Thioglycollate Induced Peritonitis

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[Abstract] Intraperitoneal (i.p.) injection of thioglycollate elicits a robust influx of neutrophils into the peritoneal cavity. The trafficking of cells is believed to be mediated by chemokines CXCL1, CXCL2, and CXCL8 (Call *et al*., 2001; Cacalano *et al*., 1994). Thus this model can be used to test the ability of neutrophils to migrate towards these chemokines in bioengineered mouse strains (*e.g.* knockout or transgenic mice) or the ability of certain molecules to inhibit the chemoattractive activities of these chemokines (*e.g.* small molecules or inhibitory antibodies). This protocol has been used by the author successfully to test the functions of a viral multi-chemokine inhibitor.

Materials and Reagents

A. Antibodies
   1. Rat anti-mouse Gr-1 PE (BD Biosciences, catalog number: 553129)
   2. Rat anti-mouse CD11b FITC (Southern Biotech, catalog number: 1560-02)

B. Other materials
   3. Mice
   4. PBS
   5. 4% sterile thioglycollate (Sigma-Aldrich, catalog number: 70157) in ddH_2O

      Note: Thioglycollate solution needs to be wrapped with aluminum foil to avoid light and be placed at room temperature to age for several weeks until it turns to brown in color. The aging process is critical to the ability of thioglycollate to induce peritonitis.

Equipment

1. 6G1/2 needle
2. 18G1/2 needle connected with a 10 ml syringe
3. BD LSR II flow cytometer

Procedure

1. Inject intraperitoneally mice with 1 ml of 4% sterile thioglycollate.
2. Two hour later, anesthetize the mice.
   The influx of neutrophils is at peak around this time point. Users need to wait for 48 h before they anesthetize the mice, should they wish to observe monocyte influx.
3. Cut a small opening at the lower abdomen to expose the underneath muscle.
   Note: Do not compromise the integrity of peritoneal cavity.
4. Slowly inject 10 ml ice cold PBS into peritoneal cavity using a 26G1/2 needle.
   Note: Some protocols suggest using PBS containing low concentrations of EDTA to achieve maximal yield of peritoneal cells.
5. Remove the needle.
6. Hold the mouse by tail and swish around for 3 min to wash peritoneal cavity extensively.
7. Lay the mouse by the side and insert an 18G1/2 needle connected with a 10 ml syringe.
8. Retrieve maximal amount of PBS by slowly pulling out the plunge.
9. Record the volume of PBS retrieved.
10. Spin down the cells at 1,200 rpm at 4 °C for 5 min.
11. Discard supernatant and resuspend cells with 100 µl of 3% FBS containing 1: 200 anti-GR-1 PE and anti-CD11b FITC.
12. Incubate at room temperature for 15 min.
13. Wash with 1 ml PBS and spin down at 1,200 RMP at 4 °C for 5 min.
14. Discard supernatant and resuspend cells with 200 µl PBS.
15. Acquire the entire 200 µl of cells on flow cytometer.
16. Calculate the numbers of Gr-1^{high}CD$11b^{high}$ cells and adjust the numbers to the volume of retrieved PBS.
    I.e. if the volume of retrieved PBS is 9 ml, then the total number of neutrophils per peritoneal cavity = (calculated number of Gr-1^{high}CD$11b^{high}$ cells x 10 ml)/9 ml

Notes

Some protocols prefer to decide cell numbers simple by counting cells using hemocytometer. The numbers obtained by such method may not accurately reflect the number of neutrophils because there are other types of cells such as B1 B cells and macrophages in peritoneal cavity.

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References

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