Hooke Laboratories

Sample IACUC Protocol

Evaluation of compound effects on EAE development in C57BL/6 mice

Introduction and use permissions

This document is offered as a sample IACUC protocol. Your institutional IACUC committee may require changes or additions to this protocol – this document is only a starting point.

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Species justification

While MS models have been described in many animal species, mouse models are as effective as any other animal models in the elucidation of the mechanisms of MS and in prediction of the success of therapeutic compounds in human patients.

Mouse models have several advantages compared to models in other species:

- The small size of mice allows the use of small amounts of test compounds.
- The greatest number of genetically modified animals exist for mouse species.
- The use of mice has low costs compared to other experimental animals.

Protocol keywords

Autoimmunity, blood collection, complete Freund's adjuvant, MOG, EAE, experimental autoimmune encephalomyelitis, injections, mouse, mice, rodents, tissue collection, MS, multiple sclerosis

Technical terms and abbreviations

CNS	Central nervous system
CFA	Complete Freund's adjuvant (mineral oil with killed M. tuberculosis)
EAE	Experimental autoimmune encephalomyelitis
IFA	Incomplete Freund's adjuvant
MS	Multiple sclerosis

Background

Experimental autoimmune (allergic) encephalomyelitis (EAE) is considered to be the best model of multiple sclerosis (MS), a chronic, often disabling disease of the human central nervous system (CNS). Over 6000 papers have been published in scientific journals on this model.

EAE is characterized by immune responses against CNS tissue and can be induced in animals by immunizing them against proteins of CNS.

Study design

Mice will be immunized with MOG_{35-55} peptide or MOG_{1-125} emulsified in complete Freund's adjuvant (CFA) by injecting them subcutaneously at two sites on the back (0.1 mL of emulsion/site).

On the same day, and again the following day, mice will receive intraperitoneal injection of pertussis toxin in PBS, at up to 600 ng/mouse/dose (0.1 mL).

Some animals develop skin lesions at the site of injection of CFA. These lesions appear to be related to the amount of killed Mycobacterium tuberculosis in the adjuvant, rather than to the volume of adjuvant – lesions do not develop if animals are injected with incomplete Freund's adjuvant. The amount of killed Mycobacterium tuberculosis used is the lowest amount that will reliably induce EAE.

No skin lesions are observed after pertussis toxin injection.

EAE will develop in mice 7-14 days after immunization (Day 0).

Animals which develop EAE will become paralyzed. Paralysis is usually chronic, with the most severe paralysis lasting 2-4 days.

Daily observation and scoring of mice starts on Day 7 and continues until the end of the study.

Scoring is on the scale of 0 to 5, according to the table below. Mice may be given "in-between" scores (i.e. 0.5, 1.5, 2.5, 3.5) when the clinical picture lies between two defined scores.

In some cases atypical EAE symptoms develop. There is no universal consensus on how to score these cases. These can occur, for example, when inflammatory lesions develop in parts of the brain affecting balance.

When one or more mice in a cage initially receive a score of 0.5 or greater, the supportive care is initiated by placing a "Transgel" tag on the cage card holder. This tag indicates to animal husbandry personnel that easily accessible food and water is to be provided by placement of pelleted or wet food and Transgel (Charles River Laboratories) on the floor of the cage. Often mice progress from level 0.5 to 1.5 or 2 overnight.

This supportive care is continued until the "Transgel" tag is removed by the person responsible for scoring mice. Supportive care may be discontinued when all mice in a cage have returned to a score of 2 or less. Usually the tag will stay on the cages throughout the study.

EAE scoring

Score	Clinical Observations
0	No obvious changes in motor functions of the mouse in comparison to non-immunized mice. When picked up by the tail, the tail has tension and is erect. Hind legs are usually spread apart. When the mouse is walking, there is no gait or head tilting.
1	Limp tail. When the mouse is picked up by the tail, instead of being erect, the whole tail drapes over your finger.
2	Limp tail and weakness of hind legs. When mouse is picked up by tail, legs are not spread apart, but held closer together. When the mouse is observed when walking, it has a clearly apparent wobbly walk.

3	Limp tail and complete paralysis of hind legs (most common). OR Limp tail with paralysis of one front and one hind leg. OR ALL of: • Severe head tilting, • Walking only along the edges of the cage, • Pushing against the cage wall, • Spinning when picked up by the tail.
4	Limp tail, complete hind leg and partial front leg paralysis. Mouse is minimally moving around the cage but appears alert and feeding. Usually, euthanasia is recommended after the mouse scores level 4 for 2 days. When the mouse is euthanized because of severe paralysis, score of 5 is entered for that mouse for the rest of the experiment.
5	Complete hind and complete front leg paralysis, no movement around the cage. OR Mouse is spontaneously rolling in the cage. OR Mouse is found dead due to paralysis. If mouse is alive, euthanize the mouse immediately if it scores 5. Once mouse is scored 5, the same score is entered for all the days for the rest of the experiment.

Criteria used to euthanize mice

If an animal scores 5, it is immediately euthanized.

If an animal is severely paralyzed (score 4 or 4.5), it will be given s.c. fluid – 1 mL of 0.9% NaCl or Ringer's solution – and will be re-evaluated at the same time (+/- 1 hour) the following day. If that animal scores 4 or higher again, it will be euthanized immediately afterwards. This is done to ensure that no animal spends more than 24 hours with a score of 4 or higher.

In addition to EAE scoring, overall clinical appearance of the mice will be used as a criterion to euthanize mice. This criterion may override the EAE scoring criterion.

In cases where mice will be bled during the experiment, approximately 0.1 to 0.2 mL of blood will be collected from the retro-orbital plexus under complete anesthesia

(isofluorane). The sample will be used to analyze cytokine levels or leukocyte levels in blood and/or concentration of a therapeutic compound in plasma or serum.

For major terminal surgery, deep surgical anesthesia will be achieved using i.p. administration of tribromoethanol. This will be done when CNS tissue needs to be collected for immunohistochemistry. Once mice are fully anesthetized, as tested by absence of any reflex withdrawal of the leg after firm pinching of the foot with forceps, perfusion will be performed, first with 5 to 10 mL of PBS, and then with 10 to 20 mL of 4% paraformaldehyde. The tissue will then be collected.

At the end of the study, animals will be euthanized using CO_2 asphyxiation.

In some studies after the animals are euthanized various organs may be collected for in vitro analysis. In this case after CO_2 asphyxiation animals may be perfused with PBS to eliminate blood from blood vessels.

Treatment

Any time during the period of disease induction, development, or even before disease is induced, mice may receive experimental drugs. These drugs may be administered via:

- Oral gavage – up to twice/day, up to 10 mL/kg, using a 1 inch long 25G gavage needle
- Intraperitoneal injection – up to once/day, up to 10 mL/kg, using a 5/8 inch 27G needle
- Subcutaneous injection – up to once/day, up to 10 mL/kg, using a 5/8 inch 27G needle
- Intravenous injection - up to once/day, up to 10 mL/kg, using a 5/8 inch 27G needle

Use of surgically manipulated mice

In some cases, surgically manipulated mice may need to be used. Surgical manipulation will be done by an outside vendor. Wound will be inspected upon arrival and then daily. Clips will be removed 7 days after the surgery.

Hazardous Agents:

Pertussis toxin, killed Mycobacteria tuberculosi – will be handled in biosafety cabinet using appropriate personal protective devices (lab coat, gloves, eye protection).

