Video Article

Imaging Effector Memory T cells in the Ear After Induction of Adoptive DTH

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Abstract

Delayed type hypersensitivity (DTH) is an immune reaction in which the main players are $CCR7^-$ effector / memory T lymphocytes. Here, we demonstrate a method for inducing and recording the progress of a DTH reaction in the rat ear. This is followed by a demonstration of the preparation of rat ear tissue for two-photon imaging of the $CCR7^-$ effector / memory T cell response.

An adoptive DTH is induced by the intraperitoneal injection of GFP-labeled Ova-specific $CCR7^-$ effector / memory T cell line (Beeton, C J. Visualized Experiments, Issue 8). Cells are then allowed to equilibrate in the rat for 48 hours before challenge by injecting one ear with saline (control ear) and the other with a 1:1 mix of Ova and Ova conjugated to Texas-Red (Ova-TR) to allow visualization of resident antigen-presenting cells.

We describe a method of tissue preparation useful for imaging the motility of cells within the deep dermal layer during an immune response, in conjunction with visualization of collagen fibers by second harmonic generation. Ear tissue is cut into 5 x 5 mm squares (slightly larger is better) and mounted onto plastic cover slips using Vetbond™, which are then secured using silicone grease in an imaging chamber and superfused by oxygen-bubbled tissue culture medium at 37°C.

Video Link

The video component of this article can be found at http://www.jove.com/video/907/

Protocol

Induction of Adoptive DTH

Please see JoVE article: Induction and Monitoring of Adoptive Delayed-Type Hypersensitivity in Rats

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Here, we have modified this protocol by using antigen (Ovalbumin) conjugated to Texas-Red in a 1:1 ratio with unlabeled antigen to allow for visualization of phagocytic antigen-presenting cells.

Ear Tissue Harvest

1. Euthanize the rat at the desired time-point by procedures outlined in your approved animal protocol. Here, we use an overdose of the anesthetic, isoflurane. This is done in a closed container located inside a well-ventilated hood. Ensure the animal is dead by either decapitation of by opening the thoracic cavity and cutting the heart.

2. Using large scissors remove the ear with a single cut, as close to the body of the animal as possible (Figure 1).

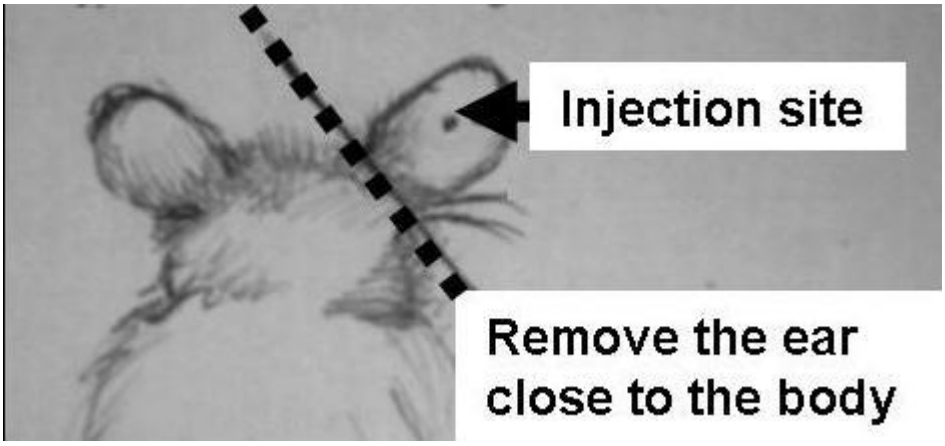

Figure 1. Removal of the rat ear

3. Place the ear in a 15 ml conical tube containing ~10 ml of ice-cold 1x PBS or RPMI-1640. Keep the tissue samples on ice until you are ready to prepare the tissue for imaging.

4. Image the tissue as soon as possible; we find, though, that 1 to 2 hours on ice has no discernable effect on cell motility in the tissue when compared to tissue harvested and imaged immediately.

Tissue Preparation for Imaging

I. Removal of Epidermal and Dermal Layer

***Note**: The deep dermal layer must be kept intact. If done correctly large blood vessels will remain intact and the cartilage of the ear will not be exposed. This is a difficult technique, especially when there is no inflammation (control conditions) and it is helpful to use a dissecting microscope.

1. Place both the tissue and media from the conical tube in a 10 cm tissue culture dish.

2. With gloved hands hold the rat ear gently at the tip of the ear (distal) with the dorsal side of the ear facing up (Figure 2).

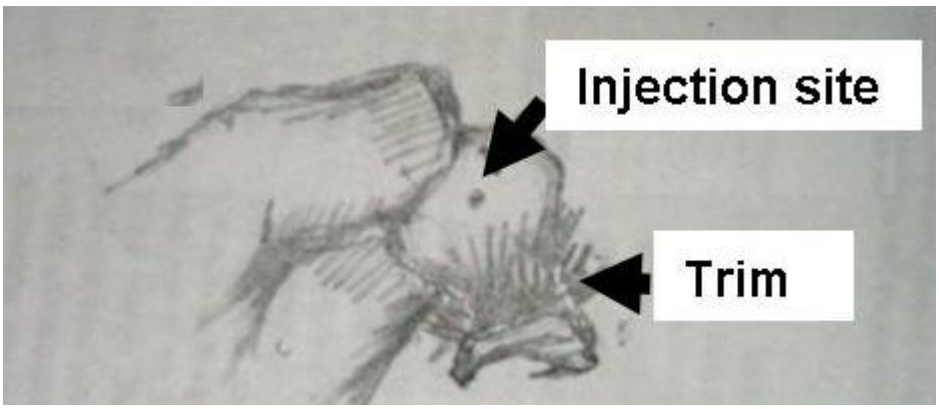

Figure 2. Trim fur off the ear

3. With your thumb on top of the ear, steady the tissue while using the tip of closed Dumont #5 scissors to separate the dermis from the deep dermis. Alternatively, if the edge of the ear has an overhang of skin/deep dermis, this can be grasped with forceps and gently pulled to separate this from the tissue underneath (Figure 3).

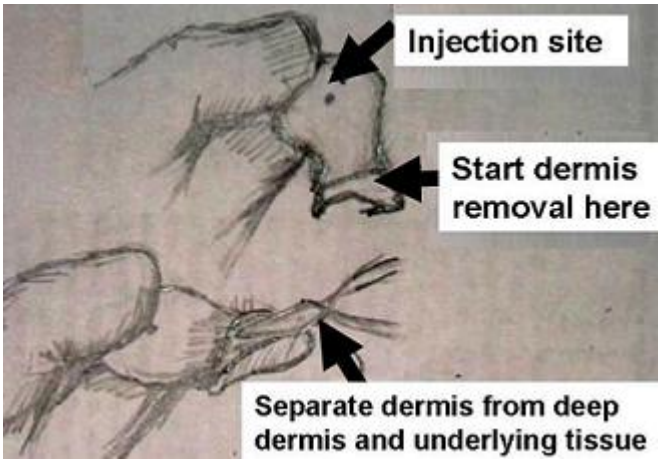

Figure 3. Removal of the upper dermis

4. Once a small area of skin has been separated from the underlying tissue, repeat the separation steps alternatively using scissors to cut between the dermal layers and forceps to gently remove and peel off the dislodged skin.

5. Expose a 5 x 5 mm or larger area of ear tissue that is at least 3 mm from the initial site of antigen injection (Figure 4).

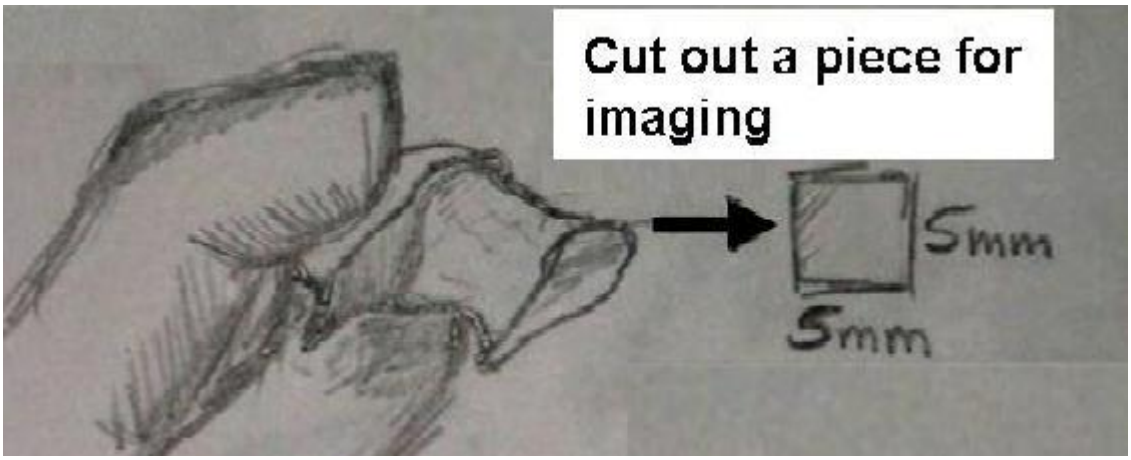

Figure 4. Tissue exposed for imaging

II. Securing Tissue to the Imaging Stage

1. Cut a plastic cover slip to a size slightly larger than prepared tissue.

2. Spread a thin layer of 3M Vetbond™ tissue-safe glue across the slide.

3. Grasp the corner of the prepared tissue (where you are not likely to image), remove from media, and dab the underside of the tissue on lens paper or Kym wipe to remove excess media.

4. Touch the edge of the tissue to the glue covered slide (the Vetbond cures immediately on wet tissue). Gently stretch the tissue flat, with the opposite end being the last to touch the slide (Figure 5)

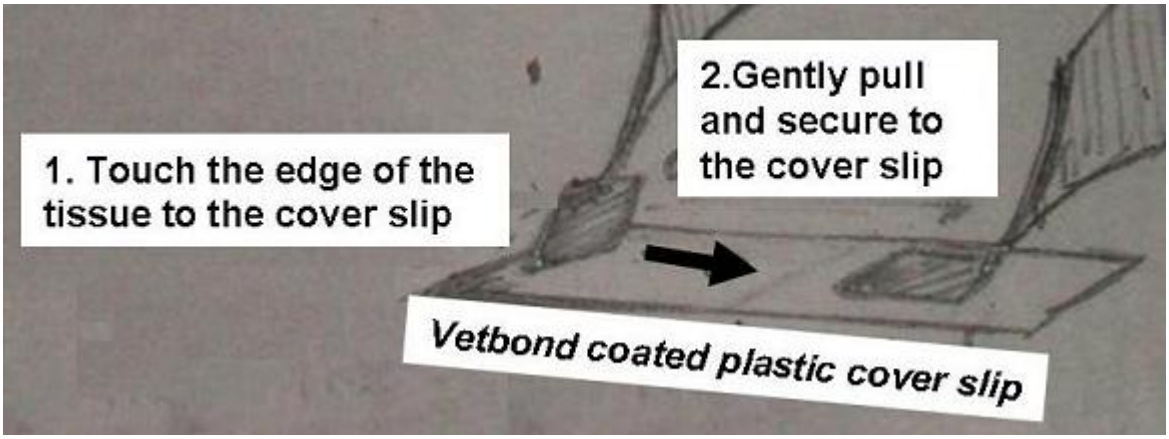

Figure 5. Mount tissue to cover slip

5. Cure the Vetbond™ by inverting the tissue sample/slide and dipping it in a reservoir of media such that the tissue and slide are face down and roughly at a 45 degree angle. Holding the tissue upside down at an angle helps to prevent excess glue from curing on the exposed tissue surface. Glue on the surface of the tissue will block the laser (Figure 6).

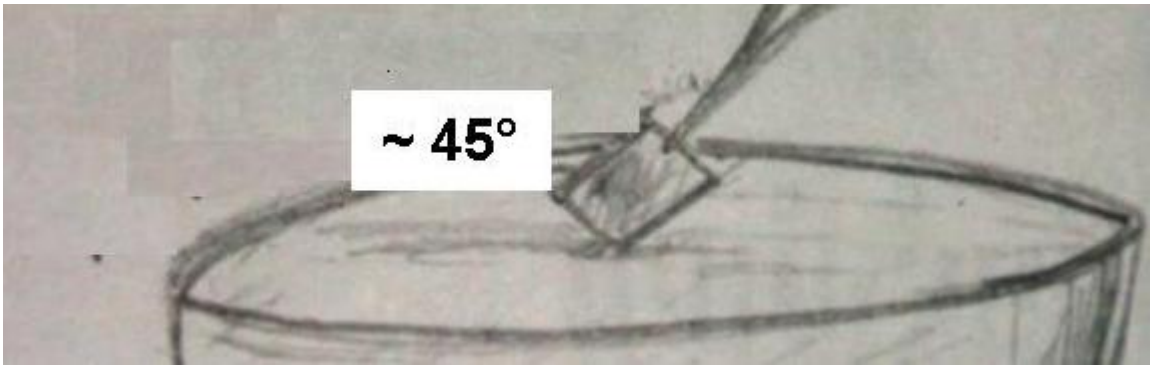

Figure 6. Cure vetbond tissue glue by dipping in media

Note: Steps 2-5 may take a little practice to master. Try practicing with discarded or extra tissue pieces before attempting for the first time.

6. Dab a small amount of silicone grease onto the bottom of the slide.

7. Place the slide in the perfusion chamber. Make sure that the perfusion media is flowing, 37°C and oxygenated before adding tissue. Gently press down on the edges of the slide making a seal with the silicon grease between the bottom of the perfusion chamber and the plastic cover slip.

III. Two-Photon Imaging

1. Using bright-field transmitted light, focus on the top of the tissue. Turn off all of lights.

2. Turn on the laser, PMTs as required by your multi-photon microscope system.

3. Second harmonic generation (SHG) in highly-ordered fibrous proteins (collagen and myosin) produces a signal at half the wavelength of the excitation laser. We use excitation at 900 nM, leading to SHG at 450 nm which can be visualized using the "blue" channel of the microscope. SHG generation is a non-linear (two-photon) process, and thus provides an optical "sectioning" effect similar to that of two-photon excitation of fluorescence. The peak efficiency of SHG occurs when the fibrils are perpendicular to the laser, while fibrils that run in parallel will not be visualized.[1]

4. Locate a good imaging area with plenty of cells, set your acquisition area such that the majority of cells are in the center and start imaging. Note: In DTH reactions, T cells and other cell infiltrates are often found to be concentrated together while other areas are devoid of infiltrating cells[2]. Therefore, it may take some time searching the tissue to find the best area to image.

5. Check for cell viability by assessing cell velocity and polarity during imaging. Use the lowest laser power that provides acceptable image quality to ensure minimal photo-damage.

6. Swap in a new tissue preparation as needed.

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