Induction and monitoring of chronic-relapsing EAE in C57BL/6 mice

The emulsion is a 1:1 mixture of antigen in aqueous solution added to supplemented complete Freund’s adjuvant. It is very important to add the antigen to the adjuvant and not the other way round!!!

There is a lot of waste when making and injecting an emulsion, always prepare 1.5 or 2 times more than what you need.

Use autoclaved mortar, pestle, glass syringes, and bridges. All plastics should be sterile. The preparation can be done on a regular laboratory bench.

1. Preparations of the supplemented adjuvant

Weigh 40 mg of *Mycobacterium tuberculosis* (Difco catalog # 231141) and place into a mortar. Make into thin powder but without pressing too hard to avoid breaking the bacteria. Add 10 ml of complete Freund’s adjuvant H37Ra (Difco catalog # 231131) and mix. This supplemented adjuvant now contains 5 mg/ml *Mycobacterium tuberculosis*. Transfer to a 50 ml tube.

2. Preparation of the peptide

Dissolve 5 mg of mMOG peptide (Anaspec catalog # 60130-5) in 2.5 ml PBS to make a 2 ml/ml solution of peptide. Prepare fresh each time.

3. Preparation of the emulsion

Place 2.5 ml of supplemented complete Freund’s adjuvant in a tube and vortex at high speed. Add the mMOG peptide drop by drop while vortexing. When all of the peptide is added, vortex for 3 more minutes. The mixture should turn white.

Put the emulsion in a 5 ml glass syringe and link it to another 5 ml glass syringe using a 18G bridge (Fisher catalog # 14-825-17L). Remove all air bubbles. Send the emulsion from a syringe to the other until it becomes hard (5-10 min). If more than 5 ml of emulsion is prepared, use several 5 ml syringes, do NOT use larger syringes.

4. Preparation of the Pertussis toxin

Dilute 10 ul of Pertussis toxin stock (Sigma catalog # P2980-50) in 1 ml of PBS.

5. Immunization of the mice

Mix the emulsion in the syringes for a few minutes and transfer to the syringe used for the injection. Inject 200 µl subcutaneously at the very base of the tail and in the flanks using 23G needles and 3 ml Luer-Lock syringes under short-term anesthesia.

Inject 200 ul diluted Pertussis toxin intraperitoneally per mouse on day 0 and on day 2.
6. Monitoring of the mice

Mice should be observed and weighed daily. Clinical signs are expected ~12 days after injection of the emulsion and peak around day 27. Weight loss often precedes clinical signs by a day.

Clinical scoring:
0: no disease
1: flaccid tail
1.5: flaccid tail and impaired righting reflex
2: impaired righting reflex and hind limb weakness
2.5: one hind leg paralyzed
3: both hind legs paralyzed with residual mobility in both legs
3.5: complete hind leg paralysis
4: complete hind limb paralysis and beginning front leg paralysis
5: moribund, difficulty breathing, does not eat or drink. Euthanize immediately.

Mice with a score of 3 or more need special care. See the protocol for EAE in DA rats for more details.