Video Article

Induction and Clinical Scoring of Chronic-Relapsing Experimental Autoimmune Encephalomyelitis

Christine Beeton, Adriana Garcia, K. George Chandy
Department of Physiology and Biophysics, University of California, Irvine

Correspondence to: Christine Beeton at cbeeton@uci.edu

URL: http://www.jove.com/index/Details.stp?ID=224
DOI: 10.3791/224
Citation: Beeton C., Garcia A., Chandy K.G. (2007). Induction and Clinical Scoring of Chronic-Relapsing Experimental Autoimmune Encephalomyelitis. JoVE. 5. http://www.jove.com/index/Details.stp?ID=224, doi: 10.3791/224

Abstract

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that commonly affects young adults. It is characterized by demyelination and glial scaring in areas disseminated in the brain and spinal cord. These lesions alter nerve conduction and induce the disabling neurological deficits that vary with the location of the demyelinated plaques in the CNS (e.g. paraparesis, paralysis, blindness, incontinence).

Experimental autoimmune encephalomyelitis (EAE) is a model for MS. EAE was first induced accidentally in humans during vaccination against rabies, using viruses grown on rabbit spinal cords. Residues of spinal injected with the inactivated virus induced the CNS disease. Following these observations, a first model of EAE was described in non-human primates immunized with a CNS homogenate by Rivers and Schwenther in 1935. EAE has since been generated in a variety of species and can follow different courses depending on the species/strain and immunizing antigen used. For example, immunizing Lewis rats with myelin basic protein in emulsion with adjuvant induces an acute model of EAE, while the same antigen induces a chronic disease in guinea pigs.

The EAE model described here is induced by immunizing DA rats against DA rat spinal cord in emulsion in complete Freund's adjuvant. Rats develop an ascending flaccid paralysis within 7-14 days post-immunization. Clinical signs follow a relapsing-remitting course over several weeks. Pathology shows large immune infiltrates in the CNS and demyelination plaques. Special considerations for taking care for animals with EAE are described at the end of the video.

Protocol

Induction and monitoring of chronic-relapsing EAE

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. Preparation of the supplemented adjuvant

Weigh 30 mg of *Mycobacterium tuberculosis* (Difco catalog # 231141) and place into a mortar. Make into thin powder 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 4 mg/ml *Mycobacterium tuberculosis*. Transfer to a tube.

2. Preparation of the spinal cord homogenate

Collect spinal cords from DA rats (age/gender indifferent) and store frozen at -80°C. Weigh the frozen spinal cords to have enough to mix 1:1 (weight:volume) with the supplemented adjuvant. Mince the spinal cords as fine as possible with a razor blade, transfer to the mortar used in section 1, and make a paste with the pestle.

3. Preparation of the emulsion

1. Place supplemented complete Freund's adjuvant in a tube. Vortex at high speed. Add the spinal cord homogenate drop by drop while vortexing. When all of the spinal cord homogenate is added, vortex for 5 more minutes. The mixture should turn light pink.
2. 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). 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.
3. The emulsion can be stored in the syringes at 4°C for 3 weeks. I recommend preparing it at least 12 hours in advance to check that it does not break down. It should remain thick and not separate in 2 phases. The color will change overnight to a light yellow or beige.

4. Immunization of the rats

1. 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 using 23G needles and 3 ml Luer-Lock syringes under short-term anesthesia.
2. The recipients are 7-9 weeks old female DA rats. We get our rats from Harlan-Sprague Dawley.

3. Rats should be observed twice daily and weighed daily. Clinical signs are expected 7-15 days after injection of the emulsion.

Clinical scoring:

0: no disease

0.5: distal limp tail

1: limp tail

2: mild paraparesis, ataxia

3: moderate paraparesis, the rats trips from time to time

3.5: one hind limb is paralyzed, the other moves

4: complete hind limb paralysis

5: complete hind limb paralysis and incontinence

6: moribund, difficulty breathing, does not eat or drink. Euthanize immediately.

Disclosures

CB and KGC are co-founders and consultants for Airmid Inc.

Discussion

There are a few important considerations for this protocol.

Genetics of DA rats will differ slightly depending on which breeder they are bought from. These differences may increase or decrease the susceptibility of rats to EAE induction. If your rats are more susceptible to EAE you will want to reduce the strength of the immunization by using non-supplemented complete Freund's adjuvant or even incomplete Freund's adjuvant. You may also consider reducing the amount of emulsion injected or diluting the spinal cord homogenate with saline before preparing the emulsion. If your rats do not develop clinical signs of EAE using the full-strength immunizing emulsion check the cleanliness of your housing facility. Rats infected with parasites (such as pinworm) will not develop EAE. You may consider housing your animals under cleaner conditions in autoclaved cages with filter-tops, and giving them acidified water ad libitum (the protocol for preparing acidified water is attached).

The emulsion must be water-in-oil so that the oil in the adjuvant completely coat the antigen you add drop by drop. An emulsion prepared the other way round (oil-in-water) will become as thick but will not be encephalitogenic.

Rats are friendly animals if they are accustomed to their handler, it is therefore a good idea to handle them gently on a daily basis before injection of the encephalitogenic emulsion. This will reduce stress and the risk of a bite.

Animals with severe EAE (score of 3 or more) need special care. Rats should never be picked by the tail but rather by the body. This is even more important for reducing stress in sick animals. It is very important to ensure that all animals have access to food and water by putting food and gel packs in the bedding and providing long-sipper tubes on the water bottles. Rats with EAE will continue eating and drinking, if an animal stop either eating or drinking consult your institute's veterinarian or euthanize the animal. Sick rats should be separated from healthy animals to ensure they are not constantly trampled, but avoid leaving rats alone since they are social animals and need company.

References

