Harvest and Culture of Mouse Peritoneal Macrophages

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[Abstract] Peritoneal macrophages are used as primary macrophages in lots of studies, mainly because they are easy to obtain. Injection of thioglycollate broth i.p. induces inflammatory responses and elicits large numbers of macrophages. This protocol can be used for harvesting resident or thioglycollate-elicited peritoneal cells. Peritoneal macrophages are non-adherent *in situ* and when they are cultured in dishes, they become adherent so that macrophages may be separated from other types of cells in peritoneal cavity.

Materials and Reagents

1. C57BL/6 mouse
2. 70% alcohol or isopropanol
3. PBS (Life Technologies, catalog number: 10010023)
4. EDTA (Life Technologies, catalog number: 15575-020)
5. RPMI 1640 (Cellgro®, catalog number: 10-041-CV)
6. 10% heat-inactivated FBS (endotoxin < 0.06 EU/ml) (Hyclone, catalog number: SH30071.03HI)
7. Penicillin-Streptomycin-Glutamine (Life Technologies, catalog number: 10378016)
8. Nonessential amino acids (Life Technologies, catalog number: 11140050)
9. Sodium pyruvate (Sigma-Aldrich, catalog number: S8636)
10. Hepes (suitable for cell culture) (Sigma-Aldrich, catalog number: H0887)
11. 2-mercaptoethanol (EMD Millipore, catalog number: ES-007-E)
12. Thioglycollate medium (BD Biosciences, catalog number: 211716)
13. Pyrogen-free water (Hyclone, catalog number: SH30529.03)
14. cRPMI medium (see Recipes)

Equipment

1. 5 ml syringe
2. 12-well plate
3. 15 ml sterile Falcon tubes

4. Needles (20 G and 25 G)
5. Centrifuge
6. 37 °C, 5% CO_2 cell culture incubator
7. 300 ml flask with an aluminum foil lid
8. Laboratory oven

Procedure

A. Harvest and culture of resident peritoneal cells
   1. Fill 5 ml of PBS with 5 mM EDTA in a 5 ml syringe with a 25 G needle.
   2. Anesthetize and sacrifice mouse with CO_2.
   3. Place mouse with abdomen up on paper towel in hood.
   4. Swab abdomen with 70% alcohol or isopropanol.
   5. Make a small incision in the center of the skin overlying the peritoneal wall.
      Note: Small incision is made on the skin to peel the skin off. Injection and extraction can be at different sites on peritoneal membrane.
   6. Firmly pull skin to expose the peritoneal wall.
   7. Insert the needle to peritoneal membrane; avoid inserting needle into guts or bladder. Inject 5 ml of PBS EDTA into peritoneal cavity.
   8. Massage abdomen for approximately 10-15 seconds.
   9. Withdraw the needle slowly. Change the 25 G needle on the syringe to a 20 G needle.
   10. Use one hand to push the fluid to one side of the peritoneum. Using the other hand, insert needle to the side of cavity with plenty of fluid and withdraw the fluid from peritoneum. Avoid fat, gut or mesentery, which may clog the needle. Try to draw as much fluid as possible. Usually, approximately 4 - 4.5 ml fluid can be recovered from one mouse.
   11. Remove needle from syringe and dispense contents into a centrifuge tube on ice.
   12. Centrifuge peritoneal cells (300 *x g*; 3 min) and collect cell pellet. Usually about 2-4 million resident peritoneal cells can be recovered from one C57BL/6 mouse using this method and about 50% are peritoneal macrophages.
   13. Resuspend cell pellet from one mouse in 1 ml of cRPMI medium, count cells.
   14. Culture peritoneal cells in a 12-well plate, 2 million/well in 1 ml cRPMI at 37 °C with 5% CO_2 for 6-18 h. During this time, peritoneal macrophages adhere to the plastic surface. The floating non-macrophages can then be washed away by adding and aspirating 0.5 ml cRPMI medium twice.
   15. The adherent macrophages are ready to use.

B. Harvest and culture thioglycollate-elicited peritoneal cells

Thioglycollate elicited peritoneal macrophages can be harvested and cultured in the same way as described in steps 1-15 with additional steps as shown below.

16. Preparation of 3% thioglycollate medium.
    a. Heat a 300 ml flask with an aluminum foil lid (180-200 °C) in a laboratory oven for at least 18 h to get rid of endotoxin.
    b. Suspend 6 grams of thioglycollate medium in 200 ml of pyrogen-free water.
    c. Autoclave (15 psi/121 °C/15 min).
    d. After cooling, aliquot to 15 ml sterile Falcon tubes. Store in a dark place at room temperature for 2 months before using. We have found that thioglycollate medium stored at room temperature for up to 2 years can still be used.
17. Inject 1 ml of aged thioglycollate i.p. per mouse. Wait for 4-5 days, harvest peritoneal cells. About 10 million macrophages can be recovered from one mouse.
    Note: The study was performed under an IACUC approved protocol (LCID 11E).

Recipes

1. cRPMI medium
   RPMI 1640 with 10% heat-inactivated FBS (endotoxin < 0.06 EU/ml)
   2 mM L-glutamine
   100 μM of nonessential amino acids
   100 U/ml penicillin
   0.1 mg/ml streptomycin
   10 μM of sodium pyruvate
   25 mM Hepes, pH 7.4
   50 μM 2-mercaptoethanol

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References

1. Lu, M., Varley, A. W. and Munford, R. S. (2013). Persistently active microbial molecules prolong innate immune tolerance *in vivo*. *PLoS Pathog* 9(5): e1003339.

