Chitin-challenged Mice Model to Study M2 Macrophages Polarization

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[Abstract] Chitin is a key component of insects, fungi, and house-dust mites. Chitin has been shown to induce M2-type immune responses *in vivo*. Intranasal or intraperitoneal (i.p.) administration of chitin particles results in infiltration of eosinophils to the local sites and activation of macrophages with a M2 phenotype. Chitin-challenged mice model can be used to induce M2 macrophages polarization and thus to analyze the M2 phenotype from isolated peritoneal cells.

Materials and Reagents

1. Mice C57BL/6 male (age 8-12 weeks)
2. Chitin (Sigma-Aldrich, catalog number: C9752)
3. 70% ethanol
4. PBS (Lonza, catalog number: BE 17-515Q)
5. 70 µm sterile cell strainers (BD Biosciences, catalog number: 352350)
6. RPMI 1640 (Lonza, catalog number: BE 12-115F)
7. 10 ml syringe
8. 21 G Needles
9. 25 G Needles
10. 15 ml conical tubes

Equipment

1. Sonication bath, P-selecta ultrasons (150 W)
2. Scissors
3. Bench-top refrigerated centrifuge

Procedure

A. Chitin administration
   1. Preparation of chitin
      a. Weight 800 ng of chitin per mouse and wash 3 times with PBS (5 min, 13,000 rpm, 16,800 *x g*).

b. Suspend chitin in 1 ml of PBS and sonicate in bath for 1 h [at the maximum setting of the *sonicator* (Ultrasons, Selecta)].
Note: Alternatively chitin can be sonicated at 25% output power three times for 5 min with a Branson sonicator.
c. Filter the suspension with 70 µm sterile cell strainers.

2. Inject 1 ml of chitin suspension i.p. per mouse using a 10 ml syringe with a 25 G needle. Wait for 2 days and harvest peritoneal cells.

B. Isolation of chitin elicited peritoneal exudate cells (PECs)

1. Sacrifice mouse with CO_2 or isofluorane (the use of cervical dislocation is not indicated in this protocol in order to avoid potential internal bleeding).
2. Clean abdomen with 70% ethanol.
3. Remove skin to expose the peritoneal wall. Practice a small incision in the skin and pull firmly.
4. Inject 10 ml of RPMI 1640 into the peritoneal cavity with a 25 G needle.
5. Massage abdomen for approximately 30 sec.
6. Recover peritoneal fluid as much as possible using a 10 ml syringe with a 21G needle. (Usually approximately 8-10 ml fluid can be recovered from one mouse.)
7. Remove needle from syringe and put fluid into a 15 ml conical centrifuge tube on ice.
8. Centrifuge peritoneal cells 5 min at 300 *x g* (1,500 rpm, 4 °C). Discard the supernatant and collect the pellet.
9. Resuspend cell pellet in 10 ml of RPMI 1640 medium and count cells. (The cell number expected to be recovered is about 5-8 millions.)
10. Analyze peritoneal cells to confirm M2 activation by performing either real-time PCR or flow cytometry techniques to detect typical M2 markers (*e.g.* Arginase-1, Ym-1, Fizz-1, mannose receptor).

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References

1. Byles, V., Covarrubias, A. J., Ben-Sahra, I., Lamming, D. W., Sabatini, D. M., Manning, B. D. and Horng, T. (2013). The TSC-mTOR pathway regulates macrophage polarization. *Nat Commun* 4: 2834.

2. Jiménez-García, L., Herránz, S., Luque, A. and Hortelano, S. (2015). Critical role of p38 MAPK in IL-4-induced alternative activation of peritoneal macrophages. *Eur J Immunol* 45(1): 273-286.
3. Satoh, T., Takeuchi, O., Vandenbon, A., Yasuda, K., Tanaka, Y., Kumagai, Y., Miyake, T., Matsushita, K., Okazaki, T., Saitoh, T., Honma, K., Matsuyama, T., Yui, K., Tsujimura, T., Standley, D. M., Nakanishi, K., Nakai, K. and Akira, S. (2010). The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* 11(10): 936-944.

