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DIFFERENTIAL LEVELS OF RESISTANCE TO DISEASE INDUCTION AND DEVELOPMENT OF RELAPSING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN TWO H-2^b-RESTRICTED MOUSE STRAINS

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Abstract

Besides the major histocompatibility complex (MHC) genes, background genes are believed to influence the encephalitogenicity of SJL(H-2^s) and B10.S (H-2^s) mice responding to myelin basic protein (MBP). A new mouse strain was constructed to study the effects of the SJL genetic background in mice responding to H-2^b-restricted neuroantigens. Although the SJL.B (H-2^b) mouse remained resistant to MBP in active EAE induction, the disease severity was uniformly higher in MOG-induced active EAE and in MBP-induced adoptive EAE when compared to those of B6 (H-2^b) mice. Treatment of mice with anti-CD25 antibodies prior to immunization caused 60% of SJL.B mice to become susceptible to MBP-induced EAE while only 14% of B6 mice were converted. In addition, MOG-induced EAE in SJL.B mice followed a remitting-relapsing disease course while B6 mice only exhibited monophasic or chronic episodes. The new SJL.B mouse strain provides a valuable tool for studying EAE resistance and remitting-relapsing disease in H-2^b mice.

Keywords

Experimental autoimmune encephalomyelitis; EAE resistance and susceptibility; SJL genetic background; Adoptive transfer of disease; anti-CD25 treatment; remitting-relapsing disease

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1. INTRODUCTION

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) and has been used as an animal model for study of the human demyelinating disease, multiple sclerosis (MS). EAE is characterized by pathologic infiltration of mononuclear cells into the CNS and by clinical manifestation of paralytic symptoms (Raine, 1984). EAE can be induced in animals by immunization with myelin antigens or by adoptive transfer of myelin antigen-primed donor T cells into naive recipients (Traugott et al., 1985; Traugott et al., 1986). Three major myelin antigens are commonly used, namely, myelin basic protein (MBP) (Zamvil et al., 1986), proteolipid protein (PLP) (Trotter et al., 1987; Tuohy et al., 1989) and myelin oligodendrocyte glycoprotein (MOG) (Johns et al., 1995). For EAE induced by MBP and PLP, SJL is a highly susceptible mouse strain that is often used as a prototype of this disease model. After developing an acute phase of the disease, these mice spontaneously undergo remission. However, these mice are also prone to spontaneous disease relapses and the remitting/relapsing cycles become a recurring feature of the disease (Kim and Tse, 1993). In contrast to SJL mice, C57BL/6 mice (B6) exhibit no disease symptoms when immunized with MBP with the same exact experimental procedures (Levine and Sowinski, 1974; Bernard, 1976; Shaw et al., 1992). These mice are loosely referred to as EAE resistant, although the basis of this unresponsiveness/resistance is not fully understood. The genes that control EAE susceptibility have been mapped to the H-2 complex (Fritz et al., 1985; Butterfield et al., 1998), but non-H-2 background genes are also implicated (Montgomery and Rauch, 1982; Linthicum and Frelinger, 1982; Butterfield et al., 1998). B10.S mice, for example, share with SJL mice the same H-2^s haplotype but are resistant to EAE induction with MBP or PLP (Korngold et al., 1986; Shaw et al., 1992; Encinas et al., 1996; Butterfield et al., 1998). The SJL genetic background, as opposed to that of the C57BL/10 (B10), apparently has influenced the level of MBP-specific encephalitogenicity in the H-2^s mice. How would the SJL genetic background modify the MBP-induced disease development in EAE resistant H-2^b mice is the subject of this report. Previously, Shaw et al. (1992) showed that EAE resistance was not simply a lack of phenotypic responsiveness, but was an actively regulated process. These investigators found that while adoptive transfer of MBP-sensitized B6 donor lymph node cells into syngeneic naive recipients failed to generate disease, subsequent challenge of the recipients with MBP emulsified in CFA (MBP-CFA) induced severe symptoms of EAE very similar to those induced in susceptible strains with adoptive transfer alone. These experiments clearly establish the important concept that MBP-specific encephalitogenic T cells exist in resistant H-2^b mice and that the lack of detectable EAE responses is mediated by other mechanisms perhaps related to the non-H-2 genetic backgrounds. Recently, Reddy et al. (2004) showed that treatment of PLP139-151-resistant B10.S mice with anti-CD25 prior to immunization rendered these mice susceptible to EAE induction, supporting the notion that regulatory T cells (Tregs) are involved in maintaining EAE resistance. In this report, a new mouse strain, SJL.B, was created by genetic backcrossing (Klein, 1975), replacing the H-2^s haplotype of SJL mice with the H-2^b haplotypes from B6 mice. It was found that the resulting SJL.B mice still retained the resistance phenotype of the H-2^b mice to MBP-specific EAE. However, the SJL genetic background of these mice generally conferred higher immunogenic and encephalitogenic responses to H-2^b-specific antigens as compared to B6 mice. Consequently, treating SJL.B mice with anti-CD25 antibodies prior to immunization rendered these mice susceptible to EAE induction with MBP, though B6 mice remained unresponsive. Furthermore, SJL.B mice (H-2^b) were found to follow a MOG-induced remitting-relapsing disease course as opposed to the monophasic or chronic form of EAE typical of B6 mice (H-2^b). These results indicate that there are differential levels of EAE resistance among EAE resistant mouse strains, supporting the argument that EAE susceptibility and resistance are not just discreet all-or-none events, but are graded levels of EAE responsiveness on a continuous spectrum.

2. Materials and Methods

2.1. Animals

Female SJL/J and C57BL/6 mice were purchased from the Frederick NCI (Frederick, MD) and the Jackson Laboratory (Bar harbor, ME). SJL.B mice, originally derived in this laboratory, were under contract for breeding at the Jackson Laboratory. Female donor mice were used between 10 to 12 weeks of age and recipient mice were of 6 weeks of age. All experimental procedures involving live animals had received prior approval from IACUC.

2.2 Antigen Peptides

Frozen guinea pig spinal cords were purchased from Rockland Inc. (Gilbertsville, PA). Myelin basic protein (MBP) was prepared according to the procedures described by Swanborg (1988) and was stored in lyophilized form until use. Myelin antigen peptides were synthesized by Genemed Synthesis (San Antonio, TX). The sequences for the peptides are: MBP₆₀₋₈₀: SHHAARTTHYGSLPQKSQR; MOG₃₅₋₅₅: MEVGWYRSPFSRVVHLYRNGK; PLP₁₇₈₋₁₉₁: NTWTTCQSIAPPSK;

2.3. Active induction of EAE

Myelin peptides were dissolved in PBS in 2mg/ml concentration. Equal volume of complete Freund adjuvant (CFA) (Difco Laboratories, Detroit, MI) was added and emulsified by passing repeatedly through a three-way stopcock between two glass syringes. Fifty μ l of the emulsion was injected at each of four sites in the flanks draining the inguinal and axillary lymph nodes. At the same time and 48 hours later, 200 ng of pertussis toxin (PT) (Hou et al., 2003) was given i.p. to the animal.

2.4. Adoptive transfer of EAE

Donor mice were immunized with myelin antigens in the same manner as described in active induction without the administration of PT. Draining lymph nodes were removed from mice ten days after immunization and teased into single cell suspensions. Cells were washed with PBS twice and put into culture at 4×10^6 /ml with 100 μ g/ml of the corresponding myelin peptides in RPMI 1640 supplemented with 10% fetal calf serum, 2mM L-glutamine, 1mM MEM sodium pyruvate, 0.1mM MEM non-essential amino acids, 100U/ml penicillin, 100 μ g/ml streptomycin, 10mM HEPES buffer and 5×10^{-5} M 2-ME culture medium. Cells were cultured in 24-well plates in 2ml volume in each well for four days at 37 °C in 5% CO₂/air incubator. Cell were harvested and washed with PBS twice, then re-suspended in PBS and adjusted to a cell concentration of 2.5×10^8 /ml. 0.2 ml of the cell suspension containing 5×10^7 cells was injected into each naïve recipient through the tail vein. In EAE resistant mice, recipients were challenged, sixteen to twenty days after cell transfer, with the priming myelin peptide in four sites in the flanks similar to the immunization procedures.

2.5. Clinical assessment of EAE

In active and passive EAE, mice were monitored for clinical signs of disease and were scored in a 0–6 scale according to the following standard: 0, no symptoms; 1, loss of tail tone; 2, weakness of one hind limb; 3, total paralysis of both hind limbs; 4, total paralysis of both fore limbs; 5, premoribund state; 6, death.

2.6. Establishment of the SJL.B mouse strain

The backcross or NX breeding system (Klein, 1975) was adopted for this purpose. This consisted of a series of backcrosses and selection of progeny expressing the H-2^b antigens. Initially, F₁s of a cross between SJL.Thy1^a (H-2^s and Thy1^a genotype) and C57BL/6 (H-2^b

and Thy1^b genotype) were backcrossed to SJL.Thy-1^a and mice in the N1 generation were screened for H-2^b expression. H-2^{s/b} heterozygous mice were selected and backcrossed again to SJL.Thy-1^a. This process was repeated for 12 generations. To fix the genotype thus derived, H-2^{s/b} heterozygous mice were intercrossed. H-2^b homozygous mice were selected and propagated by inbreeding for at least 5 generations before use. This particular line is also homozygous for Thy-1^a.

2.7. ELISPOT assay for IFN γ and IL-17 and IL-2

ELISPOT assays were conducted using ELISPOT kits from BD Biosciences (San Jose, CA). The procedures were carried out according to the manufacturer's instructions. Cells were assayed in duplicate wells on ELISPOT plates with titrating number of cells at 1×10^6 /well, 5×10^5 /well and 2.5×10^5 /well. Cells were cultured overnight with the corresponding myelin peptides. Spots were counted under a dissecting microscope and with a CTL-ImmunoSpot Analyzer (Cellular Technology, Shaker Heights, OH).

3. Results

3.1. SJL.B mice expressed the H-2^b phenotype

SJL.B mice were originally derived by crossing SJL-Thy1^a female mice to C57BL/6 male mice. For each of the 12 N generations backcrossing to SJL-Thy1^a, the mice were selected for expression of H-2^b cell surface antigens by flow cytometry. The mice used in these experiments were inbred for at least five generations. The H-2^b haplotype of the mice was verified by staining spleen cells with anti-MHC class I and Class II antibodies. As shown in figure 1, these mice distinctly expressed H-K^b, H-2D^b and H-2IA^b, but were stained negative with anti-H-2K^s, anti-H-2D^s or anti-H-2IA^s antibodies.

3.2 SJL.B mice exhibited more severe MBP-specific adoptive EAE than C57BL/6 mice

The C57BL/6 mouse has the classic characteristic of being unresponsive to EAE induction with MBP (Bernard, 1976). To test if the new SJL.B strain is susceptible to active EAE induced with MBP, B6 and SJL.B mice were immunized with whole MBP or with MBP₆₀₋₈₀ peptides (Shaw et al., 1992; Shaw et al., 1996) and given pertussis toxin (PT) on days 0 and 2. Disease development was followed for the next 30 days. No EAE was observed in either strain (data not shown). However, it was previously reported that adoptive transfer of MBP-primed B6 donor lymph node cells into naïve syngeneic recipients followed by antigenic challenge 16 days later led to development of severe adoptive EAE similar to that induced in SJL mice (Shaw et al., 1992; Shaw et al., 1996). Accordingly, B6 and SJL.B mice were immunized with MBP₆₀₋₈₀ peptides. Ten days later, draining lymph nodes were isolated and cells were cultured with MBP₆₀₋₈₀ peptides for 5 days. At the end of the culture period, cells were harvested and 5×10^7 viable cells were transferred into naïve syngeneic recipients. As shown in Table I, adoptive transfer alone did not induce EAE in B6 or SJL.B mice. None of the groups of mice showed any symptoms of EAE. Twenty days after adoptive transfer, both B6 and SJL.B mice were challenged with MBP₆₀₋₈₀. In this case, B6 mice exhibited clinical signs of adoptive EAE after challenge as previously reported. SJL.B mice, when compared to the B6 groups, also developed adoptive EAE, but exhibited a slightly earlier disease onset and a significantly more severe disease course. Thus, the SJL genetic background influenced H-2^b mice in the extent of MBP-specific adoptive disease development. This enhancement, nevertheless, is not sufficient to convert the resistant phenotype of the SJL.B mice.

3.3 SJL.B mice exhibited more severe MOG-specific active EAE than C57BL/6 mice

While B6 mice show a resistant phenotype to MBP-induced active EAE, they are nevertheless susceptible to EAE induction with MOG (Johns et al., 1995). To test the

susceptibility of SJL.B mice to other H-2^b-restricted myelin epitopes, B6 and SJL.B mice were immunized with MOG₃₅₋₅₅ and PLP₁₇₈₋₁₉₁ peptides (Tompkins et al., 2002). Pertussis toxin was given at 0 and 48 hours after immunization. Development of EAE was assessed daily. As shown in Table II, both B6 and SJL.B mice developed active EAE to the MOG₃₅₋₅₅ peptide. The B6 response was moderately severe with average disease grade of 2.4. SJL.B mice, on the other hand, again exhibited a slightly earlier disease onset and a significantly more severe disease course than B6 mice. This experiment was repeated many times and the SJL.B responses were very consistent with less variability in disease severity than B6 mice. Since PLP₁₇₈₋₁₉₁ was not a dominant epitope for H-2^b mice, the disease was less severe and the responses were more variable in both cases. It should be pointed out that SJL mice also respond to active EAE induction with PLP₁₇₈₋₁₉₁. The SJL responses are generally higher than the SJL.B responses because of the H-2^s haplotype of the SJL mice. Taken together, the data support the contention that the SJL genetic background enhances EAE susceptibility of H-2^b mice..

3.4. SJL.B mice exhibited higher inflammatory cytokine responses than B6 mice

T cell functions in immune and autoimmune responses are often mediated through production of cytokines. In EAE, inflammatory IFN γ , TNF α and IL-17 are found to be the dominant cytokines produced by CD4⁺ T cells from lymph nodes, spleens or spinal cords of mice during acute disease (Begolka et al., 1998; Mustafa et al., 1991; Hofstetter et al., 2005). Accordingly, the frequencies of encephalitogenic T cells producing IFN γ and Th17 cytokines in B6 and SJL.B mice responding to MBP₆₀₋₈₀ or MOG₃₅₋₅₅ were assessed using the ELISPOT assays. In the present study, groups of mice were immunized with the individual peptides and on days 3, 6 and 9 after immunization, isolated lymph node cells were cultured overnight with the respective antigens in IFN γ and IL-17 ELISPOT plates. Results are shown in Table III. Cytokine responses to MBP₆₀₋₈₀ in B6 and SJL.B mice are relatively low. This is not unexpected as both strains showed no detectable active EAE to this antigen. Nevertheless, SJL.B mice did show a graduate increase over time in the number of IFN γ spots. By day 9, SJL.B showed significantly higher levels of IFN γ frequencies than B6 mice ($p = 0.0088$). For responses to MOG₃₅₋₅₅, both strains showed big increases in IFN γ and IL-17 spots by days 6 and 9. The frequencies of T cells producing IFN γ and IL-17 in SJL.B mice were significantly higher than those in B6 mice by day 6 and day 9, respectively. These results reaffirm the previous observation of EAE enhancement related to the SJL genetic background.

3.5 SJL.B mice were susceptible to active EAE induction with MBP₆₀₋₈₀ if pre-treated with anti-CD25 antibodies

As stated earlier, B6 and SJL.B mice are non-responsive to active EAE induction with the peptide MBP₆₀₋₈₀. To understand the mechanisms of EAE resistance, Reddy et al. (Reddy et al., 2004) pretreated PLP₁₃₉₋₁₅₁-resistant B10.S mice with anti-CD25 antibodies before the mice were immunized with PLP₁₃₉₋₁₅₁/CFA emulsion and showed that about 30% of the mice became susceptible to EAE induction. It was concluded that CD4⁺CD25⁺ regulatory T cells mediated EAE resistance. In these experiments, the effects of anti-CD25 treatment on CD8⁺CD25⁺ regulatory T cells had not been elucidated. To test if the MBP-specific EAE unresponsiveness of B6 and SJL.B mice were due to regulatory T cells, these mice were pre-treated with anti-CD25 antibodies prior to immunization with MBP₆₀₋₈₀ emulsified with CFA. The development of active EAE was monitored. The results are shown in Table IVa. It was found that anti-CD25 treatment caused changes in the susceptibility of SJL.B mice, but not B6 mice, to active EAE induction with MBP₆₀₋₈₀. Active EAE was induced in 60% of the SJL.B mice while only two out of 14 B6 mice (14%) became responsive to EAE induction. These data are in agreement with the overall expectation that SJL.B mice are relatively more susceptible to EAE induction than B6 mice although both strains are of the

H-2^b haplotype. Interestingly, the data also raise the question why anti-CD25 treatment failed to cause changes in the susceptibility phenotype of B6 mice. To show that even EAE susceptible mouse strains harbor Treg cells, SJL mice were pretreated with anti-CD25 antibodies prior to immunization with PLP₁₃₉₋₁₅₁. It was found (Table IVb) that untreated SJL mice developed severe active EAE as expected (average disease severity = 2.8). However, anti-CD25-treated mice developed even more severe disease that many of the mice died as a result of EAE (average disease severity = 5.2). Thus, Treg cells regulate both resistant and susceptible mice in EAE development.

3.6. SJL.B mice exhibited a remitting-relapsing course of EAE

In MS, Lublin and Reingold (1996) reported that greater than 80% of MS cases exhibited the remitting-relapsing form of the disease. However, the basis of the spontaneous remission and relapsing cycles is not well understood. It is interesting to note that the major forms of MS, namely, relapsing-remitting (RR) MS, primary-progressive (PP) MS, secondary-progressive (SP) MS and progressive-relapsing (PR) MS, can be reproduced in murine EAE. SJL mice responding to PLP₁₃₉₋₁₅₁ typically exhibit the remitting-relapsing pattern (Kim and Tse, 1993). B6 mice, on the other hand, exhibit either the monophasic pattern as seen in passive EAE induced by MBP (Shaw et al., 2007) or chronic disease in active EAE induced by MOG (Buenafe and Bourdette, 2007; Stark and Cross, 2006). Whether the SJL background in SJL.B mice influences this aspect of the disease was investigated. Accordingly, SJL.B and B6 mice were immunized with MOG₃₅₋₅₅ and disease development was followed for the next 30 days. As shown in Figure 2, B6 mice exhibited a chronic form of MOG-EAE as described by others (Buenafe and Bourdette, 2007; Stark and Cross, 2006). Apparently, age is not a factor in influencing this pattern of chronic disease as 11-month old B6 included in the experiments developed similar disease as their younger counter-part. SJL.B mice, on the other hand, followed a remitting-relapsing disease course typical of SJL mice responding to EAE induction with PLP₁₃₉₋₁₅₁. The SJL genetic background thus influenced the EAE disease course of mice of the H-2^s and H-2^b haplotypes.

4. Discussion

Past efforts studying EAE tended to focus on the mechanism of disease susceptibility pertaining to antigen specificities, disease progression and related cytokines (Racke et al., 2000; Podojil et al., 2006; Jager et al., 2009). The basis of EAE resistance (Arnon, 1981), on the other hand, had received relatively little attention. This may be due to the fact that EAE resistant mice generally lack display of measurable phenotypic parameters. Nevertheless, it has long been shown that EAE resistance is a regulated process that is tightly controlled (Kim and Tse, 1993; Reddy et al., 2004; Korn et al., 2007) and much can be learned about the regulatory mechanisms. This paper expands the concept that EAE susceptibility and resistance are not all-or-none discrete entities, but are a continuous spectrum of reactivity. Traditionally, the H-2^b mouse with the B6/B10 genetic background is highly resistant to EAE induction with MBP (Levine and Sowinski, 1974; Shaw et al., 1992). By replacing the B6 genetic background of the C57BL/6 mouse (H-2^b) with that of the highly susceptible SJL mouse (H-2^s), this report shows that the SJL genetic background has influenced the immune and encephalitogenic responses of the SJL.B mouse (H-2^b). Two conclusions can be drawn. First, the H-2^b haplotypes is not an EAE permissive MHC. The SJL.B mouse remains in the EAE resistant category with respect to disease induction with MBP (Table I). It is apparent that the SJL non-H-2 genes have not brought the MBP-specific responses above the threshold required for disease induction. The mouse, nevertheless, is similar to the B6 mouse being susceptible to EAE induction with MOG (Table II). Second, the SJL.B mouse clearly mounts higher levels of responses to neuroantigens when compared to those of the B6 mouse (Tables I – III). Thus, different EAE resistant strains are regulated differentially

depending on their non-H-2 genetic backgrounds. These observations support the notion that EAE resistance is not an all-or-none event but is a relative term as exemplified by the responses of B6 and SJL.B to various neuroantigens. Similarly, susceptibility and resistance are relative terms and should be viewed as a continuous spectrum of responses above and below a certain threshold of disease induction. It is also interesting to note that the thresholds for disease induction is antigen-dependent, such that the B6 mouse can be susceptible to MOG-induced active EAE (Johns et al., 1995; and Table II) and at the same time resistant to MBP-induced active EAE (Shaw et al., 1992; and Table I).

Although the mechanism of EAE resistance is not known, studies indicated that regulatory T cells (Sakaguchi et al., 1995; Sakaguchi, 2004) played a role in this process. This was demonstrated by the pre-treatment of PLP-specific EAE resistant B10.S mice with anti-CD25 antibodies prior to immunization and the subsequent development of EAE in a portion of the treated mice (Reddy et al., 2004). This observation is confirmed in this report with MBP-specific resistant SJL.B mice, but not with B6 mice (Table IVa). The basis of this discrepancy between B6 and SJL.B mice is not known. It can be speculated that their differential levels of responses to MBP may account for the relative susceptibility of these two strains of mice to anti-CD25 pretreatment. Whether the levels of MBP responses can be correlated with the frequency of Tregs in both strains of mice is being investigated with MBP₆₀₋₈₀/IA^b tetramers. However, it remains possible that Treg cell might not be the only mechanism that controls EAE resistance.

Another feature of great interest in murine EAE is the phenomenon of EAE remission and relapse. SJL again is the prototype for the study of MBP- and PLP-induced EAE remitting-relapsing disease. B6 mice, on the other hand, are reported to exhibit either monophasic or chronic disease courses in response to MOG. The development of MOG-specific active EAE in SJL.B mice was investigated. Results show that SJL.B mice distinctly exhibit a remitting-relapsing disease course, in contrast to the monophasic or chronic disease developed in B6 mice. This is in full agreement with the finding of Skundric et al. (2003) who reported that the majority (93%) of the F1 mice of a SJL × C57BL/6 cross immunized with MOG35-55 developed remitting-relapsing disease as opposed to 76% of C57BL/6 mice developing monophasic EAE. The parental SJL genes apparently dominated the disease phenotype of the F1 mice. In SJL.B mice, the non-H-2 genetic background is totally of the SJL origin. The SJL.B mice thus provide a valuable tool, complementing the SJL mice, to further study the mechanism of remitting-relapsing EAE induced by a different myelin antigen other than PLP.

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References

- Arnon R. Experimental allergic encephalomyelitis – susceptibility and suppression. *Immunol Rev.* 1981; 55:5. [PubMed: 6165673]
- Begolka WS, Vanderlugt CL, Rahbe SM, Miller SD. Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. *J Immunol.* 1998; 161:4437–4446. [PubMed: 9780223]
- Bernard CCA. Experimental autoimmune encephalomyelitis in mice: genetic control of susceptibility. *J Immunogenet.* 1976; 3:263. [PubMed: 1109134]
- Buenafe AC, Bourdette DN. Lipopolysaccharide pretreatment modulates the disease course in experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2007; 182:32–40. [PubMed: 17055066]

- Butterfield RJ, Sudweeks JD, Blankenhorn EP, Korngold R, Marini JC, Todd JA, Roper RJ, Teuscher C. New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. *J Immunol.* 1998; 161:1860–1867. [PubMed: 9712054]
- 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]
- Fritz RB, Skeen MJ, Chou CHJ, Garcia M, Egorov IK. Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. *J Immunol.* 1985; 134:2328. [PubMed: 2579140]
- Hofstetter HH, Karulin AY, Forsthuber TG, Ott PA, Tary-Lehmann M, Lehmann PV. The cytokine signature of MOG-specific CD4 cells in the EAE of C57BL/6 mice. *J Neuroimmunol.* 2005; 170:105–114. [PubMed: 16257061]
- Hou W, Wu Y, Sun S, Shi M, Sun Y, Yang C, Pei G, Gu Y, Zhong C, Sun B. Pertussis toxin enhances Th1 responses by stimulation of dendritic cells. *J Immunol.* 2003; 170:1728–1733. [PubMed: 12574336]
- Jager A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J Immunol.* 2009; 183:7169–7177. [PubMed: 19890056]
- Johns TG, Kerlero de Rosbo N, Menon KK, Abo S, Gonzales MF, Bernard CC. Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. *J Immunol.* 1995; 154:5536–5541. [PubMed: 7537310]
- Kim C, Tse HY. Adoptive transfer of murine experimental autoimmune encephalomyelitis in SJL Thy-1 congenic mouse strains. *J Neuroimmunol.* 1993; 46:129–136. [PubMed: 7689583]
- Klein, J. *Biology of the Mouse Histocompatibility-2 Complex.* Springer-Verlag; New York: 1975. p. 33
- Korn T, Redy J, Gao W, Bettelli E, Awasthi A, Petersen TR, Backstrom BT, Sobel RA, Wucherpfennig KW, Fromm TB, Oukka M, Kuchroo VK. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. *Nat Med.* 2007; 13:423–431. [PubMed: 17384649]
- 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]
- Levine S, Sowinski R. Experimental allergic encephalomyelitis in congenic strains of mice. *Immunogenetics.* 1974; 1:352–356.
- 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 R, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology.* 1996; 46:907–911. [PubMed: 8780061]
- 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]
- Mustafa MI, Diener P, Hojeberg B, Van der Meide P, Olsson T. T cell immunity and interferon-gamma secretion during experimental allergic encephalomyelitis in Lewis rats. *J Neuroimmunol.* 1991; 31:165–77. [PubMed: 1704016]
- Podajil JR, Kohm AP, Miller SD. CD4+ T cell expressed Cd80 regulates central nervous system effector function and survival during experimental autoimmune encephalomyelitis. *J Immunol.* 2006; 177:2948–2958. [PubMed: 16920930]
- Raine CS. *Biology of disease. Analysis of autoimmune demyelination: its impact upon multiple sclerosis.* Lab Invest. 1984; 50:608. [PubMed: 6202955]
- Racke MK, Ratts RB, Arredondo L, Perrin PL, Lovett-Rack A. The role of costimulation in autoimmune demyelination. *J Neuroimmunol.* 2000; 107:205–215. [PubMed: 10854658]

- Reddy J, Illes Z, Zhang X, Encinas J, Pyrdol J, Nicholson L, Sobel R, Wucherpfennig KW, Kuchroo VK. Myelin proteolipid protein-specific CD4+CD25+ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. *Proc Nat Acad Sci*. 2004; 101:15434–15439. [PubMed: 15492218]
- Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance cause various autoimmune diseases. *J Immunol*. 1995; 155:1151. [PubMed: 7636184]
- Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Ann Rev Immunol*. 2004; 22:531. [PubMed: 15032588]
- Shaw MK, Kim C, Ho K, Lisak RP, Tse HY. A combination of adoptive transfer and antigenic challenge induces consistent murine experimental autoimmune encephalomyelitis in C57BL/6 mice and other reputed resistant strains. *J Neuroimmunol*. 1992; 39:139–150. [PubMed: 1619036]
- Shaw MK, Kim C, Hao HW, Chen F, Tse HY. Induction of myelin basic protein-specific experimental autoimmune encephalomyelitis in C57BL/6 mice: Mapping of T cell epitopes and T cell receptor Vb gene segment usage. *J Neurosci Res*. 1996; 45:690–699. [PubMed: 8892080]
- Shaw, MK.; Li, J.; Ho, PP.; Hao, H.; Lisak, RP.; Tse, HY. Induction of myelin basic protein-specific adoptive experimental autoimmune encephalomyelitis in C57BL/6 mice: Longevity of donor T cells and lack of disease relapse. In: Broglio, PV., editor. *Neuroimmunology research focus*. Nova Science Publishers, Inc., publisher; Hauppauge, N.Y: 2007. p. 175-191.
- Skundric DS, Zakarian V, Dai R, Lisak RP, Tse HY, James J. Distinct immune regulation of the response to H-2^b-restricted epitope of MOG causes relapsing-remitting EAE in H-2^{b/s} mice. *J Neuroimmunol*. 2003; 136:34–45. [PubMed: 12620641]
- Stark JL, Cross A. Differential expression of suppressors of cytokine signaling-1 and -3 and related cytokines in central nervous system during remitting versus non-remitting forms of experimental autoimmune encephalomyelitis. *Internatl Immunol*. 2006; 18:347–353.
- Swanborg, RH. Experimental allergic encephalomyelitis. In: Sabato, GD., editor. *Methods in Enzymology*. Vol. 162. Academic Press; New York: 1988. p. 413-421.
- Tompkins SM, Padilla J, Dal Canto MC, Ting JP, Van Kaer L, Miller SD. Do novo central nervous system processing of myelin antigen is required for the initiation of experimental autoimmune encephalomyelitis. *J Immunol*. 2002; 168:4173–4183. [PubMed: 11937578]
- Traugott U, Raine CS, McFarlin DE. Acute experimental allergic encephalomyelitis in the mouse: immunopathology of the developing lesion. *Cell Immunol*. 1985; 91:240. [PubMed: 2578895]
- Traugott U, McFarlin DE, Raine CS. Immunopathology of the lesion in chronic relapsing autoimmune encephalomyelitis in the mouse. *Cell Immunol*. 1986; 99:395. [PubMed: 3093105]
- Trotter JL, Clark BH, Collins KG, Wegeschiede CL, Scarpelli JD. Myelin proteolipid and the development of demyelinating disease in mice. *J Neurol Sci*. 1987; 79:173. [PubMed: 2440996]
- Tuohy VK, Lu Z, Sobel RA, Lausen RA, Lees MB. Identification of an Encephalitogenic determinant of myelin proteolipid protein for SJL mice. *J Immunol*. 1989; 142:1523. [PubMed: 2465343]
- Zamvil SS, Mitchell DJ, Powell MB, Sakai K, Rothbard JB, Steinman L. T cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature*. 1986; 324:258. [PubMed: 2431317]

Figure 1. SJL.B mice express cell surface H-2^b antigens

Spleen cells from SJL.B mice were prepared and single cell suspensions were stained with specific antibodies against class I and class II H-2^s and H-2^b antigens as indicated.

Figure 2. EAE disease course of B6, B6(old) and SJL.B mice

B6 mice of two different ages (10 weeks and 11 months old) and SJL.B mice were immunized with MOG₃₅₋₅₅. Development of EAE was monitored for 40 to 50 days. Disease severity grades were plotted against days post immunization. Data on disease course were for individual mice. Average disease severity and average day of onset for the group are shown in the insert.

Table I

Induction of MBP₆₀₋₈₀-specific adoptive EAE in C57BL/6 and SJL.B mice.

Strains	Clinical Signs of Adoptive EAE			Clinical EAE after antigenic challenge		
	Incidence	Av. Disease Severity	Av. Day of Disease Onset	Incidence	Av. Disease Severity	Av. Day of Disease Onset
B6	0/16	-	-	16/16	2.9 ± 0.17*	10.9 ± 0.28
SJL.B	0/14	-	-	14/14	4.4 ± 0.32*	8.3 ± 0.44

SJL mice were immunized with MBP₆₀₋₈₀ peptides emulsified in CFA in four sites in the flanks. Ten days later, draining lymph nodes were removed and the cells were restimulated in vitro with the priming antigen for four days. Cells were harvested and 5×10^7 viable cells were transferred into naïve syngeneic recipients through the tail vein. No EAE was observed in both groups. Twenty days after cell transfer, recipient mice were challenged with MBP₆₀₋₈₀ peptides emulsified in CFA in four sites in the flanks. Symptoms of EAE developed in both groups. Disease was further monitored for 30 days. Data were combined from two experiments. t-tests are two-tailed tests.

* mean ± SEM p = 0.02

Induction of MOG₃₅₋₅₅- and PLP₁₇₈₋₁₉₁-specific active EAE in C57BL/6 and SJL.B mice.

Table II

Peptides	Strains	Clinical signs of active EAE		
		Incidence	Av. Disease Severity	Av. Day of Dis. Onset
MOG ₃₅₋₅₅	B6	12/14	2.4 ± 0.32*	15.1 ± 0.29
	SJL.B	13/13	3.6 ± 0.14*	10.5 ± 0.27
PLP ₁₇₈₋₁₉₁	B6	9/12	1.6 ± 0.36	20.2 ± 1.55
	SJL.B	11/14	2.0 ± 0.31	13.8 ± 0.41

Mice were immunized s.c. with 200 ug of the indicated peptide emulsified in CFA in four sites in the flanks. 200 ng of pertussis toxin was injected i.v. on days 0 and 2 after immunization. The development of EAE was monitored for 30 days. Data were combined from two experiments.

* mean ± SEM p = 0.0027 t-tests are two-tailed tests.

Frequencies of IFN γ - and IL-17-producing T cells in the lymph nodes of B6 and SJL.B mice immunized with MBP₆₀₋₈₀ or MOG₃₅₋₅₅.

Table III

		Spot Forming Cells (per million cells)					
		IFN γ			IL-17		
Ag/Peptides	Strains	Day 3	Day 6	Day 9	Day 3	Day 6	Day 9
MBP ₆₀₋₈₀	B6	1 $\pm$ 1.2	12 $\pm$ 9.2	9 $\pm$ 2.9 [^]	1 $\pm$ 0.7	2 $\pm$ 0.3	1 $\pm$ 0.7
	SJL.B	4 $\pm$ 2.9	76 $\pm$ 10.4	119 $\pm$ 30.6 [^]	1 $\pm$ 0.7	8 $\pm$ 0.9	11 $\pm$ 1.8
MOG ₃₅₋₅₅	B6	23 $\pm$ 2.3	158 $\pm$ 68.7 [*]	256 $\pm$ 84.3	5 $\pm$ 1.6	133 $\pm$ 13.5	159 $\pm$ 10.2 [#]
	SJL.B	58 $\pm$ 22.5	210 $\pm$ 6.4 [*]	350 $\pm$ 60.0	59 $\pm$ 3.3	333 $\pm$ 6.9	394 $\pm$ 23.2 [#]

Mice were immunized with MBP₆₀₋₈₀ or MOG₃₅₋₅₅ peptides emulsified in CFA. Three, six or nine days after immunization, draining lymph node cells were cultured overnight with the corresponding peptide in IFN γ or IL-17 ELISPOT plates. Plates were developed following the manufacturer's instructions. Spots were counted with a dissecting microscope with appropriate dilutions. t-tests are two-tailed tests.

[^] mean $\pm$ SEM p = 0.0088.

^{*} mean $\pm$ SEM p = 0.0085.

[#] mean $\pm$ SEM p = 0.0473.

Table IV

(a) Effects of anti-CD25 treatment on development of MBP ₆₀₋₈₀ -induced active EAE in B6 and SJL.B mice.					
Strains	Treatment	d. of Treatment	Clinical signs of active EAE		
			Incidence	Av. Dis. Severity	Av. d. of Dis. Onset
B6	Isotype-match Ig	-8, -3	0/12	--	--
	Anti-CD25 (PC61)	-8, -3	2/14* (14.3%)	0.14 ± 0.10	20.5 ± 3.5
SJL.B	Isotype-matched Ig	-8, -3	0/12	--	--
	Anti-CD25 (PC61)	-8, -3	9/15* (60.0%)	1.67 ± 0.33	14.33 ± 0.91

(b) Effects of anti-CD25 treatment on development of PLP _{139,151} -induced active EAE in SJL mice.					
Strains	Treatment	d. of Treatment	Clinical signs of active EAE		
			Incidence	Av. Dis. Severity	Av. d. of Dis. Onset
SJL	Isotype-matched Ig	-8, -3	10/10	2.8 ± 0.59	11.2 ± 0.20
	Anti-CD25 (PC61)	-8, -3	10/10	5.2 ± 0.42	10.6 ± 0.22

Mice were injected i.p. with 1 mg of anti-CD25 (Clone PC61) antibodies in 0.2 ml saline or isotype-matched Ig only on days -8 and -3. On day 0, mice were immunized with 200µg of MBP₆₀₋₈₀ or PLP₁₃₉₋₁₅₁ peptides emulsified in CFA. On days 0 and 2 after immunization, mice were also given i.v. 200ng of pertussis toxin as a co-adjuvant. Mice were observed for development of active EAE.

* Fisher's Exact Test: one-tailed probability: 0.01