Video Article

Murine Skin Transplantation

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Abstract

As one of the most stringent and least technically challenging models, skin transplantation is a standard method to assay host T cell responses to MHC-disparate donor antigens. The aim of this video-article is to provide the viewer with a step-by-step visual demonstration of skin transplantation using the mouse model. The protocol is divided into 5 main components: 1) harvesting donor skin; 2) preparing recipient for transplant; 3) skin transplant; 4) bandage removal and monitoring graft rejection; 5) helpful hints. Once proficient, the procedure itself should take <10 min to perform.

Video Link

The video component of this article can be found at https://www.jove.com/video/634/

Protocol

Prior to the initiation of experiment:

- Obtain IACUC approval
- Sterilize surgical instruments and Q-tips

I. Harvest Donor Tissue

1. Euthanize donor mouse (in accordance with institution's SOP)
2. Wet hair back from base of ear with alcohol
3. Harvest donor skin by cutting ear off at base
4. Place tissue in plate on ice
5. Split ear: pinch ventral and dorsal sides of tissue at base of ear with rat-tooth forceps; gently pull apart tissue; float dorsal tissue, internal surface down, in PBS on ice; discard collagenous ventral flap

II. Prepare Recipient for Transplant

1. Anesthetize mouse using approved procedure
2. Administer analgesic for post-op pain relief (i.e. 0.05-0.1 mg/g Bupernex ip)
3. Place mouse in lateral position
4. Perform toe pinch to ensure animal is sufficiently sedated
5. Wet hair with alcohol and shave thorax
6. Wipe area clean with alcohol swab

III. Skin Transplant

1. Make ~ 1 cm incision: tent skin with rat-tooth forceps and cut ~ 0.25-0.5 cm below forceps
2. Remove donor skin from PBS, place atop graft bed (with internal surface of ear skin facing graft bed), and remove excess PBS with sterile Q-tip
3. Carefully trim away excess donor skin so that transplant lies flatly within graft bed (making sure that graft edges are not curled under and that graft does not hangover onto recipient's trunk skin)
4. Wrap recipient in sterile bandage (with the non-adhesive gauze segment placed over the skin graft)
5. Ensure that bandage is not too tight (assess animal's breathing) and that arms are freely mobile
6. Secure bandage with a single 2-0 silk suture
7. Place animal in clean cage (under heating lamp until animal has sufficiently recovered from anesthesia)
8. Monitor recipient 1-2/day for signs of distress and administer analgesic as needed for pain relief

IV. Bandage Removal (6-7 days post-transplant)

1. Anesthetize mouse using approved procedure
2. Cut and remove silk suture
3. Using blunt end scissors, carefully cut through bandage (making sure not to disrupt graft) and gently remove

4. Return mouse to clean cage
5. Check graft ~8 hrs after bandage removal for signs of scabbing or contraction. If present, graft may have failed to vascularize and should be considered a technical failure.
6. Monitor daily for signs of rejection (usually defined as ~80% necrosis of the donor tissue)
7. Allogeneic skin transplants will usually reject between d8-12 post-op

V. Helpful Hints

1. Harvesting donor tissue close to the base of the ear will: 1) maximize graft size, and 2) provide an uneven ventral overhang that will facilitate splitting
2. Always use a new razor blade for shaving recipient
3. If analgesic is to be given ip, administer prior to transplant procedure to minimize post-op handling (minimize chance of disrupting the graft)
4. If possible, remove gloves before applying the bandage. Gloves tend to stick the bandage and complicate application
5. Placing a single suture at the bandage end will minimize premature removal
6. Wrap bandage as tight as permitted and assess breathing BEFORE placing suture. If bandage is too tight, animal will stop breathing. If bandage is too loose, animal will remove it.

Discussion

As demonstrated in this video-article, the skin transplant model is a quick and easy method for monitoring allogeneic T cell responses. Allogeneic skin transplantation has a wide range of applications and has been utilized to assay the efficiency of a variety of immunosuppressive agents and tolerance induction protocols, as well as to investigate the biological effects of various blocking antibodies.

Disclosures

The authors have nothing to disclose.

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