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Experimental Autoimmune Encephalomyelitis in Mice

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Abstract

Experimental autoimmune encephalitis (EAE), the animal model of multiple sclerosis (MS), has provided significant insight into the mechanisms that initiate and drive autoimmunity. Several central nervous system proteins and peptides have been used to induce disease, in a number of different mouse strains, to model the diverse clinical presentations of MS. In this chapter, we detail the materials and methods used to induce active and adoptive EAE. We focus on disease induction in the SJL/J, C57BL/6, and BALB/c mouse strains, using peptides derived from proteolipid protein, myelin basic protein, and myelin oligodendrocyte glycoprotein. We also include a protocol for the isolation of leukocytes from the spinal cord and brain for flow cytometric analysis.

Keywords

Experimental autoimmune encephalomyelitis; Multiple sclerosis; Autoimmune disease; Mouse model; CD4⁺ T cells

1 Introduction

Multiple sclerosis (MS) is a chronic and debilitating autoimmune disease of the central nervous system (CNS), characterized by lesion formation in the white matter of the brain, spinal cord, and optic nerve (1, 2). The precise mechanisms that trigger and drive MS are not completely understood. However, it is clear that myelin antigens are key targets (3). Autoreactive T cells and other immune cells, particularly macrophages, infiltrate the CNS and cause significant damage to the myelin sheath and underlying axons, resulting in neuronal dysfunction and death (4, 5). Depending on the severity and location of the immune insult, patients may present with a range of neurological symptoms that impact physical functioning (e.g., muscle weakness, numbness or spasms, impaired balance and coordination, fatigue, incontinence) or mental capacity (e.g., memory loss, depression, cognitive difficulties) (6).

The frequency and severity in which MS symptoms occur differs greatly between individuals. Four main categories are used to classify the clinical course of MS in patients (7). Relapsing-remitting MS is the most common clinical pattern observed, and is characterized by recurrent attacks (relapses) followed by periods of remission in which little or no permanent neurological sequelae are evident. Secondary progressive MS describes the clinical course in which patients initially present with relapsing-remitting disease, but

⁴CFA can be prepared up to 24 h in advance and stored it in polystyrene tubes or glass syringes at 4 °C until use.

develop significant deficits that increase over time. Primary progressive MS describes the clinical course in which patients show progressive worsening of symptoms without relapse or remission phases. Progressive-relapsing MS is characterized by a steadily worsening disease state, in which patients undergo relapses without complete remission (7).

The animal model of MS, Experimental Autoimmune Encephalomyelitis (EAE), aims to replicate the clinical symptoms of disease in vivo, and has been induced in a range of species, including mice, rats, and hamsters (8). Two different methods of EAE induction have been described. Subcutaneous immunization of mice with an emulsion of myelin protein/peptide and complete Freund's adjuvant (CFA) is referred to as "active EAE," and models the induction and effector stages of disease (9). This process results in the direct priming of myelin epitope-specific CD4⁺ T cells in vivo, which migrate to the CNS and mediate autoimmune responses. In comparison, "adoptive EAE" models the effector phase of disease only. Activated CD4⁺ T cells are isolated from the draining lymph nodes of immunized mice, restimulated with the initiating myelin protein/peptide in vitro for several days, and then injected into naïve recipients. Disease is typically accelerated, more severe and uniform in comparison to active EAE, with higher incidence (9).

Depending on the initiating protein/peptide, animal species and strain used for the induction of EAE, different clinical etiologies may be mimicked (Table 1). For example, induction of disease in the SJL/J mouse strain using the proteolipid protein (PLP) 139–155 peptide induces relapsing remitting disease (Fig. 1). In comparison, immunization of C57BL/6 mice with the myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide results in a chronic disease course (Fig. 2). Immunization of BALB/c and C57BL/6 mice with the PLP 180–199 peptide also induces chronic disease, but causes relapsing-remitting disease in the SJL/J strain (Fig. 3). The development of transgenic and humanized mice strains, outlined in Table 2, have also proved to be invaluable tools in understanding the processes that drive autoimmunity. This chapter aims to provide a detailed protocol for inducing EAE in the mouse. Here, we focus on whole proteins and peptides derived from PLP, MOG, and myelin basic protein (MBP). We include the protocols to induce both active and adoptive disease, and the methodology used to isolate single-cell suspensions of leukocytes from brain tissue.

2 Materials

2.1 Active Induction of EAE

1. Female SJL/J, C57BL/6, or BALB/c mice at 6–8 weeks old (Jackson Laboratories).
2. Small animal clippers (e.g., model A5, blade size 50; Oster).
3. Incomplete Freund's Adjuvant (IFA; Difco).
4. *Mycobacterium Tuberculosis* H37Ra, inactivated and desiccated (Difco).
5. Desired myelin protein or peptide (see Table 1).
6. Phosphate-buffered saline (PBS).
7. Pertussis Toxin, if required (see Table 1; List Biologicals).
8. 15 mL polystyrene test tubes.

9. 18 and 25 G needles.
10. 1 mL glass tuberculin syringes with Luer-Lok (VWR).

2.2 Adoptive Induction of EAE

1. Materials listed in Section 2.1.
2. Dissection forceps and scissors.
3. 100 μ m sieves (Becton Dickinson).
4. 1 mL syringes.
5. 50 mL conical polystyrene tubes.
6. Recombinant mouse IL-12, if required (R&D Systems).
7. 175 cm² culture flasks.
8. 37 °C, 6 % CO₂ tissue culture incubator.
9. 30.5 G needles.
10. Complete Roswell Park Memorial Institute (cRPMI): 440 mL of calcium-free, L-glutamine-free RPMI medium, 5 mL of 100 $\times$ L-glutamine, 5 mL of 100 $\times$ Penicillin-Streptomycin, 500 μ L of 55 μ M 2-mercaptoethanol, and 50 mL of Fetal Bovine Serum (FBS).

2.3 Isolation of CNS Infiltrating Leukocytes

1. Institution-approved anesthetic.
2. Dissection forceps and scissors.
3. 30 mL syringes with Luer-Lok.
4. 21.5 G needles.
5. PBS.
6. Non-treated plastic petri dishes, 60 mm.
7. 5 mL syringes.
8. 18 G needle.
9. Stainless steel wire mesh, 200–300 μ m.
10. 15 mL polystyrene tubes.
11. 50 mL conical polystyrene tubes.
12. Liberase, low Thermolysin concentration (Roche).
13. DNase I (Sigma).
14. Percoll (GE Healthcare).

15. 10× no calcium, no magnesium, no phenol red Hank's Balanced Salt Solution (HBSS).
16. 10× no calcium, no magnesium HBSS.
17. 0.5 M EDTA, pH 8.
18. FBS.

3 Methods

3.1 Active Induction of EAE

1. Shave the back of the mice using the small animal clippers (*see* Note 1).
2. Prepare complete Freund's adjuvant (CFA) by combining 50 mL IFA and 200 mg *M. tuberculosis* H37Ra, resulting in a final concentration 4 mg/mL *M. tuberculosis* (*see* Note 2).
3. Prepare an emulsion of CFA and desired protein/peptide (Table 1) by mixing 1 mL of CFA with 1 mL of desired peptide/protein diluted in PBS. Repeatedly draw up and expel the liquid from a 1 mL glass syringe into a 15 mL polystyrene test tube, using an 18 G needle (*see* Notes 3–5).
4. Slowly draw up the emulsion into a new 1 mL glass syringe using an 18 G needle, taking care not to introduce air bubbles. Replace the 18 G needle with a 25 G needle.
5. Inject 100 µL of emulsion subcutaneously into the shaved backs of the mice, distributing evenly over three injection sites. One injection should be placed on the midline of the back just below the shoulders, and two on either side of the midline on the lower back.
6. Refer to Table 1 to determine if pertussis toxin is needed for the mouse strain and initiating protein/peptide you are using. If required, dilute pertussis toxin to 1 µg/mL in sterile PBS (*see* Note 6). Inject 200 µL of diluted pertussis toxin (200 ng per mouse) into the peritoneal cavity or intravenously on the day of disease induction, and again 48 h after induction (*see* Note 7).

¹It is recommended to shave the back of the mice at least 24 h before inducing disease. The animals will then be easier to handle on the day of induction.

²It is critical to purchase IFA and *M. tuberculosis* separately and prepare CFA at a final concentration of 4 mg/mL. Commercially available CFA only contains 1 mg/mL *M. tuberculosis*.

³The lowest concentration of a given myelin protein/peptide that can be used to reliably induce EAE may vary by source and batch number. The source and age of the mice used may also alter the disease course from that expected. Thus, the concentrations of protein/peptide indicated in Table 1 should be used as a guide only and should be confirmed by the individual investigator before conducting large-scale experiments.

⁵To test the consistency of the emulsion, a small droplet should be expelled onto the surface of water in beaker. If the emulsion is stable, the droplet will remain in a bead on the water surface. If the droplet disperses across surface, further emulsification is required.

⁶It is recommended to dissolve the lyophilized pertussis toxin in sterile PBS at least 24 h before injection and store at 4 °C until use.

⁷We inject 200 ng of pertussis per mouse on day 0 and day 2 post-immunization where indicated on Table 2. However, some papers report injection of up to 500 ng of pertussis per mouse. The individual investigator should determine the optimal dose for the protein/peptide and mouse strain used.

7. Monitor mice every day to observe the development of clinical symptoms, which usually occur between day 10 and 28 post-induction (see Figs. 1, 2, and 3). Symptoms are measured using the scoring system shown in Table 3.

3.2 Adoptive Induction of EAE

1. Immunize female donor SJL/J, C57BL/6, or BALB/c mice with the desired myelin protein/peptide as detailed in Section 3.1.
2. Prepare cRPMI.
3. Between day 7 and day 14 post-induction (see Table 4), euthanize the mice and remove the draining lymph nodes (inguinal, brachial, and axillary). Place the lymph nodes into cRPMI.
4. Place a 70 μ m filter into a non-treated culture plate. Generate a single-cell suspension by pressing the lymph nodes in cRPMI through the filter with the plunger from a 1 mL syringe.
5. Transfer the cRPMI solution containing the cells into a 50 mL polystyrene tube and centrifuge for 10 min at $300 \times g$.
6. Remove the supernatant and raise the pellet in 1 mL of cRPMI and gently resuspend using a pipette. Add another 19 mL of cRPMI to increase the total volume to 20 mL.
7. Count the number of viable white blood cells using trypan blue exclusion of dead cells on a hemocytometer slide.
8. Culture the cells at a concentration of 8×10^6 cells per mL in cRPMI in 175 cm² flasks (up to 30 mL total volume per flask). Add in the required amount of initiating protein/peptide to restimulate the cells (see Table 4; Notes 8–⁹). Add in 10 ng/mL IL-12 if necessary (see Table 4; Notes 10–¹¹). Incubate for 72 h in a 37 °C, 6 % CO₂ tissue culture incubator (*see* Note 12).
9. Harvest the cells by transferring into 50 mL conical tubes and centrifuging at $300 \times g$ for 15 min. Count the number of live blasts by using trypan blue exclusion. Blasts will appear larger than other leukocytes in the culture and should comprise at least 10–30 % of the culture.

⁸The amount of peptide needed to effectively restimulate T cells in vitro may vary by protein/peptide source and batch number. The source and age of the mice used may also affect the amount of protein/peptide needed for effective restimulation. Thus, the concentrations of protein/peptide indicated in Table 4 should be used as a guide only and should be confirmed by the individual investigator before conducting large-scale experiments.

⁹Con A may be used at a dose of 1 μ g/mL to stimulate T cells for 48 h in vitro in place of specific myelin protein/peptide for the induction of adoptive EAE. Con A activation, however, will reduce the frequency of myelin epitope-specific T cells in the culture, thus an increased number of total cells will need to be injected into recipient mice. The individual investigator will need to titrate these numbers in vivo to determine the lowest number of cells required to achieve severe and reliable EAE.

¹⁰In addition to IL-12, IL-2 may also be added into the media at a concentration of 10 ng/mL to enhance T cell activation and proliferation. The individual investigator should confirm the optimum concentration of these cytokines before conducting large-scale experiments.

¹¹IL-23 may be added to the media at a concentration of 10 ng/mL to induce a Th17-skewed T cell phenotype. The individual investigator should confirm the optimum concentration of IL-23 before conducting large-scale experiments.

¹²We typically incubate cells in vitro for 3 days before transferring into recipients. Most papers report incubation periods of 3–4 days. The individual investigator should determine the optimal incubation time for the protein/peptide and mouse strain used.

10. Wash the cells twice in PBS and raise to the required concentration in PBS. Inject the recommended number of blasts in a final volume of 200 μ L (see Table 4; Note 13) into recipient mice using a 1 mL syringe and 30.5 G needle via intravenous tail vein. Alternatively, blasts may be injected into the peritoneum.
11. Inject 200 μ L of diluted pertussis toxin (200 ng per mouse) into the peritoneal cavity or intravenously on the day of disease induction, and again 48 h after induction if necessary (refer to Table 4).

3.3 Isolation of CNS Infiltrating Leukocytes

1. Induce deep anesthesia by injecting mice with approved anesthetic (e.g., Nembutal). Ensure deep anesthesia is achieved by testing reflex responses of the footpads.
2. Draw up PBS into a 30 mL syringe with a 21.5 G needle.
3. Using surgical scissors and forceps, expose the chest cavity by making an incision at the diaphragm and cutting upwards through the rib cage.
4. Using the forceps, carefully hold the heart in place. Make a small incision in the right atrium of the heart.
5. Immediately insert the 30 mL syringe with 21.5 G needle into the left atrium of the heart. To ensure correct placement, put a small amount of pressure on the syringe plunger. An efflux of dark red blood should immediately flow from the right atrium (*see* Note 14). Slowly perfuse the animal by continuing to apply pressure on the plunger (*see* Notes 15, ¹⁶).
6. Remove the head of the mouse by carefully cutting under the skull, through the neck. Using scissors or a scalpel, cut the skin and cutaneous muscle by making a single incision along the midline length of the head, from the posterior aspect of the skull to the nose of the mouse. Using the forceps, fold back the two flaps of skin to expose the skull.
7. Carefully cut through the top of the skull along the midline, from the posterior aspect of the skull to the nose. Make two incisions from the posterior aspect of the skull to the eye sockets, and use the forceps to remove the pieces of skull from the top of the head. Carefully remove the brain from the cranial cavity and place in a 50 mL tube containing 20 mL of PBS.
8. Using a scalpel or scissors, make a long incision through the midline of the mouse (from the neck to the base of the tail). Peel the skin back to expose the spinal

¹³The lowest number of cells that are needed to reliably induce EAE may vary by myelin protein/peptide source and batch number. The source and age of the mice used may also alter the disease course from that expected. The numbers listed in Table 4 should be used as a guide only. The individual investigator will need to titrate these numbers in vivo to determine the lowest number of cells required to achieve severe and reliable EAE.

¹⁴If the needle is not placed correctly, dark red blood will not flow from the right atrium and the lungs may inflate. Loss of red coloration of the liver is a good indicator of correct perfusion. The authors recommend practicing this technique before conducting large-scale experiments.

¹⁵Perfusions should be conducted slowly (over a period of at least 3–5 min per mouse) to avoid tissue damage.

¹⁶If the mouse is not well perfused after the initial procedure, the syringe may be refilled with 30 mL of PBS and perfusion repeated.

column. Identify where the base of the spinal column attaches to the pelvis, and make a perpendicular cut through the spine at this point. Cut along each side of the column to the neck to remove the column.

9. Attach an 18 G needle to a 5 mL syringe that has been filled with PBS. Hold the column in a petri dish with forceps, and insert the needle into the spinal column at the caudal end. Push the plunger of the syringe to expel PBS into the spinal column. Initial resistance should be felt, followed by release, in which the spinal cord will be flushed from the column. Transfer spinal cord into a 50 mL tube containing 20 mL PBS.
10. Carefully pass the brain and spinal cord through the stainless steel wire mesh in the 60 mm petri dishes using the plungers taken from 10 mL syringes. Rinse all tissue off the mesh with additional PBS. Transfer back into 50 mL tubes and centrifuge for 20 min at $300 \times g$ at 4 °C. Remove the supernatant.
11. Prepare digestion enzyme mix by adding 1 g DNase I (50 µg/mL final concentration) and 800 U Liberase (40 U/mL final concentration) to 20 mL PBS (*see* Note 17). Raise cells in 2 mL of digestion enzyme mix and incubate for 30 min at 37 °C.
12. Prepare 500 mL FACS buffer by adding 10 mL FCS (2 % final concentration) and 2 mL 0.5M EDTA (2 mM final concentration) to 488 mL PBS.
13. Add 30 mL FACS buffer to samples and pass through a 70 µM sieve into a new 50 mL tube. Centrifuge for 10 min at $300 \times g$ at 4 °C. Wash cells again twice in FACS buffer.
14. Prepare 30 % Percoll by adding 5 mL 10 % HBSS (with phenol red) and 15 mL Percoll to 35 mL of water. Prepare 70 % Percoll by adding 5 mL 10 % HBSS (without phenol red) and 35 mL Percoll to 15 mL of water (*see* Note 18).
15. Raise the CNS samples in 5mL of 30 % Percoll and transfer to a 15 mL tube. Underlay samples with 5 mL of 70 % Percoll. Centrifuge the samples at $1,000 \times g$ for 25 min at room temperature with no brake.
16. Using a pipette, remove the myelin layer from the top of the gradient. Using a new pipette tip, collect the mononuclear cells at the interface between the 30 and 70 % Percoll interface and transfer into a new 15 mL tube containing 5 mL FACS buffer. Centrifuge for 10 min at $300 \times g$ at 4 °C. Wash cells again twice in FACS buffer.
17. Count live cells using trypan blue exclusion.
18. Single cells can now be further processed for the desired assay, e.g., flow cytometric analysis.

¹⁷The protocol listed here using Liberase and DNase is optimized for the isolation of total leukocytes. Different enzymatic digestion may be performed on the CNS tissues to isolate different target cell populations. For example, we have found that digesting the CNS using Accutase (Millipore) in place of Liberase and DNase is optimal for the isolation of oligodendrocyte progenitor cell isolation.

¹⁸The use of HBSS with and without phenol red for the 30 % Percoll and 70 % Percoll solutions, respectively, will increase the ease of which to see the interface between the two gradients and identify the mononuclear cell layer here.

Abbreviations

CNS	Central nervous system
EAE	Experimental autoimmune encephalomyelitis
IFA	Incomplete Freund's adjuvant
MBP	Myelin basic protein
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
PLP	Proteolipid protein
TCR	T cell receptor

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Fig. 1. Induction of EAE in the SJL/J mouse using PLP₁₃₉₋₁₅₁. Immunization of SJL/J mice with 50 µg of the PLP₁₃₉₋₁₅₁ peptide results in a relapsing-remitting course of EAE

Fig. 2.
Induction of EAE in the C57BL/6 mouse using MOG₃₅₋₅₅. Immunization of C57BL/6 mice with 200 μ g of the MOG₃₅₋₅₅ peptide results in a chronic course of EAE

Fig. 3.
Induction of EAE in the BALB/c, C57BL/6, and SJL/J mouse using PLP₁₈₀₋₁₉₉.
Immunization of BALB/c and C57BL/6 mice with 200 µg of the PLP₁₈₀₋₁₉₉ peptide results
in a chronic course of EAE, but causes relapsing-remitting disease in SJL/J mice

Table 1

Induction of active experimental autoimmune encephalomyelitis in mice

Mouse strain	Myelin peptide/protein	Sequence	Peptide/protein dose (µg)	Pertussis toxin	Clinical course
BALB/c	Whole PLP (10)	From : bovine	200	Yes	Chronic
	PLP ₁₈₀₋₁₉₉ (10)	WTTCQSIAPPSKTSASIGSL	200	Yes	
C57BL/6	MOG ₃₅₋₅₅ (11)	MEVGWYRSPFSRVVHLYRNGK	200	Yes	Chronic
	PLP ₁₇₈₋₁₉₁ (12)	NTWTTTCQSIAPPSK	200	Yes	
	PLP ₁₈₀₋₁₉₉ ^a	WTTCQSIAPPSKTSASIGSL	200	Yes	
SJL/J	Whole MBP (13)	From: mouse, rat, or bovine	200–400	Yes	Relapsing-remitting
	MBP ₈₄₋₁₀₄ (14)	VHFFKNIVTPRTPPPSQGKGR	200 ^b	Yes	
	MBP ₈₉₋₁₀₁ (15)	VHFFKNIVTPRTP	200	Yes	
	MOG ₉₂₋₁₀₆ (16, 17)	DEGGYTCFFRDHSYQ	200 ^b	Yes	
	Whole PLP (18)	From: rat or bovine	200	Yes	
	PLP ₅₇₋₇₀ (19)	YEYLINVIHAFQYV	100	Yes	
	PLP ₁₀₄₋₁₁₇ (20, 21)	KTTCGKGLSATVT	50	Yes	
	PLP ₁₃₉₋₁₅₁ (22)	HSLGKWLGHDPKF	50	No	
	PLP ₁₇₈₋₁₉₁ (23)	NTWTTTCQSIAPPSK	200	No	
	PLP ₁₈₀₋₁₉₉ ^a	WTTCQSIAPPSKTSASIGSL	200	Yes	
ABH	Whole MOG (16)	From: rat	200 ^b	Yes	Chronic-relapsing
	MOG ₈₋₂₂ (16, 24)	PGYPIRAL VGDEQED	200 ^b	Yes	
	PLP ₅₆₋₇₀ (24)	DYEYLINVIHAFQYV	100 ^b	Yes	
PL/J, B10.PL	Whole MBP (25)	From: rat or guinea pig	200	Yes	Chronic/acute monophasic
	MBP _{Ac1-11} (26, 25)	Ac-ASQKRPSQRSK	100	Yes	
	MBP ₃₅₋₄₇ (27)	TGILDTSIGRFFSG	200	Yes	
	MOG ₃₅₋₅₅ (28)	MEVGWYRSPFSRVVHLYRNGK	200	Yes	
	PLP ₄₃₋₆₄ (29)	EKLIEITYFSKNYQDYEYLINVI	150	Yes	

Mouse strain	Myelin peptide/protein	Sequence	Peptide/protein dose (µg)	Pertussis toxin	Clinical course
C3H/HeJ	Whole PLP (18)	From: rat or bovine	200	Yes	Chronic/atypical
	PLP ₁₉₀₋₂₀₉ (19, 30)	SKTSASIGSLCADARMYGVL	100	Yes	
	PLP ₂₁₅₋₂₃₂ (31, 19)	PGKVCGSNLLSICKTAEFQ	100	Yes	

^aTerry, Harp, and Miller, unpublished data, Fig. 2

^bMice require a second immunization on day 7

Table 2

Transgenic mouse models of experimental autoimmune encephalomyelitis

Background	TCR specificity	Cell-inducing disease	% of spontaneous disease	Age of onset (in weeks)	Clinical course
B10.PL/TCR tg B10.PL/TCR tg × RAG-1 ^{-/-}	MBP _{Ac1-11} (32, 33)	CD4	14-44 % 100 %	5-20 6-20	Chronic/AM Chronic
C57BL/6 HLA-DR2/TCR tg DR2/TCR tg × RAG-2 ^{-/-}	huMBP ₈₄₋₁₀₂ (34)	CD4	4 % 100 %	ND 7-15	Variable
C57BL/6 HLA-DR15/TCR tg DR15/TCR tg × RAG-2 ^{-/-}	huMBP ₈₅₋₉₉ (35)	CD4	60 % 80-100 %	16-24 5-16	Chronic
SJL/J 5B6	PLP ₁₃₉₋₁₅₁ (36)	CD4	40-60 %	6 and older	Chronic
C57BL/6 2D2	MOG ₃₅₋₅₅ (37)	CD4	4-15 % 30-40 %	10-20 10-52	Chronic Optic neuritis
C57BL/6 2D2 × IgH _{MOG}	MOG ₃₅₋₅₅ (38, 39)	CD4 B cells	50-60 %	4-10	Chronic with lesions only in spinal cord and optic nerve
SJL/J TCR1640	MOG ₉₂₋₁₀₆ (40)	CD4 B cells	60-90 %	8-23	RR on female PP on male
C57BL/6 B7.2 expressed on microglia and T cells	ND (41, 42)	CD8	100 %	12-30	Chronic
C57BL/6 ODC-OVA × OT-I	OVA ₂₅₇₋₂₆₄ (43)	CD8	90-100 %	1-3	Chronic/lethal
C57BL/6 HLA-A3/TCR tg	huPLP ₄₅₋₅₃ (44)	CD8	4 %	ND	Motor deficit
NOD IC6 × IgH _{MOG}	MOG ₃₅₋₅₅ (45)	CD4 CD8 B cells	45-80 %	12-18	RR to chronic

AM acute monophasic, RR relapsing-remitting, PP primary progressive, tg transgenic, ODC oligodendrocytes, ND not defined

Table 3

Clinical scoring of mice with experimental autoimmune encephalomyelitis

Clinical score	Clinical symptoms
0	Mouse shows no symptoms of disease (asymptomatic)
1	Mouse has a limp tail (complete flaccidity, absence of curling at the tip) or hind limb weakness (waddling gait, mouse's hind limbs fall through the top of a wire cage), not both
2	Mouse has both a limp tail and shows hind limb weakness
3	Mouse has partial paralysis of the hind limbs (can no longer maintain posture of the rump, but can still move one or both limbs to an extent)
4	Mouse shows complete hind limb paralysis (complete loss of movement of the hind limbs, all movement is the result of the mouse dragging on the forelimbs) ^a
5	Moribund (death caused by EAE), mice are euthanized for humane reasons

^a Mice at this stage of disease are given soft gel food on the cage floor, long sipper tubes, and daily saline injections to prevent dehydration

Table 4

Induction of adoptive experimental autoimmune encephalomyelitis^a

Mouse strain	Myelin peptide/protein	Donor immunization period (days)	In vitro peptide/protein dose (µg/mL)	No. of blasts transferred (×10 ⁶)	Disease type and severity	Pertussis
BALB/c	PLP ₁₈₀₋₁₉₉	10-14	20 ^b	5-10	Chronic, Moderate	Yes
C57BL/6	Whole MOG	10-14	50 ^b	20	Chronic, Moderate	Yes
	MOG ₃₅₋₅₅	10-14	10 ^b	20	Chronic, Moderate	Yes
	PLP ₁₇₈₋₁₉₁	10-14	20	10-20	Chronic, Moderate	Yes
	PLP ₁₈₀₋₁₉₉	10-14	20	5-10	Chronic, Moderate	Yes
SJL/J	Whole MBP	7-14	50-100	40-60	RR, Moderate	Yes
	MBP ₈₄₋₁₀₄	7-14	50	10-20	RR, Moderate	Yes
	Whole PLP	7-14	50-100	5-10	RR, Severe	Yes
	PLP ₁₃₉₋₁₅₁	7-14	20	1-5	RR, Severe	No
	PLP ₁₇₈₋₁₉₁	7-14	20	10-20	RR, Severe	No
	PLP ₁₈₀₋₁₉₉	10-14	20 ^b	5-10	RR, Severe	Yes

RR relapsing-remitting

^aThese parameters have been modified from protocols as previously described for induction of disease in BALB/c (10, 46), C57BL/6 (11, 47-50), and SJL/J (51-58, 20) strains

^bIL-12 should be added at a dose of 20 ng/mL