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Antigen induced arthritis in mice

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Protocol status: Working

We use this protocol and it's working

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Abstract

Antigen-induced arthritis model of rheumatoid arthritis. The Udalova lab (University of Oxford) created this protocol, which Dr George Goodwin (King's College London) adapted.

Expected results: Inflammation and swelling in the injected knee joint.

Attachments



Figure 1. AIA inject...

532KB

Materials

REAGENTS

Incomplete Freund's Adjuvant (IFA) - #263910, Difco

Mycobacterium tuberculosis (Mtb) - #231141, Difco

Methylated Bovine Serum Albumin (mBSA) - #A1009, Sigma Aldrich

D-PBS - #D8537, Sigma Aldrich

M110022 blunt fill needle (for mixing)

Equipment

Heating blanket

Isoflurane anaesthetic rig + oxygen concentrator

Electric shaver

70% Ethanol swab

26 G needle

1ml syringes

Hamilton Syringe with 30g removable needle

Troubleshooting



CFA Preparation (3.3mg/ml)

- 1 In a fume hood, add 30ml Incomplete Freund's Adjuvant (3 vials) and 100mg Mycobacterium tuberculosis (1 vial) to a mortar and grind with a pestle until the powder is fine.
- 2 Transfer to a 50ml Falcon tube and store at 4°C indefinitely.
- 3 Vortex well before use.

mBSA preparation (40mg/ml)

- 4 Add 40mg mBSA to 1ml dH₂O in an Eppendorf.
- 5 Mix on a rotating wheel until fully dissolved.
- 6 Aliquot and store at -20°C (avoid excessive freeze/thaw).

Mice

- 7 Perform experiments in male C57BL/6 mice (RRID:IMSR_JAX:000664), aged approx. 12 weeks

AIA IMMUNISATION (Day 0)

- 8 Prepare the CFA/mBSA emulsion in a 5ml bijou for immunisations: 500ul CFA at 3.3mg/ml 475ul D-PBS 25µl mBSA at 40mg/ml (This is plenty for the immunisation of 4 mice – scale up as required - always prepare excess as you lose volume when the emulsion thickens).
- 9 Mix the solution on ice using a 1ml syringe (without a needle) by rapidly moving the syringe plunger up and down to draw up the solution and expel back into the bijou. Do this for 10mins until the solution emulsifies (turns white) and thickens.

****This is very important – you need the solution to thicken so that it stays under the skin following injection, as if it runs out of the injection site it can irritate the skin and cause ulcers.****

- 10 Transport to the animal unit on ice.
- 11 Load 1ml syringe with the mBSA+CFA emulsion (100µl per mouse), ensuring that there are no bubbles – check carefully as the bubbles are slow moving when the emulsion is thick.
- 12 Prepare the isoflurane anaesthetic rig, oxygen concentrator and heating blanket.
- 13 Set oxygen concentrator flow rate to 1/L min.
- 14 Place mouse into isoflurane induction chamber and set vaporiser to 4% until deep level of anaesthesia is achieved.
- 15 Remove mouse from chamber and place mouse on the heating blanket with the nose of the mouse into the nose cone.
- 16 Shave fur at the base of the tail and the right flank (See Figure 1 for approximate locations).

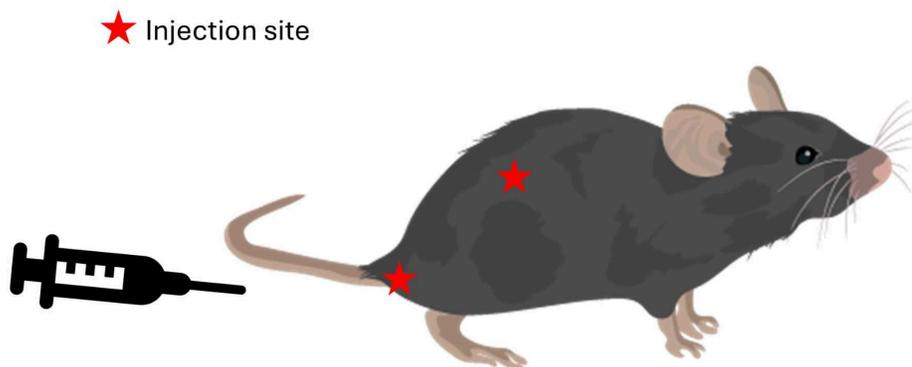


Figure 1: AIA injection sites



- 17 Remove excess fur with tape and then wipe the injection site with a 70% ethanol swab.
- 18 Ensure that the depth of anaesthesia is adequate by checking front paw pedal reflexes.
- 19 Inject 100µl mBSA+CFA per mouse – 50µl sub-cutaneously at the base of the tail (insert needle where the hairline meets the tail), and 50µl sub-cutaneously on the right flank (just to the right of midline) with an orange needle (Figure 1).

****Be prepared to apply more force to the syringe than a usual injection due to the thickness of the emulsion. You should see a 'mound' of the emulsion under the skin when complete, this is most clear at the base of the tail.****

- 20 Remove mouse from nose cone and return to its home cage.

****Check for ulceration daily at the injection sites.**** Ulcers can be treated by applying Sudocrem with a cotton bud. Check the severity limits are maintained during the procedure. If the ulcer looks raw, red, wet, is greater than 4mm in diameter and does not heal for a period of 48 hours despite Sudocrem application, the mouse will need to be culled.

AIA CHALLENGE (Day 7)

- 21 Prepare 80mg/ml mBSA for the challenge in a 1.5ml tub. Also prepare 0.9% sterile saline in a 1.5ml tube.
- 22 Transport to the animal unit on ice.
- 23 Turn on oxygen cylinder or oxygen concentrator and set the flow of oxygen is 1 litre/min.
- 24 Turn on heating blanket.
- 25 Place mouse into isoflurane induction chamber and set vaporiser to 4% until deep level of anaesthesia is achieved.



- 26 Remove mouse from box and, in a separate area, use a shaver to remove the fur from around the knee joint you intend to operate on.
- 27 Wipe skin around knee joint with 70% ethanol swab.
- 28 Place the mouse belly up and hold the leg at the ankle with the knee in a slightly bent position.
- 29 Run the side of the needle up and down the front of the leg until it rests in the 'groove' running from the lateral to medial meniscus. Press with the side of the needle to indent the skin slightly so that you can visualise the location of the ridge.
- 30 Using a 30g removable needle attached to a 10ul Hamilton syringe, insert the needle from the front (with the needle perpendicular to the front of the knee) about 2-2.5mm depth. You will know that the needle has entered the articular space as you will feel a slight 'give' as the needle passes into the space rather than hitting bone.
- 31 Inject 2.5µl of mBSA (200ug) or 0.9% sterile saline into the knee joint.

** Note that we have observed leakage when injecting volumes >5µl into the knee joint and so we inject 2.5µl to ensure that the solution stays within the joint capsule.**
- 32 Return mouse to its home cage.

Protocol references

Weiss M, Byrne AJ, Blazek K, Saliba DG, Pease JE, Perocheau D, Feldmann M, Udalova IA. IRF5 controls both acute and chronic inflammation. Proc Natl Acad Sci U S A. 2015 Sep 1;112(35):11001-6. doi: 10.1073/pnas.1506254112. Epub 2015 Aug 17. PMID: 26283380; PMCID: PMC4568217.