

Allergen Sensitization and Challenge to Ovalbumin

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[Abstract] This protocol describes the sensitization and challenge of mice with ovalbumin for use as an acute murine model of asthma. This protocol induces reproducible airway inflammation and remodelling, and bronchial hyperresponsiveness to methacholine as measured by barometric plethysmography, as well as by the Flexivent® technique in Balb/c mice.

Materials and Reagents

1. Sterile saline (0.9% NaCl) (B. Braun)
2. Chicken egg albumin (ovalbumin, grade V) (Sigma-Aldrich, catalog number: A-5503)
3. Aluminium hydroxide (Sigma-Aldrich, catalog number: 23918-6)
4. Ketamine (Imalgene®, Merial)
5. Xylazine (2%) (Rompun®, Bayer)
6. Ovalbumin (see Recipes)
7. Anaesthetics (see Recipes)

Equipment

1. "1 ml" sterile syringes (Terumo, catalog number: SS+01T1)
2. "25G" 0.5 mm sterile needles (Terumo, catalog number: NN-2516R)
3. Sterile tips (Starlab, catalog number: S1111-0800)
4. Precision pipette (20 µl)
5. End-over-end rotator (SB2, Stuart)
6. 15-ml Falcon tube

Procedure

Summary:

- Nine week-old male Balb/c mice are sensitized on days 0 and 7 by intraperitoneal

- (i.p.) injections of 50 μ g ovalbumin adsorbed with 2 mg aluminium hydroxide (alum) in saline.
- Mice are challenged on days 18, 19, 20 and 21 by intranasal (i.n.) instillations of 10 μ g ovalbumin in saline; control animals receive i.n. instillations of saline alone; i.n. administrations must be carried out under anesthesia (ketamine and xylazine diluted in sterile saline).
 - Assessments are performed 18-24 h after the last i.n. challenge.
1. Allergen sensitization
 - a. Freshly prepare a suspension containing 0.5 mg/ml of ovalbumin and 20 mg/ml of alum in sterile saline (0.9% NaCl): Weigh 80 mg alum in a 5 ml tube, add 1 ml from a 2 mg/ml ovalbumin aliquot, and 3 ml of sterile saline (0.9% NaCl).
 - b. Gently homogenize the suspension in a rotator for 4 h at 4 °C so that ovalbumin may adsorb on alum. The mixture appears as a white suspension, susceptible to rapidly settle down to the bottom of the tube. It should be homogenized by rapid reversals (3-4) of the tube/syringe just before use.
 - c. Before use, bring suspension to room temperature (18-25 °C) on the rotator.
 - d. Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers.
 - e. Use "1 ml" syringes and "25G" needles to inject i.p. 100 μ l suspension per mouse. *Note: Gently homogenize the suspension by 3-4 repeated reversals of the tube/syringe between each use so that the suspension does not drop at the bottom of the tube/syringe).*
 2. Allergen challenge
 - a. Before use, bring solutions (anaesthetics, 0.4 mg/ml ovalbumin aliquot (1 aliquot per 24 mice), and saline) to room temperature (18-25 °C). Vortex for 4 sec at the highest speed to mix.
 - b. Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers.
 - c. Use "1 ml" syringes and "25G" needles to inject i.p. 100 μ l per mouse (25 μ g) of the anesthetic solution (at room temperature).
 - d. Wait until the mouse is anesthetized; check that vibrissae do not move any more.
 - e. Hold the mouse in your hand so that its head is up and its rear legs are down. Administer 12.5 μ l of the 0.4 mg/ml ovalbumin solution in each nostril for sensitized mice, and saline alone for control mice, by use of sterile tips (*note: Solution must be administered drop by drop, slowly and very carefully*).
 - f. Keep the mouse in your hand in a vertical position at least for 1 min, and check that the mouse breathes normally.

Note: If the mouse does not breathe normally, perform a thorax massage by pressing the rib cage several times, quickly but carefully.

- g. Place the mouse in decubitus on a heated blanket until complete recovery.

Recipes

1. Ovalbumin

Prepare a sterile 2 mg/ml solution of ovalbumin as follows: Weigh 80 mg ovalbumin and dissolve it in 40 ml cold sterile saline in a 50-ml Falcon tube. Vortex for 5 min at 2,000 rpm to mix and distribute 30 ml of the solution as 1.1 ml aliquots into 1.5 ml microtubes; this will result in ovalbumin aliquots for 24 mice. Immediately store frozen at -20 °C for up to 2 months.

Take the remaining 10 ml of the 2 mg/ml ovalbumin solution, dilute it 1/5 in sterile saline resulting in a 0.4 mg/ml solution, and distribute as 1-ml aliquots into microtubes (1 aliquot per 24 mice). Immediately store aliquots frozen at -20 °C for up to 2 months.

2. Anaesthetics

In a 15-ml Falcon tube, add 1.5 ml of a 100 g/l commercial solution of ketamine (Imalgene® 1000), 0.5 ml of a 20 g/l commercial solution of xylazine (Rompun® 2%) and 10 ml of sterile saline (0.9% NaCl). The prepared solution contains 12.5 mg/ml ketamine base and 0.83 mg/ml xylazine base from hydrochloride. Inject 100 µl of the anaesthetic solution per mouse (25 g), *i.e.* a 4 ml/kg.

The administrated dose is 50 mg/kg ketamine and 3.3 mg/kg xylazine. This preparation may be stored at 4 °C for 10 days.

Notes

Other protocols based on this one may be implemented by modification of either the mouse strain, the dose or sequence of administration of ovalbumin, or the allergen (house dust mite or cockroach extracts for instance), with a sensitization step followed by challenges to the allergen. However, using standardized procedures for allergen sensitization and challenge would allow better reproducibility and comparison between results in the literature when studying airway inflammation and remodelling, and generation of bronchial hyper responsiveness.

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References

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