Escherichia coli Outer Membrane Vesicle Immunization Protocol and Induction of Bacterial Sepsis

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[Abstract] Outer membrane vesicles (OMVs) are spherical bilayered phospholipids of 20-200 nm in size produced from all Gram-negative bacteria and Gram-positive bacteria investigated to date. OMVs, which resemble the outer membrane and periplasm in composition, are proinflammatory and immunogenic facsimiles, and therefore could activate both innate and adaptive immunity. Here, we describe the OMVs immunization protocol and bacteria challenge protocol to induce bacterial sepsis in mice.

Materials and Reagents

1. Five-week-old male C57BL/6 or BALB/c mice or a variety of transgenic/knockout mice, body weight 18-20 g
2. Phosphate buffered saline (PBS) (Gibco®, catalog number: 70013-032)
3. *Escherichia coli* (*E. coli*) derived OMVs
4. *E. coli* (Isolated from the peritoneal lavage fluid of cecal ligation and puncture-operated mice)
5. Luria-Bertani broth (LB) medium (Merck KGaA, catalog number: 1.10285.0500)

Equipment

1. 1.5 ml microtube
2. Ultra-fine-II insulin syringe 1 ml 31 G (0.25 mm x 8 mm) (BD, catalog number: 328820)

Procedure

A. Vaccine preparation
   1. Prepare a suspension containing 10 μg/ml of *E.coli* OMVs in PBS.
      Notes:

a. *The total amount of the sample depends on the number of mice in the immunized group. For each mouse, 100 µl of sample will be given. Add extra 100 µl to compensate for any loss of sample during each injection. i.e. If N = 5 per group, make a total of 600 µl for each group.*

b. *Refer to the protocol "Preparation of Outer Membrane Vesicle from Escherichia coli" (Kim et al., 2013b) for preparing OMVs.*

2. Make three aliquots of the above OMVs sample in each 1.5 ml microtube and store at -80 °C until use.

B. Immunization

1. At day 0, label the cage (or mouse) to distinguish between immunized and sham.
2. Bring one of the OMVs sample aliquot to room temperature and mix by vortexing before use.
3. Hold the mouse in your hand by the dorsal skin so that the head of the mice is pointing the top and its rear legs are down. Maintain the tail with the fingers as in Figure 1 below.

Figure 1. Intraperitoneal injection

4. Use sterile 1 ml syringe to intraperitoneally inject 100 µl of the OMVs suspension per mouse of OMVs-immunized group.
5. Use new sterile 1 ml syringe to intraperitoneally inject 100 µl of PBS per mouse of sham-immunized group.
6. Repeat the steps B2-5 at day 7 and 14.

C. Bacteria challenge for induction of sepsis

1. At day 21, prepare *E. coli* (5 x 10^9 CFU/ml) in PBS after culturing *E. coli* in LB broth for 200 rpm at 37 °C until OD_{600}=1.0.

   Note: The total amount of the sample depends on the number of mice that will be challenged. For each mouse, 200 µl of the sample will be given. Add extra 100 µl to

compensate for any loss of sample during each injection. i.e. For a total of 10 mice, make 2,100 µl of the sample.

2. Hold the mouse in your hand by the dorsal skin so that the head of the mice is pointing the top and its rear legs are down. Maintain the tail with the fingers.
3. Use sterile 1 ml syringe to intraperitoneally inject 200 µl of the *E. coli* suspension per mouse.
4. Monitor the survival of mice every 12 h and record.
   Note: The symptoms for sepsis will appear after about 6-8 h of challenge. Sacrifice the mice after 5 days of monitoring. Please refer to the reference article for survival results.

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