pathology, suggesting that the susceptibility to disease induction differs between antigens (Korngold et al., 1986). These results have led to the conclusion that B10.S mice are resistant to EAE. However, these studies have focused primarily on the development of clinical signs and did not follow animals for a long enough period of time after sensitization, typically less than 30 days (Table 1).

To test whether the differences in disease course and pathology between SJL/J and A.SW mice are due to the distal portion of the H-2 complex or genes outside the MHC, B10.S mice, whose MHC haplotype is *H-2^s*, were sensitized with MOG₉₂₋₁₀₆ in complete Freund's adjuvant (CFA). Only 30% of B10.S mice sensitized with MOG₉₂₋₁₀₆ developed clinical signs of EAE, regardless of whether *Bordetella pertussis* (BP) was given or not. However, most B10.S mice developed extensive CNS disease pathology (93%). B10.S mice that displayed clinical signs of disease developed a monophasic or RR disease course, with the majority of mice exhibiting classic, paralytic, signs of disease and one third of the mice exhibiting ataxic signs. Overall mice that showed clinical signs of disease had more demyelination, inflammation, perivascular cuffing and axonal damage in the CNS compared to asymptomatic mice, but only weak correlations were found between any of these factors and the extent of clinical disease (clinical score). This indicates that none of these factors alone are responsible for the lack of clinical signs. Interestingly, the ratio of B cells to T cells in lymphocytic infiltrates was found to correlate with clinical score, suggesting that the balance between B and T cells contributes to the expression of, or the lack of, clinical signs.

Materials and Methods

Animals and EAE induction

B10.S, SJL/J and A.SW mice were purchased from the Jackson Laboratory (Bar Harbor, ME). A.SW and B10.S mice were bred at the Comparative Medicine Center at the University of Utah. Four to six week old mice were sensitized subcutaneously in the base of the tail with 100 nmol of MOG₉₂₋₁₀₆ peptide (DEGGYTCFFRDHSYQ) (Peptide Core Facility, University of Utah, Salt Lake City, UT) (Amor et al., 1994; Papenfuss et al., 2004) in CFA containing 2 mg/ml *Mycobacterium tuberculosis* H37 Ra (Difco Laboratories, Detroit, MI) with or without intravenous injection of 5×10^9 BP cells (Michigan Department of Public Health, Lansing, MI). Mice were weighed and observed for clinical signs for a minimum of 3 months.

Classical EAE signs were assessed according to the following criteria: 0 = no clinical disease; 1 = loss of tail tonicity; 2 = mild hind leg paresis; 3 = moderate hind leg paralysis; 4 = complete paraplegia; and 5 = quadriplegia, moribund state or death (Tsunoda et al., 1998).

Ataxic signs of EAE were assessed according to the following criteria: 0 = no clinical disease; 1 = mice turned their heads or bodies to one side with or without a waddling gait; 2 = mice leaned to one side and fell while walking; 3 = mice continuously rolled by twisting their bodies or rotated laterally in a circle; 4 = mice could not stand but would lie on their sides with or without rolling; and 5 = moribund state or death (Greer et al., 1996; Tsunoda et al., 2000).

Histology

Mice were euthanized using isoflurane, and blood was obtained by cardiac puncture. The mice were then exsanguinated and perfused with phosphate-buffered saline (PBS) followed by a

buffered 4% paraformaldehyde solution, and brains and spinal cords were removed from the mice. Brains were divided into five coronal slabs, and spinal cords into 10 to 12 transverse slabs. Tissues were then embedded in paraffin. Four-micrometer thick tissue sections were stained with Luxol fast blue for myelin visualization. Brain and spinal cord sections were scored for meningitis, demyelination and perivascular cuffing as described previously (Tsunoda et al., 2000). Briefly, brain sections were scored for meningitis (0 = no meningitis; 1 = mild cellular infiltrates; 2 = moderate cellular infiltrates; 3 = severe cellular infiltrates), perivascular cuffing (0 = no cuffing; 1 = 1 to 10 lesions; 2 = 11 to 20 lesions; 3 = 21 to 30 lesions; 4 = 31 to 40 lesions; 5 = more than 40 lesions) and demyelination (0 = no demyelination; 1 = mild demyelination; 2 = moderate demyelination; 3 = severe demyelination). The scores were then combined for a maximum score of 11 per mouse brain. Spinal cord segments were divided into four quadrants consisting of the anterior funiculus, the posterior funiculus and each lateral funiculus. Each quadrant containing meningitis, perivascular cuffing or demyelination was given a score of 1 in that pathologic class. The total number of positive quadrants for each pathologic class was determined, then divided by the total number of quadrants present on the slide and multiplied by 100 to give the percent involvement for each pathologic class (Tsunoda et al., 2000).

Immunohistochemistry

B cells, T cells, and Ig deposition were detected by the avidin-biotin peroxidase complex (ABC) technique, using biotin conjugated anti-mouse CD45R/B220 antibody (1:3,000 dilution, BD PharMingen, San Diego, CA), anti-CD3 antibody (following trypsinization, 1:32 dilution, Dako Corporation, Carpinteria, CA) and biotin-conjugated mouse IgG (H + L) antibody (1:200 dilution, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) (Tsunoda et al., 2000). Complement deposition was detected using an anti-C1q antibody (1:1,000 dilution, Dako Corporation). Regulatory T cells were visualized using an antibody against forkhead box P3 (FoxP3) (1:100 dilution, BioLegend, San Diego, CA) (Roncador et al., 2005). Axonal damage was visualized by the ABC method with SMI 311 (1:5,000 dilution, Sternberger Monoclonal, Inc., Baltimore, MD), a cocktail of monoclonal antibodies (SMI 32, 33, 37, 38, and 39) against nonphosphorylated neurofilament protein (NFP) (Tsunoda et al., 2003). Spinal cords were scored for axonal damage in a manner similar to that used for scoring pathology. Spinal cord sections were divided into four quadrants consisting of the anterior funiculus, the posterior funiculus and each lateral funiculus. Each quadrant containing axonal damage was given a score of 1. The total number of positive quadrants was determined, then divided by the total number of quadrants present on the slide and multiplied by 100 to give the percent involvement for axonal damage.

Cloning and expression of recombinant mouse MOG (rmMOG)

The cDNA encoding mouse MOG on the plasmid pcDNA3.1 (Bourquin et al., 2000) was a kind gift from Drs. Andreas Holz and Hartmut Wekerle (Max-Planck-Institute of Neurobiology, Martinsried, Germany). This plasmid was propagated using standard molecular biology techniques (Sambrook et al., 1989). The extracellular domain region of MOG minus the signal sequence (amino acids 30–150) was subcloned using the cDNA as template in a nested PCR reaction; the first PCR product, encoding the MOG gene minus the signal sequence, was used as template in a second PCR reaction. The primers used in the second PCR reaction had restriction enzyme sites engineered into them. The PCR product and the prokaryotic expression vector, pET43.1a (EMD Biosciences, La Jolla, CA), were digested with the restriction enzymes *XhoI* and *NdeI*. The digested PCR product and vector were purified, ligated and propagated in *E. coli* using standard techniques (Sambrook et al., 1989). The sequence for the extracellular domain (minus the signal sequence) of MOG, covalently linked to a histidine tag, was confirmed by sequencing (DNA Sequencing Core Facility, University of Utah, Salt Lake City, UT). The resultant rmMOG clone was transformed into BL21(DE3) cells, grown

in bacterial culture and induced with isopropyl β -D-thiogalactoside (IPTG) for expression in bacterial cells using standard techniques (Sambrook et al., 1989). Two hours after induction, the cell cultures were pelleted by centrifugation and the recombinant protein was isolated using the Ni-NTA Spin Kit (Qiagen, Chatsworth, CA) according to the manufacturer's recommendations. Briefly, the pellet was solubilized in 6M GuHCl (denaturing conditions), the protein solution was applied to the Ni column and washed and eluted with 8M urea. To maintain the solubility of the rmMOG, the protein eluent was dialyzed against 20mM Na acetate buffer, pH3. The production of the rmMOG protein was confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the protein concentration was determined by means of the Bio-Rad Protein assay (Bio-Rad, Hercules, CA).

ELISA for MOG antibody

Blood was collected from MOG sensitized mice at the time of sacrifice. Enzyme-linked immunosorbent assays (ELISAs) were performed to measure the level of serum MOG antibody (Tsunoda et al., 2000). Briefly, 96-well flat-bottom Nunc-Immuno MaxiSorp™ plates (Nalge Nunc International, Rochester NY) were coated overnight with 10 μ g/ml MOG₉₂₋₁₀₆ peptide or rmMOG. After blocking with 10% Cosmic calf serum (CCS) (HyClone, Logan, UT) and 0.2% Tween 20, serial dilutions of sera were added to the plates and incubated for 90 min at room temperature. After washing, a peroxidase-conjugated anti-mouse IgG (γ) (Caltag Laboratories, Burlingame, CA), anti-mouse IgM (Stressgen Biotechnologies, Victoria, BC, Canada), anti-mouse IgG1 (BD PharMingen), IgG2b (BD Pharmingen) or anti-mouse IgG2c (Abcam, Cambridge, MA) (Martin et al., 1998) were added for 90 min. Immunoreactive complexes were detected with *o*-phenylenediamine dihydrochloride (Sigma-Aldrich, St. Louis, MO) and were read at 492 nm in a Titertek Multiskan Plus MK II spectrophotometer (Flow Laboratories, McLean, VA).

Results

Clinical course and clinical signs of EAE in B10.S mice

The clinical course of EAE was determined by monitoring changes in weight and development of clinical signs of disease (described in the Methods). Thirty percent of B10.S mice sensitized with MOG₉₂₋₁₀₆ manifested clinical signs of EAE, albeit at a much later time (83.9 days $\pm$ 14.9) compared to A.SW or SJL/J mice (26 and 45 days, respectively) (Tsunoda et al., 2000). Of these, the majority (two-thirds) developed classical signs of EAE, including atony of the tail and flaccid paralysis, but complete paraplegia with incontinence occurred in a few mice. However, one third of the B10.S mice that developed clinical signs of disease displayed the ataxic form of disease including turning the head and/or body to one side and falling on their side while walking, due to the degree to which they were leaning their bodies to one side. B10.S mice that displayed clinical signs of disease developed a monophasic (Fig. 2A) or RR disease course (Fig. 2B) with a mean maximum clinical score of 2.2 ± 0.2 .

Neuropathology

To determine whether differences in the presence and/or distribution of lesions in the CNS were responsible for the development of clinical signs of disease, we assessed neuropathology. Despite the fact that only 30% of B10.S mice sensitized with MOG₉₂₋₁₀₆ displayed clinical signs of disease, lesions were present in the CNS (brain and/or spinal cord) of 93% of mice. All of the mice that had clinical signs also had lesions in their CNS. More severe lesions were detected in the brains rather than in the spinal cords of B10.S mice sensitized with MOG₉₂₋₁₀₆. Only 13 of 61 mice (23%) had brains free of lesions. Lesions were observed in the brains of all but one of the mice that developed clinical signs (93%). Interestingly, most asymptomatic mice had lesions in the brain (71%) (Table 2).

The severity of the lesions in asymptomatic mice was significantly less than that observed in mice that displayed clinical signs of disease (Brain score: symptomatic = 3.4 ± 0.5 , asymptomatic = 2.0 ± 0.3 ; $P < 0.05$, *t*-test) (Fig. 3A). In the brain, most mice developed mild to moderate meningitis composed of mononuclear cells (MNCs), particularly around the hippocampus. Mice that displayed clinical signs of disease had more meningitis in their brains than asymptomatic mice, but this difference did not reach statistical significance (Meningitis score: symptomatic = 1.8 ± 0.3 , asymptomatic = 1.1 ± 0.2 ; $P = 0.05$, *t*-test). Half of the B10.S mice sensitized with MOG₉₂₋₁₀₆ developed inflammatory demyelinating lesions in the brain, particularly in the cerebellar white matter. Demyelination was also seen in the lateral optic tract, optic nerve, brainstem (Fig. 4A), internal capsule, and crus cerebri. In most cases, inflammatory cells in the demyelinating lesions were MNCs. Some large demyelinating lesions contained both MNCs and polymorphonuclear cells (PMNs). Significantly more demyelination was detected in the brains of mice that displayed clinical signs than asymptomatic mice (Demyelination score: symptomatic = 1.3 ± 0.3 , asymptomatic = 0.5 ± 0.1 ; $P < 0.01$, *t*-test). No significant difference in perivascular cuffing was observed between mice with or without clinical signs (Cuffing Score: symptomatic = 0.3 ± 0.1 , asymptomatic = 0.3 ± 0.1 ; $P = 0.72$, *t*-test). Interestingly, five B10.S mice (8%) developed mild to moderate enlargement of the third and lateral ventricles (hydrocephalus), which is rarely reported in EAE. To examine whether clinical scores correlated with brain pathology we plotted clinical score versus meningitis ($r = 0.24$, $P = 0.08$), perivascular cuffing ($r = -0.12$, $P = 0.36$), demyelination ($r = 0.51$, $P < 0.0001$) and overall lesions in the brain ($r = 0.33$, $P < 0.05$). To our surprise, we did not find a strong association between clinical scores and brain pathology (Fig. 5A–D).

Seventy-five percent of B10.S mice sensitized with MOG₉₂₋₁₀₆ had lesions in the spinal cord. Lesions were observed in the spinal cords of all but one of the mice that developed clinical signs (93%), and 68% of asymptomatic mice (Table 2). The severity of the lesions in asymptomatic mice was significantly less than that observed in mice that displayed clinical signs of disease (Spinal cord score: symptomatic = 43.0 ± 6.5 , asymptomatic = 10.7 ± 1.9 ; $P < 0.001$, *t*-test) (Fig. 3B). Demyelinating lesions were observed in all areas of white matter in the spinal cord, the anterior funiculus, lateral funiculus and posterior funiculus (Fig. 6A). The gracile fascicle of the posterior funiculus was most frequently involved, while the cuneatus fascicle was preserved (Tsunoda et al., 1999). Significantly more demyelination was observed in the spinal cords of mice that had clinical signs of disease than asymptomatic mice (Demyelination score: symptomatic = 28.9 ± 6.7 , asymptomatic = 2.9 ± 1.0 ; $P < 0.001$, *t*-test) (Fig. 3B). Inflammatory cells were composed of MNCs in most mice, but some mice had PMN infiltration. Mice that developed clinical signs of disease had significantly more meningitis in their spinal cords than asymptomatic mice (Meningitis score: symptomatic = 31.8 ± 4.7 , asymptomatic = 10.1 ± 1.8 ; $P < 0.001$, *t*-test) (Fig. 3B). No significant difference in perivascular cuffing was observed between mice with or without clinical signs (Cuffing score: symptomatic = 0.2 ± 0.2 , asymptomatic = 0.1 ± 0.1 ; $P = 0.54$, *t*-test) (Fig. 3B). To determine whether clinical scores correlated with spinal cord pathology, we plotted clinical score versus meningitis ($r = 0.56$, $P < 0.0001$), perivascular cuffing ($r = 0.17$, $P = 0.22$), demyelination ($r = 0.69$, $P < 0.0001$) and overall lesions in the spinal cord ($r = 0.67$, $P < 0.0001$) (Fig. 7A–D). Although correlation with meningitis, demyelination and overall score reached statistical significance, no strong association was found between any pathology score and clinical score.

T cell infiltration in the CNS

To evaluate the phenotype of the cellular infiltrates in the CNS of MOG₉₂₋₁₀₆ sensitized B10.S mice, we stained and enumerated T and B cells using immunohistochemistry against CD3 and B220, respectively (Fig. 4B, C and Fig. 6C, D). CD3⁺ T cells comprised approximately 30% of meningeal infiltrates, 28% of MNCs in perivascular cuffs and 25% of infiltrates in

parenchymal demyelinating lesions in the CNS of MOG₉₂₋₁₀₆ sensitized B10.S mice (Fig. 8B). Interestingly, asymptomatic B10.S mice had a significantly higher frequency of T cells in the meningeal infiltrates in the brain compared to mice that developed clinical signs of disease ($P < 0.05$, t -test). This led us to question whether the increased number of T cells in asymptomatic mice reflected an increased number of regulatory T cells. FoxP3 immunostaining was performed to identify regulatory T cells in the CNS lesions. Regulatory T cells were detected at similar, albeit low, frequencies ($< 1\%$ of lymphocytic infiltrates) in CNS lesions in B10.S mice sensitized with MOG₉₂₋₁₀₆ regardless of the development of clinical signs (Fig. 9A). More FoxP3⁺ regulatory T cells were detected in lesions in the brain and spinal cord of A.SW mice with PP-EAE (Fig. 9C) than in B10.S mice with RR-EAE (Fig. 9A), but none were detected in the lesions of SJL/J mice with RR-EAE (Fig. 9B). These results indicate that the frequency of FoxP3⁺ regulatory T cells in the CNS is not associated with the lack of development of clinical signs in B10.S mice.

B cell infiltration in the CNS

B cells comprised approximately 32% of meningeal infiltrates, 15% of lymphocytes in perivascular cuffs and 11% of the infiltrates in parenchymal demyelinating lesions in the CNS of B10.S mice sensitized with MOG₉₂₋₁₀₆. Overall B10.S mice that developed clinical signs had a higher percentage of B cells in cellular infiltrates, compared to mice that did not develop clinical signs. Symptomatic mice had significantly more B220⁺ cells in the meninges in the brain compared to asymptomatic mice ($P < 0.01$, t -test) (Fig. 8A). Although correlation of clinical score with the frequency of B220⁺ cells in MNC infiltrates reached statistical significance ($P < 0.01$ in the brain, t -test), associations between B cell infiltration and clinical score in the brain ($r = 0.59$) and spinal cord ($r = 0.49$) were not strong. Interestingly, the ratio of total B220⁺ B cells to CD3⁺ T cells in the meninges in the CNS demonstrated a significantly strong correlation with clinical score ($r = 0.90$, $P < 0.0001$) (Fig. 8C). This suggests that the balance between B and T cells contributes to the expression or suppression of clinical signs.

CNS-infiltrating B cells were often found clustered in large aggregates within the meninges (Fig. 6D), suggestive of the development of lymphoid follicles within the inflamed meninges of B10.S mice sensitized with MOG₉₂₋₁₀₆. Ectopic follicle-like structures have been reported in patients with autoimmune diseases including Sjogren's syndrome, Hashimoto's thyroiditis, rheumatoid arthritis and MS (Hjelmström, 2001). These structures have been proposed to be responsible for maintaining the autoimmune process through localized production of pathogenic autoantibodies (Hjelmström, 2001; Magliozzi et al., 2004; Prineas, 1979). Follicle-like structures have also been observed in mice with both adoptive transfer and actively induced EAE (Magliozzi et al., 2004; Raine et al., 1984).

Immunoglobulin deposition in the CNS

Since antibody plays an important role in MOG₉₂₋₁₀₆ induced EAE (Tsunoda et al., 2000), we examined the extent of Ig deposition in the CNS, using immunohistochemistry against the heavy and light chains of Ig. In most B10.S mice, we found Ig deposition in the meninges, vessels and choroid plexus. Ig deposition was observed in inflammatory demyelinating lesions in the brain (Fig. 4D) and spinal cord (Fig. 6E), and was accompanied by deposition of complement C1q (Fig. 6F). Substantial numbers of surface Ig⁺ cells were detected in the meninges adjacent to the lesions. B220 immunostaining suggests that most of the Ig⁺ cells in the meninges are surface Ig⁺ B cells.

MOG₉₂₋₁₀₆ sensitization appeared to induce distinct antibody responses in individual mice. For example, intense Ig deposition in demyelinating lesions was present in a few mice, and one mouse had Ig deposition in normal-appearing white matter (NAWM), such as the corpus callosum and anterior commissure, and two segments of the normal appearing gray and white

matter of the spinal cord. Interestingly, another mouse had neuron specific Ig staining around the perivascular cuff in the tegmentum of the brainstem, suggesting autoantibody responses (epitope spreading or cross-reaction of natural antibody) against neurons (data not shown).

Anti-MOG antibody response

Using ELISA, we found that B10.S mice developed substantial humoral responses against MOG₉₂₋₁₀₆ in all Ig isotypes examined, including IgM, IgG(γ), IgG1, IgG2b and IgG2c (Fig. 10A). No significant difference in MOG₉₂₋₁₀₆ serum antibody titer was detected in mice that developed clinical signs of disease as compared to those that did not. B10.S mice sensitized with MOG₉₂₋₁₀₆ also developed humoral responses which reacted with rmMOG in all Ig isotypes examined (Fig. 10B). B10.S mice that developed clinical signs tended to have higher reactivity with rmMOG than mice that did not display clinical signs for all of the isotypes tested. Interestingly, B10.S mice that developed clinical signs had significantly more anti-rmMOG IgM ($P < 0.05$) and IgG1 ($P < 0.05$), compared to mice that did not develop clinical signs. However, the titer of these antibodies did not correlate with the development of clinical signs (data not shown).

Axonal damage in MOG₉₂₋₁₀₆ induced EAE

Axonal damage was monitored using a cocktail of antibodies against nonphosphorylated NFP (SMI 311). Injured axons as well as normal neuron bodies and dendrites react with SMI 311, whereas normal axons do not (Sternberger and Sternberger, 1983; Ulfing et al., 1998). Axonal torpedo formation was observed in the cerebellum and beading (Tsunoda et al., 2003) was present in longitudinal sections of spinal cord in B10.S mice with monophasic or RR-EAE, SJL/J mice with RR-EAE and A.SW mice with PP-EAE (Fig. 11). Axonal damage was detected in the anterior and lateral funiculi and the ventral root exit zone (VREZ), but less frequently in the posterior funiculus (Fig. 6B), of the spinal cord. B10.S mice that showed clinical signs of disease had significantly more axonal damage in spinal cords than mice that did not develop clinical signs of disease (Axonal damage score: symptomatic: 23.7 ± 4.8 , asymptomatic = 16.9 ± 3.0 ; $P < 0.05$, t -test) (Fig. 7E). However, there was no significant correlation between axonal damage and clinical signs ($r = 0.44$, $P = 0.02$) (Fig. 7F).

Comparison of MOG₉₂₋₁₀₆ sensitization in B10.S, A.SW and SJL/J mice

Thirty percent of B10.S mice sensitized with MOG₉₂₋₁₀₆ had clinical signs of EAE with a delayed time of onset and a lower mean maximum clinical score compared to A.SW or SJL/J mice (Table 3). The severity and/or extent of brain pathology in symptomatic B10.S mice was less than that observed in A.SW mice with PP-EAE, but greater than that observed in SJL/J mice with RR-EAE (Table 4). Symptomatic B10.S mice had similar pathology in the spinal cords to that observed in SJL/J mice with RR-EAE, in all aspects but cuffing. In contrast, symptomatic B10.S mice had lower levels of cuffing, demyelination and overall lesions compared with A.SW mice with PP-EAE (Table 4).

Since T cells predominate in CNS infiltrates in many classic forms of EAE, T cells are believed to act not only as the initiators of CNS inflammation, but also as one of the effector cells of demyelination. However, in MOG₉₂₋₁₀₆ induced EAE, while CD3⁺ T cells are the dominant lymphocytes in both meningeal and perivascular infiltrates in SJL/J mice with RR-EAE, T cells comprise only a small percentage of infiltrates in meningeal and perivascular spaces and are virtually absent in parenchymal demyelinating lesions in MOG₉₂₋₁₀₆ sensitized A.SW mice with PP-EAE (Tsunoda et al., 2000). The frequency of T cells in MNC infiltrates in the CNS of B10.S mice sensitized with MOG₉₂₋₁₀₆ was higher than that of A.SW mice, but lower than that of SJL/J mice (Table 3). We also compared the presence of FoxP3⁺ regulatory T cells in the CNS of B10.S mice with A.SW and SJL/J mice with EAE (Fig. 9). More FoxP3⁺ regulatory

T cells were detected in lesions in the CNS of A.SW mice than in B10.S mice, however, FoxP3⁺ T cells were absent from the lesions of SJL/J mice (Table 3).

No significant difference was observed in the percentage of B cells in the meningeal infiltrates of MOG₉₂₋₁₀₆ sensitized SJL/J mice with RR-EAE and A.SW mice with PP-EAE, in contrast to the significant difference in the number of T cells. B cells comprise approximately 10% of meningeal infiltrates, but are virtually absent in perivascular cuffs and parenchymal demyelinating lesions in MOG₉₂₋₁₀₆ sensitized A.SW and SJL/J mice (Tsunoda et al., 2000). In contrast, B10.S mice had substantially more B cells in the meningeal infiltrates (32%) (Table 3). Previously, we reported that A.SW mice that developed PP-EAE had higher titers of anti-MOG₉₂₋₁₀₆ antibodies in their serum and more Ig deposition in their CNS than SJL/J mice that developed RR-EAE (Tsunoda et al., 2000). B10.S mice developed a humoral response against MOG₉₂₋₁₀₆ that was lower than that observed in A.SW mice, but higher than that observed in SJL/J mice (Table 3).

Axonal damage was observed in the brains and spinal cords of B10.S mice with monophasic or RR-EAE, SJL/J mice with RR-EAE, and A.SW mice with PP-EAE (Table 3). Increased axonal damage was detected in the brains of A.SW mice with PP-EAE compared to SJL/J mice with RR-EAE and B10.S mice with monophasic or RR-EAE. In contrast, more axonal damage was detected in the spinal cords of SJL/J mice with RR-EAE compared to A.SW mice with PP-EAE and B10.S mice with monophasic or RR-EAE.

Discussion

A model in which RR- and PP-EAE can be induced in two different strains of *H-2^s* mice, SJL/J and A.SW respectively, using the same encephalitogenic peptide MOG₉₂₋₁₀₆ has been described (Tsunoda et al., 2000). Although SJL/J and A.SW mice are both of the MHC *H-2^s* haplotype, the “s” haplotype can be further subdivided to clarify differences in the genotype in the distal portion of the MHC locus, particularly the class Ib, where Qa1 and Tla antigens, and MOG are encoded (Fischer Lindahl, 1997; Stroynowski, 1990) (Fig. 1). According to this nomenclature, the haplotype of SJL/J mice is *s2*, while that of A.SW is *s*. In the current study, we examined whether the differences in disease course and pathology between SJL/J and A.SW mice were due to the distal portion of the H-2 complex or genes outside the MHC by sensitizing B10.S mice, whose MHC haplotype is *H-2^s*, with MOG₉₂₋₁₀₆. Prior to our current study, B10.S mice were considered to be resistant to EAE, and, in agreement with this, only 30% of B10.S mice sensitized with MOG₉₂₋₁₀₆ developed clinical signs of EAE. However, most B10.S mice developed extensive CNS pathology (93%). B10.S mice that developed clinical signs had either a monophasic or RR disease course, with about two-thirds of the mice exhibiting classic, paralytic signs of disease and one-third of the mice exhibiting ataxic signs. These results lead us to conclude that the H-2 complex is involved in susceptibility to MOG₉₂₋₁₀₆ induced EAE, but that genes outside the MHC determine the course of the disease.

The relationship between H-2 haplotype and susceptibility of mice to EAE has been under investigation for over three decades, yielding conflicting results. Levine and Sowinski (Levine and Sowinski, 1974) concluded that the MHC complex alone did not control susceptibility to EAE since not all strains bearing the same H-2 haplotype displayed the same degree of susceptibility or resistance to disease induction. However, they did not exclude the possibility that H-2 is one of a combination of genes that control susceptibility to EAE (Levine and Sowinski, 1974). Lando et al. (1997) provided additional support for susceptibility being governed by multiple genes by demonstrating that F1 hybrids of the susceptible SJL/J strain with various resistant strains did not inherit susceptibility to EAE in a simple Mendelian manner, but instead were more susceptible than the highly susceptible SJL/J parental strain.

In our study, we found that only 30% of B10.S mice sensitized with MOG₉₂₋₁₀₆ developed clinical signs, and at a much later time point, mean onset 84 days, as compared to A.SW and SJL/J mice (26 and 45 days, respectively) (Tsunoda et al., 2000). Despite the reduced incidence of clinical signs, the majority of MOG₉₂₋₁₀₆ sensitized B10.S mice had neuropathology. Thus upon examination, lesions characteristic of EAE were detected in mice that had no demonstrable clinical signs of EAE. These findings are reminiscent of clinically silent MS.

Clinical signs of disease have classically been used as the measurement of disease susceptibility in EAE, but reports of histological signs of disease in the absence of clinical signs exist (Lennon and Byrd, 1973; Tsunoda et al., 2005). In addition, it is likely that MS is preceded by an asymptomatic period of unknown duration prior to the onset of clinical symptoms, and that an unidentified factor(s) is responsible for the transition from clinically silent MS to clinically definite MS. Several factors have been suggested to be responsible for the lack of clinical symptoms despite the presence of lesions suggestive of MS. These include the location of the lesions, the size of the lesions, preservation of axons, neuronal redundancy and remyelination. However, no single factor has been found which can completely explain the lack of development of clinical symptoms (Mews et al., 1998). Presence of lesions in asymptomatic patients and mice suggests that a subset of inflammatory and/or demyelinating changes do not cause overt clinical signs of disease. It has been suggested that both the size and the location of these lesions is responsible for the lack of clinical signs of disease. In humans, lesions have predominantly been detected in periventricular regions which are proposed to be clinically silent regions (Engell, 1989; Mews et al., 1998). In addition, the lack of lesions in regions most likely to cause clinical signs of disease, such as infratentorial and spinal cord lesions, could also explain the absence of clinical signs (Filippi et al., 1995; Koopmans et al., 1989; Mews et al., 1998). In this study, we found that neither the size nor the location of demyelinated lesions could explain the lack of development of clinical signs of EAE in seventy percent of the B10.S mice sensitized with MOG₉₂₋₁₀₆. Demyelinated lesions were detected in the spinal cord and infratentorial regions in mice with or without signs. Periventricular lesions were not detected.

Overall, mice that developed clinical signs also had more demyelination, inflammation, perivascular cuffing and axonal damage in their CNS compared to asymptomatic mice, but no strong correlations were found between any of these factors and clinical score. This indicates that none of these factors alone are likely responsible for the lack of clinical signs. B10.S mice sensitized with MOG₉₂₋₁₀₆ had higher numbers of B cells in CNS infiltrates compared to both SJL/J mice and A.SW mice with MOG₉₂₋₁₀₆ induced EAE. Interestingly, B10.S mice that developed clinical signs of EAE had a higher frequency of B cells in CNS infiltrates compared to asymptomatic B10.S mice. In contrast, asymptomatic B10.S mice had a higher frequency of T cells compared to symptomatic B10.S mice. The increased frequency of T cells in asymptomatic mice was shown not to be the result of an increased frequency of FoxP3⁺ regulatory T cells. The ratio of B cells to T cells in lymphocytic infiltrates was found to correlate with clinical score, suggesting that the balance between these cells contributes to the expression or suppression of clinical signs. There are several ways in which B cells could contribute to the development of clinical signs of disease, including the production of pathogenic autoantibodies, presentation of antigen(s) to T cells, costimulation of T cells and production of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , lymphotoxin, IL-6 and IL-1, as well as anti-inflammatory cytokines, including IL-10 and transforming growth factor (TGF)- β , which could influence the disease course in MS and EAE [reviewed in (Lin and Constantinescu, 2005; Meinl et al., 2006)]. Cells with a B cell-like phenotype have been shown to have direct cytotoxic effects on virus infected cells (Holmes et al., 1986; Welsh et al., 1986), which suggests another possible mechanism by which B cells could contribute to the development of overt signs of clinical disease.

Another possible interpretation of the absence of clinical signs of EAE despite the presence of inflammatory demyelinating lesions in the CNS is that the current methods for assessing clinical deficits are based on gross motor defects and provide little or no information on sensory deficits, or other pathways that may be influenced by lesions in the brain rather than spinal cord. The results presented in this study emphasize the need for better ways of assessing clinical deficits in mouse models of MS.

The detection of reactivity to rmMOG, in addition to the MOG₉₂₋₁₀₆ peptide, suggests that these mice might be producing antibodies that can react with other epitopes of MOG in addition to MOG₉₂₋₁₀₆. There are several studies that suggest that only antibodies against conformational epitopes act as the target of pathogenic, demyelinating responses (von Büdingen et al., 2002; von Büdingen et al., 2004). MOG₉₂₋₁₀₆ specific autoantibodies themselves have previously been shown to be pathogenic and to induce demyelination (Jansson et al., 1991). However, it is possible that antibody-mediated demyelination in this model/strain also involves antibodies against other epitopes of MOG which could develop through epitope-spreading.

The MOG₉₂₋₁₀₆ model is useful for investigating the relationship between inflammation, demyelination and axonal damage in the different disease courses and/or development of overt clinical signs in EAE and MS, since different patterns of inflammation, demyelination, axonal loss and disease course can be induced using the same encephalitogenic peptide. In addition MOG₉₂₋₁₀₆ sensitization of B10.S mice provides a model for investigating the subclinical or perhaps preclinical phase of EAE and MS, which could aid in understanding which MS-associated autoantibodies and other biomarkers might predict the development of overt clinical disease. It could also prove useful in prognosis and treatment of patients or potential patients depending on their levels of inflammation, demyelination and axonal loss. For example, treatments that modulate the ratio of B cells to T cells could potentially prevent clinically silent MS from converting to clinically definite MS.

In summary, we demonstrate that B10.S mice, which were previously considered to be resistant to EAE, develop lesions characteristic of EAE in their CNS after sensitization with MOG₉₂₋₁₀₆ and a subset of these mice exhibit clinical signs of disease. The majority of B10.S mice sensitized with MOG₉₂₋₁₀₆ remain asymptomatic despite lesions in the CNS, a finding similar to that described for clinically silent MS. Our data demonstrates that yet another type or course of disease can be induced in mice with an MHC *H-2^s* haplotype, using a single encephalitogenic peptide, MOG₉₂₋₁₀₆, providing further support for the usefulness of the MOG₉₂₋₁₀₆ model in examining the spectrum of disease in the EAE model for MS.

Acknowledgments

The authors would like to thank Drs. Andreas Holz and Hartmut Wekerle for their generous gift of the MOG cDNA, Nikki J. Kirkman, BS, for many helpful discussions and Faris Hasanovic, J. Wes Peterson, Bryan A. Moore, Victor Gappmeier and Daniel G. Smith for excellent technical assistance. We are grateful to Ms. Kathleen Borick for preparation of the manuscript. This work was supported by NIH grant 5R01NS040350.

References

- Amor S, Groome N, Linington C, Morris MM, Dornmair K, Gardinier MV, Matthieu J-M, Baker D. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J. Immunol* 1994;153:4349–4356. [PubMed: 7525700]
- Arnold R. Experimental allergic encephalomyelitis--susceptibility and suppression. *Immunol. Rev* 1981;55:5–30. [PubMed: 6165673]

- Billiau A, Heremans H, Vandekerckhove F, Dijkmans R, Sobis H, Meulepas E, Carton H. Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN- γ . *J. Immunol* 1988;140:1506–1510. [PubMed: 3126227]
- Binder TA, Clark RB, Goldschneider I. Relative susceptibility of SJL/J and B10.S mice to experimental allergic encephalomyelitis is correlated with high and low responsiveness to myelin basic protein. *J. Neuroimmunol* 1991;35:31–43. [PubMed: 1720137]
- Bourquin C, Iglesias A, Berger T, Wekerle H, Linington C. Myelin oligodendrocyte glycoprotein-DNA vaccination induces antibody-mediated autoaggression in experimental autoimmune encephalomyelitis. *Eur. J. Immunol* 2000;30:3663–3671. [PubMed: 11169409]
- Chen F, Shaw MK, Li J, Lisak RP, Tse HY. Adoptive transfer of myelin basic protein-induced experimental autoimmune encephalomyelitis between SJL and B10.S mice: correlation of priming milieus with susceptibility and resistance phenotypes. *J. Neuroimmunol* 2006;173:146–154. [PubMed: 16480778]
- Encinas JA, Lees MB, Sobel RA, Symonowicz C, Greer JM, Shovlin CL, Weiner HL, Seidman CE, Seidman JG, Kuchroo VK. Genetic analysis of susceptibility to experimental autoimmune encephalomyelitis in a cross between SJL/J and B10.S mice. *J. Immunol* 1996;157:2186–2192. [PubMed: 8757345]
- Engel T. A clinical patho-anatomical study of clinically silent multiple sclerosis. *Acta Neurol. Scand* 1989;79:428–430. [PubMed: 2741673]
- Filippi M, Campi A, Mammi S, Martinelli V, Locatelli T, Scotti G, Amadio S, Canal N, Comi G. Brain magnetic resonance imaging and multimodal evoked potentials in benign and secondary progressive multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 1995;58:31–37. [PubMed: 7823064]
- Fischer Lindahl K. On naming H2 haplotypes: functional significance of MHC class Ib alleles. *Immunogenetics* 1997;46:53–62. [PubMed: 9148789]
- Gilbert JJ, Sadler M. Unsuspected multiple sclerosis. *Arch. Neurol* 1983;40:533–536. [PubMed: 6615282]
- Greer JM, Sobel RA, Sette A, Southwood S, Lees MB, Kuchroo VK. Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein. *J. Immunol* 1996;156:371–379. [PubMed: 8598487]
- Hjelmström P. Lymphoid neogenesis: de novo formation of lymphoid tissue in chronic inflammation through expression of homing chemokines. *J. Leukoc. Biol* 2001;69:331–339. [PubMed: 11261778]
- Holmes KV, Welsh RM, Haspel MV. Natural cytotoxicity against mouse hepatitis virus-infected target cells. I. Correlation of cytotoxicity with virus binding to leukocytes. *J. Immunol* 1986;136:1446–1453. [PubMed: 3003198]
- Jansson L, Olsson T, Höjeberg B, Holmdahl R. Chronic experimental autoimmune encephalomyelitis induced by the 89–101 myelin basic protein peptide in B10RIII (H-2^f) mice. *Eur. J. Immunol* 1991;21:693–699. [PubMed: 1707002]
- Jemison LM, Williams SK, Lublin FD, Knobel RL, Korngold R. Analysis of cerebral microvessel endothelial cell junctional permeability in experimental allergic encephalomyelitis susceptible and resistant mice. *J. Neuroimmunol* 1991;36
- Koopmans RA, Li DK, Grochowski E, Cutler PJ, Paty DW. Benign versus chronic progressive multiple sclerosis: magnetic resonance imaging features. *Ann. Neurol* 1989;25:74–81. [PubMed: 2913931]
- Korngold R, Feldman A, Rorke LB, Lublin FD, Doherty PC. Acute experimental allergic encephalomyelitis in radiation bone marrow chimeras between high and low susceptible strains of mice. *Immunogenetics* 1986;24:309–315. [PubMed: 3781573]
- Lando Z, Teitelbaum D, Arnon R. Genetic control of susceptibility to experimental allergic encephalomyelitis in mice. *Immunogenetics* 1997;9:435–442.
- Lennon VA, Byrd WJ. Role of T lymphocytes in the pathogenesis of experimental autoimmune encephalomyelitis. *Eur. J. Immunol* 1973;3:243–245. [PubMed: 4123774]
- Levine S, Sowinski R. Experimental allergic encephalomyelitis in congenic strains of mice. *Immunogenetics* 1974;1:352–356.
- Lin, X.; Constantinescu, CS. B cells and antibodies in experimental autoimmune encephalomyelitis. In: Lavi, E.; Constantinescu, CS., editors. *Experimental Models of Multiple Sclerosis*. New York: Springer Science + Business Media; 2005. p. 269–281.

- Linthicum DS, Frelinger JA. Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. *J. Exp. Med* 1982;156:31–40. [PubMed: 6806429]
- Lublin FD, Knobler RL, Doherty PC, Korngold R. Relapsing experimental allergic encephalomyelitis in radiation bone marrow chimeras between high and low susceptible strains of mice. *Clin. Exp. Immunol* 1986;66:491–496. [PubMed: 3568447]
- Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. [National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis]. *Neurology* 1996;46:907–911. [PubMed: 8780061]
- Magliozzi R, Columba-Cabezas S, Serafini B, Aloisi F. Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol* 2004;148:11–23. [PubMed: 14975582]
- Maron R, Hancock WW, Slavina A, Hattori M, Kuchroo V, Weiner HL. Genetic susceptibility or resistance to autoimmune encephalomyelitis in MHC congenic mice is associated with differential production of pro- and anti-inflammatory cytokines. *Int. Immunol* 1999;11:1573–1580. [PubMed: 10464178]
- Martin RM, Brady JL, Lew AM. The need for IgG2c specific antiserum when isotyping antibodies from C57BL/6 and NOD mice. *J. Immunol. Methods* 1998;212:187–192. [PubMed: 9672206]
- Meinl E, Krumbholz M, Hohlfeld R. B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation. *Ann. Neurol* 2006;59:880–892. [PubMed: 16718690]
- Mews I, Bergmann M, Bunkowski S, Gullotta F, Brück W. Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. *Mult. Scler* 1998;4:55–62. [PubMed: 9599334]
- Montgomery IN, Rauch HC. Experimental allergic encephalomyelitis (EAE) in mice: Primary control of EAE susceptibility is outside the H-2 complex. *J. Immunol* 1982;128:421–425. [PubMed: 7054282]
- Papenfuss TL, Rogers CJ, Gienapp I, Yurrita M, McClain M, Damico N, Valo J, Song F, Whitacre CC. Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. *J. Neuroimmunol* 2004;150:59–69. [PubMed: 15081249]
- Prineas JW. Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science* 1979;203:1123–1125. [PubMed: 424741]
- Raine CS, Barnett LB, Brown A, Behar T, McFarlin DE. Neuropathology of experimental allergic encephalomyelitis in inbred strains of mice. *Lab Invest* 1980;43:150–157. [PubMed: 7401630]
- Raine CS, Mokhtarian F, McFarlin DE. Adoptively transferred chronic relapsing experimental autoimmune encephalomyelitis in the mouse. Neuropathologic analysis. *Lab. Invest* 1984;51:534–546.
- Reddy J, Illes Z, Zhang X, Encinas J, Pyrdol J, Nicholson L, Sobel RA, Wucherpfennig KW, Kuchroo VK. Myelin proteolipid protein-specific CD4⁺CD25⁺ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* 2004;101:15434–15439. [PubMed: 15492218]
- Roncador G, Brown PJ, Maestre L, Hue S, Martínez-Torrecuadrada JL, Ling KL, Pratap S, Toms C, Fox BC, Cerundolo V, Powrie F, Banham AH. Analysis of FOXP3 protein expression in human CD4⁺CD25⁺ regulatory T cells at the single-cell level. *Eur. J. Immunol* 2005;35:1681–1691. [PubMed: 15902688]
- Sambrook, J.; Fritsch, EF.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory; 1989.
- Segal BM, Shevach EM. IL-12 unmasks latent autoimmune disease in resistant mice. *J. Exp. Med* 1996;184:771–775. [PubMed: 8786337]
- Siva A. The spectrum of multiple sclerosis and treatment decisions. *Clin. Neurol. Neurosurg* 2006;108:333–338. [PubMed: 16378680]
- Sternberger LA, Sternberger NH. Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments *in situ*. *Proc. Natl. Acad. Sci. USA* 1983;80:6126–6130. [PubMed: 6577472]

- Stroynowski I. Molecules related to class-I major histocompatibility complex antigens. *Annu. Rev. Immunol* 1990;8:501–530. [PubMed: 2188672]
- Thompson AJ, Polman CH, Miller DH, McDonald WI, Brochet B, Filippi M, Montalban X, De Sá J. Primary progressive multiple sclerosis. *Brain* 1997;120:1085–1096. [PubMed: 9217691]
- Tsunoda I, Kuang L-Q, Igenge IZM, Fujinami RS. Converting relapsing remitting to secondary progressive experimental allergic encephalomyelitis (EAE) by ultraviolet B irradiation. *J. Neuroimmunol* 2005;160:122–134. [PubMed: 15710465]
- Tsunoda I, Kuang L-Q, Libbey JE, Fujinami RS. Axonal injury heralds virus-induced demyelination. *Am. J. Pathol* 2003;162:1259–1269. [PubMed: 12651618]
- Tsunoda I, Kuang L-Q, Theil DJ, Fujinami RS. Antibody association with a novel model for primary progressive multiple sclerosis: Induction of relapsing-remitting and progressive forms of EAE in *H2^S* mouse strains. *Brain Pathol* 2000;10:402–418. [PubMed: 10885659]
- Tsunoda I, Kuang L-Q, Tolley ND, Whitton JL, Fujinami RS. Enhancement of experimental allergic encephalomyelitis (EAE) by DNA immunization with myelin proteolipid protein (PLP) plasmid DNA. *J. Neuropathol. Exp. Neurol* 1998;57:758–767. [PubMed: 9720491]
- Tsunoda I, Tolley ND, Theil DJ, Whitton JL, Kobayashi H, Fujinami RS. Exacerbation of viral and autoimmune animal models for multiple sclerosis by bacterial DNA. *Brain Pathol* 1999;9:481–493. [PubMed: 10416988]
- Ulfig N, Nickel J, Bohl J. Monoclonal antibodies SMI 311 and SMI 312 as tools to investigate the maturation of nerve cells and axonal patterns in human fetal brain. *Cell Tissue Res* 1998;291:433–443. [PubMed: 9477300]
- Urban JL, Kumar V, Kono DH, Gomez C, Horvath SJ, Clayton J, Ando DG, Sercarz EE, Hood L. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell* 1988;54:577–592. [PubMed: 2456857]
- von Büdingen HC, Hauser SL, Fuhrmann A, Nabavi CB, Lee JI, Genain CP. Molecular characterization of antibody specificities against myelin/oligodendrocyte glycoprotein in autoimmune demyelination. *Proc. Natl. Acad. Sci. U. S. A* 2002;99:8207–8212. [PubMed: 12060766]
- von Büdingen HC, Hauser SL, Ouallet JC, Tanuma N, Menge T, Genain CP. Frontline: Epitope recognition on the myelin/oligodendrocyte glycoprotein differentially influences disease phenotype and antibody effector functions in autoimmune demyelination. *Eur. J. Immunol* 2004;34:2072–2083. [PubMed: 15259004]
- Welsh RM, Haspel MV, Parker DC, Holmes KV. Natural cytotoxicity against mouse hepatitis virus-infected cells. II. A cytotoxic effector cell with a B lymphocyte phenotype. *J. Immunol* 1986;136:1454–1460. [PubMed: 3003199]
- Willenborg DO, Staykova MA. Cytokines in the pathogenesis and therapy of autoimmune encephalomyelitis and multiple sclerosis. *Adv. Exp. Med. Biol* 2003;520:96–119. [PubMed: 12613575]
- Zhao M-L, Xia J-Q, Fritz RB. Experimental allergic encephalomyelitis in susceptible and resistant strains of mice after adoptive transfer of T cells activated by antibodies to the T cell receptor complex. *J. Neuroimmunol* 1992;40:31–39. [PubMed: 1381381]

Fig. 1.
Depiction of the MHC locus and comparison of the genotypes of A.SW, SJL/J and B10.S strains of mice.

Fig. 2. Clinical course of MOG₉₂₋₁₀₆ induced EAE in B10.S mice. Mice were sensitized with MOG₉₂₋₁₀₆ emulsified in CFA, and observed for clinical signs. Mice that displayed clinical signs developed either monophasic disease (A), or a relapsing-remitting disease (B). Shown is the clinical course of a representative mouse of each group from four independent experiments.

Fig. 3.

Neuropathology scores in B10.S mice sensitized with MOG₉₂₋₁₀₆. B10.S mice were sensitized with MOG₉₂₋₁₀₆ emulsified in CFA. Only 30% of the mice developed clinical signs of disease. **A.** Mice that displayed clinical signs of disease had significantly higher brain pathology scores than asymptomatic mice. **B.** B10.S mice that displayed clinical signs also had significantly higher meningitis, demyelination and overall scores than asymptomatic mice. No difference in perivascular cuffing was observed between the groups. *, $P < 0.05$; ***, $P < 0.001$, t -test. Data shown are mean + standard error of the mean (SEM) from four independent experiments.

Fig. 4.

Demyelination, T cell infiltration, B cell infiltration and immunoglobulin deposition in consecutive sections from the brain of B10.S mice sensitized with MOG₉₂₋₁₀₆. **A.** Inflammatory demyelinating lesions (arrows) were detected in the brain by Luxol fast blue staining. **B, C.** T cell and B cell infiltrates were visualized by immunohistochemical staining using anti-mouse CD3 and B220, respectively. **D.** Immunoglobulin deposition was detected in the meninges near the demyelinated area by immunohistochemical staining using anti-mouse IgG (H+L). Magnification $\times 189$.

Fig. 5.

Correlation of brain pathology with clinical scores in B10.S mice sensitized with MOG₉₂₋₁₀₆. We scored brain pathology and compared it with the clinical score observed in mice sensitized with MOG₉₂₋₁₀₆. No strong correlations were observed between meningitis (A), perivascular cuffing (B), demyelination (C) and overall neuropathology (D) in the brains of B10.S mice sensitized with MOG₉₂₋₁₀₆ and clinical score. The data are from four independent experiments.

Fig. 6.

Demyelination, axonal damage, T cell infiltration, B cell infiltration, immunoglobulin deposition and complement deposition in consecutive sections from the spinal cord of B10.S mice sensitized with MOG₉₂₋₁₀₆. **A.** Inflammatory demyelinating lesions (arrows) were detected in the spinal cord by Luxol fast blue staining. **B.** Axonal damage (arrow) was detected by immunohistochemistry against nonphosphorylated neurofilament protein using the SMI 311 antibody cocktail. **C, D.** CD3⁺ T cell and B220⁺ B cell infiltrates were visualized using immunohistochemical staining. **E, F.** Immunoglobulin and complement deposition (arrows) were detected in the meninges or near the demyelinated area by immunohistochemical staining using anti-mouse IgG (H+L) and anti-mouse C1q, respectively. Magnification $\times 128$.

Fig. 7.

Correlation of spinal cord pathology with clinical scores in B10.S mice sensitized with MOG₉₂₋₁₀₆. No strong correlations were observed between meningitis (A), perivascular cuffing (B), demyelination (C), overall neuropathology (D) and axonal damage (F) in the spinal cords of B10.S mice sensitized with MOG₉₂₋₁₀₆ and clinical score. E. Significantly more axonal damage was observed in mice that developed clinical signs of disease compared to those that did not (*, $P < 0.05$, t -test). The data, shown as mean + SEM, are from four independent experiments.

Fig. 8.

T and B cell infiltration and correlation with clinical score in B10.S mice sensitized with MOG₉₂₋₁₀₆. T cells and B cells were detected by immunohistochemistry using antibodies against CD3 and B220, respectively. **A.** Symptomatic mice had significantly more B220⁺ cells in the meninges in their brains compared to asymptomatic mice (**, $P < 0.01$, t -test). **B.** In contrast, asymptomatic B10.S mice had a significantly higher frequency of T cells in the meningeal infiltrates in the brain compared to mice that developed clinical signs of disease (*, $P < 0.05$, t -test). Data shown are mean + SEM. **C.** Interestingly, the ratio of total B220⁺ B cells to CD3⁺ T cells in the meninges in the CNS demonstrated a significantly strong correlation with clinical score ($r = 0.90$, $P < 0.0001$).

Fig. 9. FoxP3⁺ regulatory T cells in the spinal cords of mice with MOG₉₂₋₁₀₆ induced EAE. Regulatory T cells were visualized by immunohistochemistry using antibody against FoxP3. FoxP3⁺ regulatory T cells (arrows) were detected in the spinal cords of B10.S (**A**) and A.SW (**C**) mice with MOG₉₂₋₁₀₆ induced EAE, but were absent in SJL/J mice with EAE (**B**). Magnification $\times 270$.

Fig. 10.

ELISA analyses revealed a robust humoral response against MOG₉₂₋₁₀₆ and recombinant mouse MOG (rmMOG) in the serum of B10.S mice sensitized with MOG₉₂₋₁₀₆. Plates were coated with MOG₉₂₋₁₀₆ (**A**) or rmMOG (**B**) and serum antibody titer determined using peroxidase-conjugated anti-mouse IgM, IgG(γ), IgG1, IgG2b and IgG2c antibodies. Symptomatic mice had significantly more IgM and IgG1 antibodies reactive with rmMOG in their serum compared to asymptomatic mice (*, $P < 0.05$, t -test). Data shown are mean + SEM.

Fig. 11.

Axonal damage in the CNS of B10.S (**A, D, G**), SJL/J (**B, E, H**), and A.SW mice (**C, F, I**) with MOG₉₂₋₁₀₆ induced EAE. Axonal damage was detected by immunohistochemistry against nonphosphorylated neurofilament protein using the antibody cocktail, SMI 311. (**A, B, C**) In the brain, more axonal damage was detected in the A.SW mice (**C**), compared with B10.S (**A**) and SJL/J mice (**B**). Many spindle shaped structures, torpedoes (arrows), were seen in the granular layer (g) of the cerebellum of A.SW mice (**C**), while no or few torpedoes were seen in B10.S (**A**) or SJL/J mice (**B**), respectively. The torpedo is a focal swelling of the axon of Purkinje cells (open arrow). (**D-F**) In transverse sections of spinal cords, more axonal swelling was detected in SJL/J mice (**E**), compared with B10.S (**D**) and A.SW mice (**F**). (**G-I**) Beading of axons was seen in longitudinal sections of spinal cords in all three mouse strains. Magnification: A-F, $\times 128$, G-I, $\times 192$.

Table 1

Historical overview of EAE induction in B10.S mice

Antigen	Source	Injection site	Clinical signs			Histological signs			Onset (days)	Observation period	Reference
			A.SW	B10.S	SJL/J	A.SW	B10.S	SJL/J			
SCH	mouse	lymph nodes	6/6	0/4	—	6/6	0/4	—	—	20 days	(Levine and Sowinski, 1974)
	mouse	foot pad	0/6	0/4	—	0/6	0/4	—	—	20 days	(Levine and Sowinski, 1974)
	mouse	foot pad	0/26	0/11	13/27	3/20	0/6	14/14	—	—	(Lando et al., 1997)
	C3H mouse	foot pad	5/5	2/2	5/5	3/4	2/2	4/4	15.8, 15.0, 11.8	—	(Raime et al., 1980)
	mouse	foot pad	80%	29/89	112/235	80%	31/89	190/235	—	28 days	(Montgomery and Rauch, 1982)
	mouse	foot pad	0/20	0/9	7/10	0/20	2/9	—	—	—	(Linthicum and Frelinger, 1982)
	SJL	s.c. in flank	—	4/25	17/19	—	13/19	14/14	>40, 19.2	40 days	(Korngold et al., 1986)
	B10.S	s.c. in flank	—	0/17	9/11	—	0/9	6/7	19.6	40 days	(Korngold et al., 1986)
MBP	guinea pig	s.c. in flank	—	0/20	20/20	—	—	—	7.6	40 days	(Segal and Shevach, 1996)
MBMP + IL-12	porcine	foot pad	—	1/15	6/15	—	1/15	6/15	18, 15	18 days	(Binder et al., 1991)
	guinea pig	s.c. in flank	—	20/20	—	—	—	—	8.2	40 days	(Segal and Shevach, 1996)
PLP ₁₃₉₋₁₅₁	peptide	s.c. in flank	—	2/53	73/74	—	—	—	—	—	(Encinas et al., 1996)
MOG ₉₂₋₁₀₆	peptide	foot pad	—	0/20	12/16	—	—	—	14.4	30 days	(Maron et al., 1999)
	peptide	s.c. at base of tail	14/14	—	15/17	14/14	—	15/17	45.1, 26.4	160 days	(Tsunoda et al., 2000)
	peptide	s.c. at base of tail	—	18/61	—	—	57/61	—	83.9	≥ 100 days	This study

SCH - spinal cord homogenate, MBP - myelin basic protein, IL-12 - interleukin-12, PLP - myelin proteolipid protein, MOG - myelin oligodendrocyte glycoprotein, s.c. - subcutaneous, EAE - experimental autoimmune encephalomyelitis.

Table 2
CNS neuropathology in B10.S mice sensitized with MOG₉₂₋₁₀₆

	Meningitis	Cuffing	Demyelination	Any lesions
Overall				
Brain	70%	30%	50%	77%
Spinal cord	73%	9%	45%	75%
Total CNS	88%	36%	62%	93%
Symptomatic				
Brain	87%	33%	73%	93%
Spinal cord	93%	13%	87%	93%
Total CNS	100%	33%	87%	100%
Asymptomatic				
Brain	63%	29%	41%	71%
Spinal Cord	68%	7%	27%	68%
Total CNS	83%	37%	54%	90%

CNS – central nervous system

Table 3

, histological and immunological comparison between B10.S, A.SW and SJL/J mice with MOG₉₂₋₁₀₆ induced EAE

Maximum Clinical Score ^a	Clinical Course	Disease Type	Demyelination	T cell Infiltration	FoxP ⁺ T cell Infiltration	B cell Infiltration	CNS Ig Deposition	Serum Anti-MOG Ig	Axonal Damage
2.2 ± 0.2	RR or monophasic	Paralytic+ ataxic	Plaque-like	±	±	+++	+++	++	+
4.7 ± 0.2	PP	Ataxic	Plaque-like	±	+++	±	+++	+++	++
3.8 ± 0.5	RR	Paralytic+ ataxic	Perivascular	++	-	+	+	+	++

lapsing-remitting, PP – primary-progressive.

mean (SEM)

Table 4
Comparison of neuropathology of MOG₉₂₋₁₀₆ induced EAE between *H-2^s* strains

Strain	Brain Pathology ^a	Spinal Cord Pathology ^a		
		Meningitis	Cuffing	Demyelination
B10.S	3.4 ± 0.5	31.8 ± 4.7	0.2 ± 0.2	28.9 ± 6.7
A.SW	5.7 ± 0.8	34 ± 9.6	8 ± 2.0	50 ± 11.2
SJL/J	1.8 ± 0.2	34 ± 13.9	10 ± 7.5	31 ± 15.5
				Overall
				43 ± 6.5
				54 ± 13.8
				43 ± 14.9

^aMean ± SEM