Mouse Bone Marrow (BM) Isolation & Culture
Generation of eosinophils from bone marrow-derived cells
(Adapted from the Berdnikovs Lab)

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Part I. Isolation of bone marrow

Materials:

- Adult mouse (at least 8 weeks old)
- Dissection instruments (blunt-end scissors and forceps)
- 70% ethanol for sterilization
- Sterile hood
- RPMI-1640 medium (Gibco, cat# 72400-047)
- Petri dish
- 5-ml syringe
- 27G needle
- BM medium (Components all from Gibco: RPMI-1640, 20% FBS [cat. no. 26140-079], 1% penacillin-streptomycin [cat. no. 15140-122], 1% sodium pyruvate [cat. no. 11360-070], 1% non-essential amino acid solution [cat. no. 11140-050], 2.5% HEPES [cat. no. 25-060-CI], 1% L-glutamine [cat. no. 25030-081], and 0.1% β-mercaptoethanol [cat. no. 21985-023])
- 15-ml and 50-ml conical tubes
- 100-μm cell strainers
- Trypan blue (ThermoFisher cat# 15250061)
- Hemacytometer and light microscope
- T75 cell culture flasks

Experimental Protocol:

1. Spray dissection instruments with ethanol in the hood and fill a petri dish with about 10 ml of RPMI-1640 medium.
2. Euthanize mouse according to animal care protocol and spray heavily with ethanol prior to introducing to the sterile culture hood.

3. Cut into the top layer of skin above the peritoneal cavity and cut around the hip joint. Peel skin down from the body and over the feet and remove completely. (Cutting the ends of the feet at this point may be necessary.) Repeat for both legs.
4. Remove legs from torso without cutting or snapping the bones. To do so, turn mouse over and cut between the femur and pelvis, starting near the spine. Avoid cutting into the head of the femur. Dispose of mouse body.
5. Remove muscle and other connective tissue from the bones using scissors and forceps. The legs can be sprayed with ethanol which should make it easier to pull away some of this tissue. Separate the tibia from the femur by cutting carefully between them at the knee joint to sever the ligaments; avoid cutting into either bone.
6. Ethanol-spray the bones liberally and place into the RPMI-filled Petri dish. Prepare 5-ml syringe with 27G needle filled with RPMI from the dish.
7. **For the tibia:** Cut through the bone towards the foot where it narrows, then similarly close to the top. Hold the tibia with broad forceps to avoid crushing it and insert the needle towards the top. Flush the marrow out of the bone, repeating if necessary. **For the femur:** Repeat this procedure by cutting as close to the ends of the bone as possible.
8. Transfer bone marrow-containing RPMI to a 15-ml conical. Spin down at 300 x *g* for 5 min at 4°C.
9. Decant supernatant and break up bone marrow by flicking the tube or vortexing. Resuspend in 10 ml of BM medium.
10. Filter out pieces of bone and other tissue by passing the resuspended bone marrow through a 100-µm cell strainer into a 50-ml conical.
11. Count the cells using a hemocytometer and Trypan blue. (About 45-60×10^6 cells are expected from two legs)

Part II. Bone marrow culture and eosinophil differentiation

Materials:

- Mouse cytokines (Flt3L, SCF, and IL-5 [Peprotech; cat#'s 250-31L, 250-03, and 215-15, respectively])

- Fluorophore-labeled antibodies against Siglec-F (BD Pharmingen, cat. no. 552126) and CCR3 (BD Pharmingen, cat. no. 557974)
- A cell viability stain such as DAPI

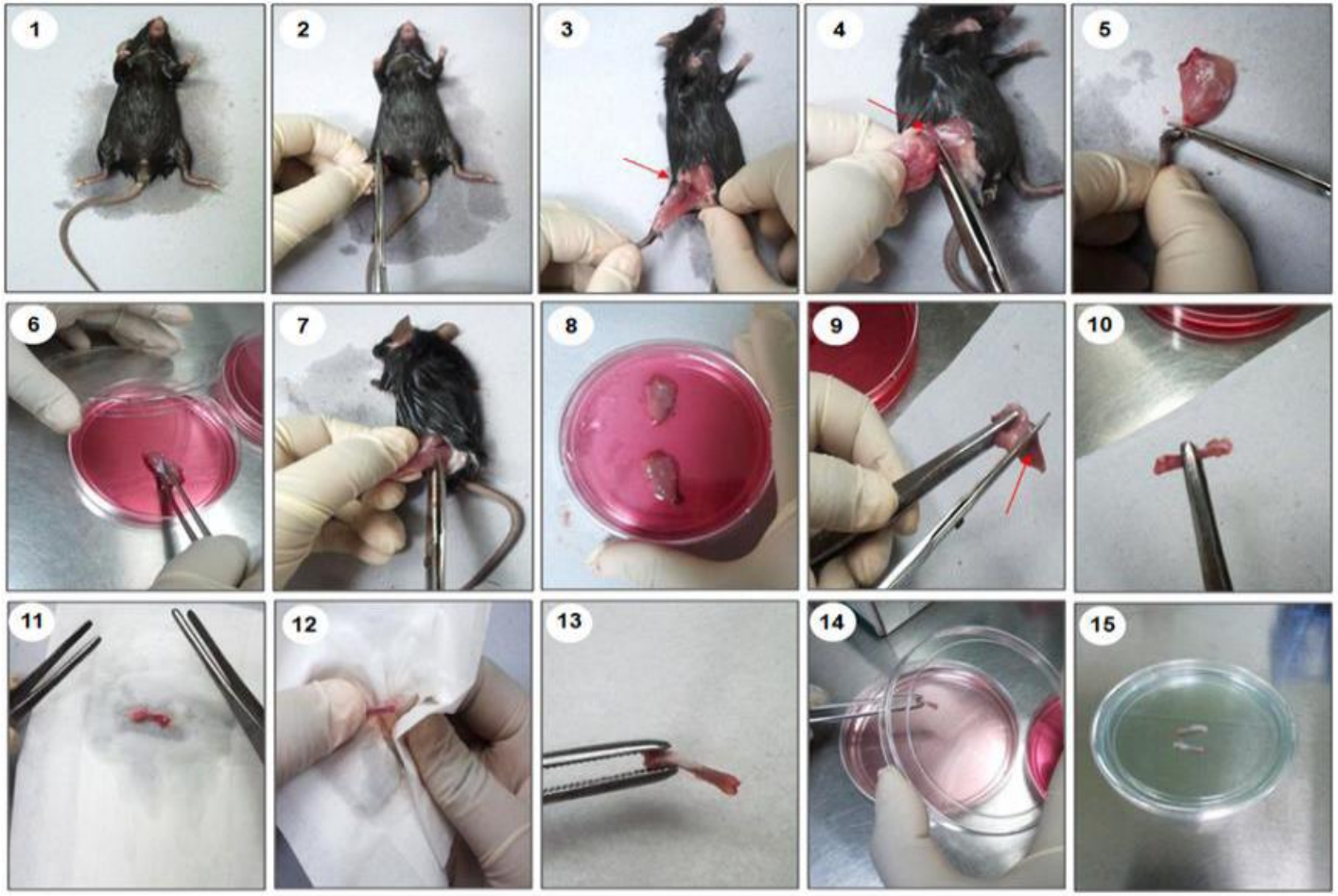
Experimental Protocol:

12. Add BM medium to a cell density of 1×10^6 cells/ml. Add recombinant mouse Flt3L (final concentration: 100 ng/ml) and SCF (final concentration: 100 ng/ml) and split equally between three T75 flasks. Incubate at 37°C in an atmosphere of 5% CO_2 for 4 days.
13. After 4 days, remove cells from flasks, spin down in 15-ml conicals (300 x *g* for 5 min at 4°C), remove supernatant, and resuspend in the same volume of BM medium containing 10 ng/ml mouse IL-5. ***Refresh medium in this manner every second day.***
14. Follow the differentiation of the cells by staining for Siglec-F and CCR3 and determine viability using DAPI or another cell viability stain. Analyze by flow cytometry. (The cell numbers will keep decreasing until about d10 and will then rebound to about 15×10^6 cells/flask.) Use in functional studies when the eosinophils have reached the appropriate level of maturity (around d12).

Generating eosinophils from murine bone marrow: why?

- The extremely short circulating half-life (~8-18h) and very low percent of eosinophils in the circulation makes it difficult to purify enough eosinophils for most studies.
- There are IL-5 transgenic mice (≈50% eos in blood and spleen) but these mice may not be ideal for all needs.
- A clever approach (Dyer & Rosenberg, JI 2008) is to generate eosinophils from bone marrow precursors. This allows one to generate as many mouse eosinophils as needed, including from bone marrow of strains deficient in various proteins such as signaling molecules.
- In Saturday's training session we will demonstrate how to isolate stem cell-like bone marrow precursors from mouse femurs.

Isolation of murine bone marrow



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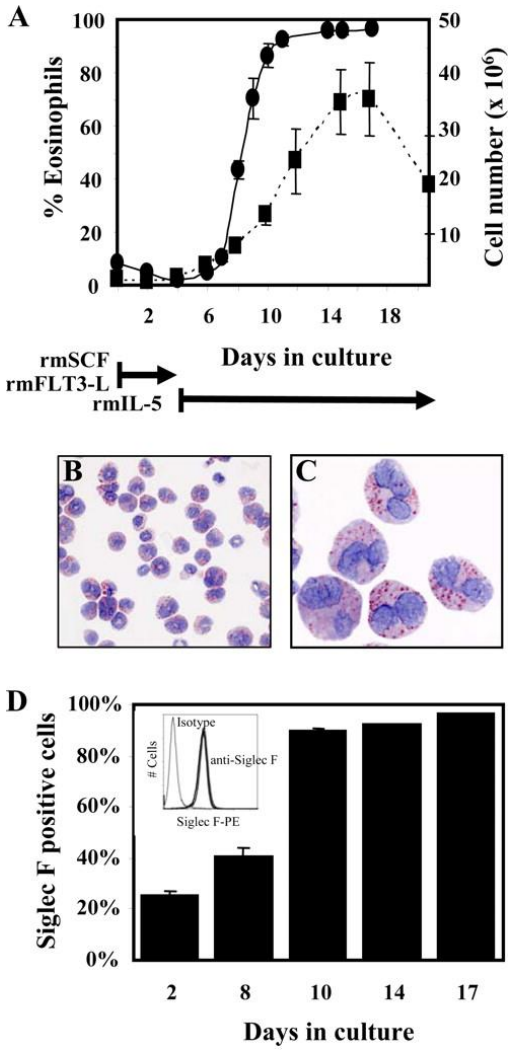
Ex vivo culture of mouse Bone Marrow Eos.

Two stages of mouse cytokines are used. First, Flt3L and SCF are added to expand immature precursors early in the culture, then the cells are switched into IL-5 to cause eosinophil maturation.



Kimberly D. Dyer et al. J Immunol 2008;181:4004-4009