Video Article

In situ Imaging of the Mouse Thymus Using 2-Photon Microscopy

Ena Ladi, Paul Herzmark, Ellen Robey
Department of Molecular and Cell Biology, University of California, Berkeley

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Abstract

Two-photon Microscopy (TPM) enables us to image deep into the thymus and document the events that are important for thymocyte development. To follow the migration of individuals in a crowd of thymocytes , we generate neonatal chimeras where less than one percent of the thymocytes are derived from a donor that is transgenic for a ubiquitously express fluorescent protein. To generate these partial hematopoetic chimeras, neonatal recipients are injected with bone marrow between 3-7 days of age. After 4-6 weeks, the mouse is sacrificed and the thymus is carefully dissected and bissected preserving the architecture of the tissue that will be imaged. The thymus is glued onto a coverslip in preparation for ex vivo imaging by TPM. During imaging the thymus is kept in DMEM without phenol red that is perfused with 95% oxygen and 5% carbon dioxide and warmed to 37°C. Using this approach, we can study the events required for the generation of a diverse T cell repertoire.

Protocol

Adoptive Transfer

Inject neonates with 50-100ul of bone marrow using an insulin syringe 2-4 times during the first 3-7 days of life, with a final donor-to-host ratio of 1:1. Aim for an injection site below the sternum and rib cage, but above the gut and stomach. Insert the needle far enough to penetrate the peritoneum, careful not to puncture the diaphragm. It is not uncommon for some of the bone marrow to be lost during the injection. You can reduce the loss by loosening your grip on the pup as you inject so as to allow the skin to expand and, then, slowly removing the needle.

Dissection of Thymus

Make an incision through the midline and cut or tear the skin away to reveal the rib cage. Hold on to the sternum and cut through the peritoneum and diaphragm. Cut up the sides of the rib cage to reveal the thoracic cavity. The thymus lies on top of the heart. So as to preserve the structure of the thymus, hold on to surrounding tissue as you dissect out the thymus. Rinse the thymus with PBS to keep it from drying out during the dissection. Gently tease away excess tissue on the surface of the thymic capsule and bisect the two lobes.

Preparing Tissue for Imaging

Use veterinary tissue adhesive to glue the thymus no to a square coverslip. Use a pipetteman to put a fraction of a microliter of adhesive. Spread the glue to the approximate length and width of the thymus. Steady the thymus against the coverslip and drag it into place.

Two-Photon Imaging

The tissue is submerged in DMEM without phenol red that is perfused with 95% oxygen and 5% carbon dioxide and warmed to 37°C. Two-photon excitation was achieved using a Spectra-Physics MaiTai laser tuned to 900nm, and CFP, GFP, YFP and Texas Red emission light was separated using a 495, 515, 560 dichroic mirrors and collected using PMT detectors.

Discussion

Two-photon imaging in the thymus enables us to examine in living, intact organs the interactions and migrations that underlie selection events within the thymus.

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References

1. Kyewski. Seeding of thymic microenvironments defined by distinct thymocyte-stromal cell interactions is developmentally controlled. J Exp Med. 166(2), 520-38 (1987).

2. Ladi, E., Yin, X., Chtanova, T., and Robey, E.A. Thymic microenvironments for T cell differentiation and selection. Nat Immunol. 7(4):338-43 (2006).

3. Witt C.M., Raychaudhuri, S., Schaefer, B., Chakraborty A.K., and Robey, E.A. Directed Migration of Positively Selected Thymocytes Visualized in Real Time. PLoS Biol. 3(6):e160 (2005).

