



OVA-Induced Allergic Airway Inflammation Mouse Model

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Abstract

Asthma is a worldwide public health issue, affecting the sufferer's quality of life. Many researchers are extensively studying the cellular processes involved in the affected airways. Experimental asthma using animals has been performed for a long time, mainly applying murine models due to well-known advantages. The aim of this study is to present an allergic airway inflammation protocol in mice. Basically, the allergic airway inflammation is induced by intraperitoneal sensitization and intratracheal challenge with ovalbumin (OVA). The model provided here mimics acute asthma characteristics including excessive mucus production, airway hyperresponsiveness, and eosinophilic airway inflammation.

Key words Allergy, Asthma, Ovalbumin, Airway inflammation

1 Introduction

Asthma is a chronic inflammatory airway disease. Its pathophysiology is complex and many aspects are not well understood. The disease affects around 300 million people worldwide and is likely to rise to 400 million by 2025. Morbidity and mortality are high as asthma represents one in accounts for 1 out of 250 deaths worldwide [1]. Thus, it is necessary to advance the knowledge in this field in order to discover new treatment approaches.

For many reasons, clinical studies are not enough to understand the biological mechanism of asthma and animal models successfully reproduced many of allergic airway inflammation characteristics described below. Experimental asthma was performed for the first time in 1937 using Guinea pigs [2]. In the 1990s, the first murine models were introduced due to some advantages compared to other animals such as easy breeding,

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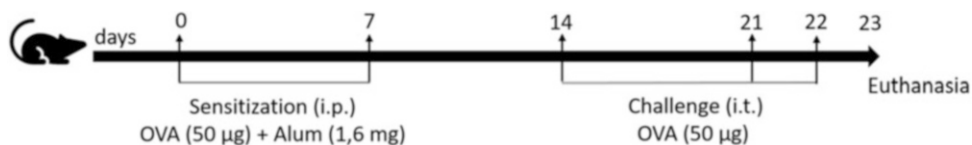


Fig. 1 The experimental design of OVA-induced allergic airway inflammation mouse model

availability of specific molecular and cellular reagents, and wide mouse strain variations [3].

Typically, the allergic airway inflammation is induced by sensitizing and challenging animals to a foreign antigen. Distinct adaptive immune behavior in airway inflammation are described in mice, some of these patterns are categorized as T helper type 1 (Th1), type 2 (Th2), and type 17 (Th17) [4]. Evidence in the literature has shown that cytokines produced by Th2 cells lead to allergic asthma phenotype [5]. Furthermore, allergic airway inflammation is characterized by an influx of lymphocytes, monocytes, and mainly eosinophils into the lung, excesses mucus production, airway hyperresponsiveness, and elevated IgE levels in serum [6, 7].

Clinically relevant antigens and allergens in humans such as extracts prepared from house dust mite (HDM), birch pollen or *Aspergillus* have already been used in mouse models of allergic asthma. However, the majority of studies were performed by employing chicken egg ovalbumin (OVA). Substantial variations of the method consist in the use of different allergens and adjuvants even different entry routes for antigen delivery [8]. The method for murine allergic airway inflammation (Fig. 1) described here utilizes the antigen OVA and the adjuvant aluminum hydroxide (alum) which are delivered intraperitoneally (i.p.) during the sensitization phase. The challenge phase is subsequently performed by intratracheal (i.t.) delivery.

2 Materials

2.1 Equipment

1. Analytical balance.
2. Platform shaker.
3. Micropipette.
4. Vortex mixer.
5. Vertical grid support.
6. Rubber band.
7. Tweezers with rubber coated tips.
8. 1 mL syringe with needle.

2.2 Reagents

1. Ovalbumin (Sigma-Aldrich, St.Louis, MO).
2. Inject Alum Adjuvant (aluminium hydroxide; Thermo Scientific; Rockford, IL, USA).
3. Sterile phosphate buffered saline (PBS).
4. Ketamine (0.1 g/mL) and xylazine (0.02 g/mL) anesthesia solution in sterile PBS.

3 Methods (See Note 1)

3.1 Allergen Sensitization

1. Prepare a fresh solution containing 1 mg/mL of OVA in sterile PBS [the volume necessary for each animal is 50 μ L (50 μ g)] (*see Note 2*).
2. Use 1.6 mg Alum Adjuvant (40 μ L per animal - *see Note 3*)
3. Mix these two solutions.
4. Add sterilized PBS to make a final volume of 200 μ L per animal.
5. Maintain the OVA plus Alum solution shaking for 20 min (*see Note 4*).
6. Inject 200 μ L i.p. on day 0 and again on day 7.

3.2 Anesthesia and Intratracheal Delivery

1. Prepare a solution containing 1 mg/mL OVA in sterile PBS.
2. Anesthetize mice i.p. using 100 μ L/kg anesthesia solution.
3. Prepare a pipette with 50 μ L (50 μ g) of OVA solution.
4. Place the animal at a 90° angle (*see Note 5*).
5. Hook the front incisor teeth with a rubber band in the vertical grid support, to keep the trachea straight (Fig. 2).
6. Using the tweezers with rubber coated tips, gently grasp the animal's tongue and move it away to expose the trachea region (Fig. 2).
7. Using the other hand, insert the tip as parallel as possible to the trachea and inject 50 μ L OVA solution (Fig. 2).
8. Hold the mouse in the same position after administration for about 1 min (*see Note 6*).
9. Observe the gasp. The gasp and the fast breathing are signs that the procedure worked.
10. The inflammation peak is 24 h after last challenge and can be checked by bronchoalveolar lavage (BAL) (Fig. 3 [9]).
11. Also, airway hyperresponsiveness, mucus production and serum IgE level can be measured [9].

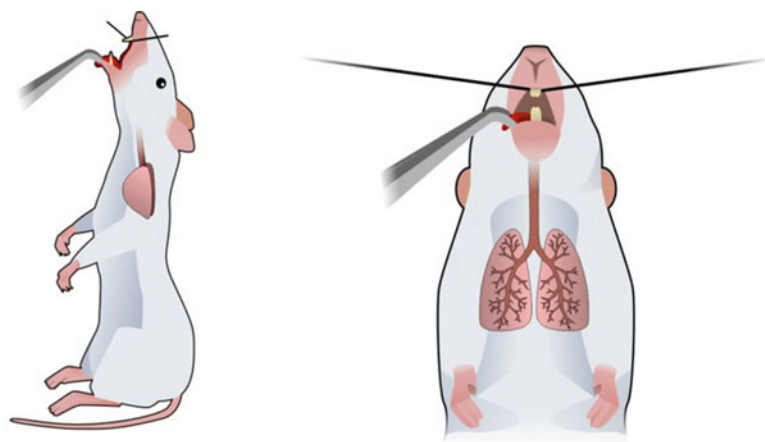


Fig. 2 Animal position during the challenge. Mice should be placed angled at 90° in order to facilitate OVA solution exposition. The tongue needs to be moved out of the way to allow the access to the trachea. For the OVA solution administration procedure. The tip should be placed as parallel as possible to the trachea

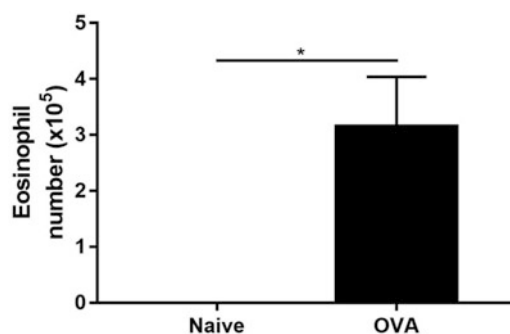


Fig. 3 Eosinophil number in mice bronchoalveolar lavage (BAL) OVA-induced allergic airway inflammation (see **Note 1**)

4 Notes

1. Ensure that all procedures are approved by the appropriate institution. In this study, the local Animal Care Committee of the Federal University of Sao Paulo approved the experiments.
2. OVA should be grade V due to purity. High levels of purity are required to generate an adequate immunological response.
3. For precise pipetting, the Alum always needs to be homogenized. Alum tends to precipitate.
4. This step is important because it promotes OVA molecule incorporation and avoids precipitation during i.p. injection.

5. In this way, the solution is able to reach the lungs.
6. The mouse should not be completely anesthetized. It should maintain its ability to react and breathe.

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References

1. Global Initiative for Asthma (GINA) (2016) Global strategy for asthma management and prevention. <https://ginasthma.org/wp-content/uploads/2016/04/GINA-Appendix-2016-final.pdf>
2. Kallos P, Kallos L (1984) Experimental asthma in guinea pigs revisited. *Int Arch Allergy Appl Immunol* 73(1):77–85
3. Kips JC, Anderson GP, Fredberg JJ, Herz U, Inman MD, Jordana M et al (2003) Murine models of asthma. *Eur Respir J* 22(2):374–382
4. Lambrecht BN, Hammad H (2015) The immunology of asthma. *Nat Immunol* 16(1):45–56. <https://doi.org/10.1038/ni.3049>
5. Walter MJ, Holtzman MJ (2005) A centennial history of research on asthma pathogenesis. *Am J Respir Cell Mol Biol* 32(6):483–489
6. Quarcio D, Pavlovic S, Joachim RA (2009) Stress and airway reactivity in a murine model of allergic airway inflammation. *Neuroimmunomodulation* 16(5):318–324
7. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD (2015) Asthma. *Nat Rev Dis Primers* 1:15025. <https://doi.org/10.1038/nrdp.2015.25>
8. Corazza N, Kaufmann T (2012) Novel insights into mechanisms of food allergy and allergic airway inflammation using experimental mouse models. *Allergy* 67(12):1483–1490
9. Reddy AT, Lakshmi SP, Reddy RC (2012) Murine model of allergen induced asthma. *J Vis Exp* (63):e3771. <https://doi.org/10.3791/3771>