Title: Isolation of Murine Macrophages: Peritoneal

Location: Old CCRC Tripp Lab

Approval Date: 10 September 2004

Supersedes Date: 

Materials:

- Lab coat
- Gloves
- Donor Mice
- 3% proteose peptone or 3% Brewer thioglycollate medium
- Fetal bovine serum (FBS) heat-inactivated
- Phosphate Buffered Saline (PBS)
- DMEM
- 37°C, 5% CO₂ incubator
- Diff-Quik solutions
- Centrifuge
- 25 gauge needles
- 6- and 30- cc syringes
- Forceps and surgical scissors
- 50mL tubes on ice
- Cytospin filter cards
- Pipettes
- Pipeteman
- Pipette Aid
- Pipetteman tips
- Avertin
- Hemacytometer

Overview:
Murine peritoneal macrophages suitable for use in protocols outlined elsewhere in this chapter are recruited and isolated as described below. Although the peritoneal cavity provides an accessible site for harvest of fair numbers of resident macrophages, in vivo manipulations prior to cell harvest can enhance macrophage yield. However, such inflammatory induction will also alter the physiologic characteristics of the cells collected. Generally, the normal mouse peritoneal cavity will yield mature, resident macrophages and the inflamed peritoneal cavity will yield immature, inflammatory macrophages recruited from the circulating and marginal pool.

Procedure:

Harvest peritoneal cells

1a. To collect resident peritoneal cells: Euthanize untreated mice by CO₂ asphyxiation. Alternatively, if CO₂ asphyxiation is not written into the animal use proposal (AUP) Follow method described in RTLP-CP1 (Collection of Bronchoalveolar Cells).

1b. To collect inflammatory macrophages: Fill 6-ml syringe with 1.0 ml of 3% proteose peptone. Attach 25-G needle and inject solution into peritoneal cavity of each mouse. Allow inflammatory response to proceed for 3 days and euthanize by decapitation or CO₂ asphyxiation. Alternatively, inject 1.0 ml of 3% Brewer thioglycollate medium into peritoneum 5 to 7 days prior to cell harvest (this will yield a larger number of inflammatory macrophages).
NOTE: Chronic, endemic infectious diseases, such as those caused by Sendai virus and mouse hepatitis virus, have a profound effect on macrophage physiology and will affect cell responsiveness and capacity for function.

2. Wet the abdomen of each mouse with 70% alcohol to sterilize the area.

3. Make a midline incision with sterile scissors. Retract abdominal skin with forceps to expose the intact peritoneal wall.

4. Attach 30-cc syringe to 19-G needle and fill with harvest medium. Push on syringe plunger to allow a small amount of medium to pass through the needle as the needle penetrates the peritoneum to avoid hitting the intestines. With bevelled end of needle facing up, insert needle through peritoneal wall at the midline. Inject 10 ml harvest medium into each mouse.

   *Inject and collect fluid from three mice using a single 30-cc syringe. Passing a small amount of medium through the needle serves to eliminate any air bubbles.*

5. Using the same syringe and needle, insert needle bevelled end down into peritoneum. Raise needle slightly to cause tenting of peritoneal wall. Withdraw peritoneal fluid slowly.

   *Expect fluid recovery of ~8 ml/mouse.*

6. Remove needle from syringe and dispense pooled peritoneal fluid to 50-ml polypropylene centrifuge tubes on ice.

   **Count cells and adjust cell concentration**

7. Remove 20-µl sample and count cells using a hemacytometer.

8. Transfer 0.2 ml peritoneal fluid to a cytocentrifuge container to prepare a cell smear for differential stain. Follow manufacturer's instructions for collecting cells using cytocentrifuge. Cytospin 6 min at 600 rpm. Remove slide and allow to air dry.


   *Follow manufacturer's instructions for Diff-Quik staining.*

10. Centrifuge peritoneal lavage fluid in 50-ml tubes 10 min at 400 x g (1000 rpm in H1000B), 4°C.

11. Discard supernatant and resuspend cell pellet by gently tapping bottom of tube. Adjust to appropriate cell concentration in harvest medium.
Expect 2-3 x 10^6 total peritoneal cells from an untreated mouse, containing ~50% to 70% macrophages. Proteose peptone-treated mice should yield 3-4 x 10^6 macrophages per mouse. Thioglycollate-treated mice will yield ~10^7 macrophages per mouse. Both inflammatory agents recruit young, immature macrophages into peritoneum. Thioglycollate-elicited cells may be better than proteose peptone-elicited cells for generation of an oxidative burst in response to PMA but not as responsive to cytokines for increased NO generation (Hoover and Nacy, 1984).

Cells are now ready for phenotypic analysis or functional assays.