

Chapter 32

Methods in Assessment of Airway Reactivity in Mice

Matthew Gold and Marie-Renee Blanchet

Abstract

Due to the wealth of reagents and transgenic strains available, mice have become one of the most commonly used model organisms for the study of allergic airway inflammation. One of the major hallmarks of the asthma phenotype in humans is reversible airflow obstruction, or airway hyper-responsiveness. However, the ability to confidently obtain useful physiological responses from such a small animal has presented a large technological challenge in murine studies. Recent advances have provided the technology to obtain lung mechanics through either the forced oscillation technique or plethysmography. Here we describe the utility of these measurements in mouse models of allergic airway inflammation and anaphylaxis.

Key words Asthma, Mouse, Airway responsiveness, Airway resistance, Methylcholine, flexiVent

1 Introduction

One of the major characteristics of the asthmatic response in humans is reversible airflow obstruction, or airway hyper-responsiveness (AHR). AHR is defined by an increased sensitivity of the airways to a constrictor agonist [1]. It is a phenomenon well described in asthmatic patients, and can be caused by stimulation of the airway smooth muscle by a variety of molecules such as histamine, prostaglandins, and leukotrienes [2, 3]—all released by inflammatory cells present in the lung environment. In humans, AHR is normally measured using the PC20 (provocative dose challenge causing a 20 % decrease of the FEV₁ (forced expiratory volume in 1 s)). In this test, increasing doses of either methacholine (MCh) or allergens are administered via nebulization to a patient. After each dose, the FEV₁ is determined: The MCh dose causing a 20 % decrease of the FEV₁ (PC20) is then calculated and used in the diagnosis of mild, moderate, or severe airway responsiveness or asthma.

While techniques used for testing AHR in humans are widespread, fairly straightforward, and well described, testing of lung functions in mice is more challenging. In the aim to obtain a

valid measurement of the response of airways to bronchoconstrictors, the first tests done on rodents were made using whole-body plethysmographs in the 1970s [4]. Plethysmography has become popular for evaluating lung function in mice, mainly due to its ease of use and noninvasive nature, which allows the monitoring of animals throughout disease and treatment regimens. However, this noninvasive approach cannot accurately measure lung mechanics in small rodents such as mice. Instead, these methods yield an arbitrary quantity called the enhanced pause (Penh) [5, 6]. Although Penh is a measure of the shape and duration of expiratory flows, it is not a true measure of lung mechanics. Although Penh may be proportional to airway resistance, invasive procedures are required to accurately calibrate the Penh in each experimental setup and design. In recent years, more accurate testing of the pulmonary functions of rodents became available, notably through the development of complete systems designed for lung function analysis in small rodents including the flexiVent (SCIREQ, Montreal, Canada) and Buxco instrument (Buxco Research Systems, Wilmington, NC, USA). These instruments are capable of measuring a variety of parameters such as airway resistance, compliance, and elastance [7, 8]. In mouse models of asthma and mast cell-dependant airway hyper-responsiveness, the parameter most widely used to determine airway responsiveness is airway resistance.

1.1 Airway Resistance in Mice

Airway resistance is undoubtedly the gold standard for measurement of AHR in mouse models of asthma. Using the flexiVent or Buxco apparatus, airway resistance is easily measurable at baseline (naïve mice without respiratory challenge). For the purpose of measuring an increase in reactivity of the lung in mouse models of asthma, resistance measurements can also be obtained in response to increasing doses of methacholine (MCh), administered either intravenously (IV) or via nebulization. In this technique, mice are anesthetized, paralyzed to block any skeletal muscle interference with the measurements, and tracheotomized with an 18-gauge (G) solid catheter (such as a blunt 18G needle). The flexiVent or Buxco apparatus provides for a set breathing rate and tidal volume. Following the intravenous administration of MCh, the airway resistance is measured every 10 s until the maximum resistance is obtained and noted. Results are typically expressed as an increase in resistance compared to baseline for each dose of MCh and these data can be compared between genotypes or experimental mice groups. This technique is sensitive enough to detect differences in airway resistance between genetically modified mice and their wild-type littermates [9, 10] and also sensitive enough to detect the effect of potential anti-inflammatory or bronchodilator drugs [11]. Increases in airway resistance have also been utilized to measure direct activation of lung-resident mast cells in a model of passive anaphylaxis [12].

Here we provide an overview of the technique used to measure airway resistance in mice. This technique can be used on naive mice as well as on mice that have undergone treatment with ovalbumin or house dust mite antigen to detect increases in airway responsiveness.

2 Materials

2.1 Surgical Preparation, Anesthesia, and Paralyzation

1. C57Bl/6J, Wsh (Kit^{W-sh}/HNihrJaeBsmJ (JAX Stock#005051)), W/W^v (WBB6F1/J-Kit^W/Kit^{W-v} (JAX Stock#100410)), or other mouse strains. All experiments must be conducted in accordance with the institutional and national guidelines (*see Note 1*).
2. Injectable anesthesia:
 - (a) Avertin stock solution: Dissolve 10 g of 2,2,2-tribromoethanol with 10 mL of *tert*-amyl alcohol for a stock solution. Store at 4 °C protected from light.
 - (b) 2.5 % working avertin solution: Prior to use, dilute stock solution 1:40 with PBS. Add the avertin stock solution dropwise to pre-warmed PBS (37 °C) with constant stirring to avoid formation of a precipitate. Store at 4 °C protected from light.
 - (c) Institutional guidelines will influence the choice of anesthetic. Avertin can be replaced with other drugs such as ketamine/xylazine or pentobarbital/xylazine.
3. Pancuronium bromide (stock 10 mg/mL in PBS or saline, stored at 4 °C).
4. Tracheal catheter.
 - (a) Prepare the tracheal catheter using an 18G needle.
 - (b) Saw off the bevel until the needle is approximately 1.2–1.4 cm long.
 - (c) Sand down the tip until it is dull to the touch (*see Note 2*).
5. Surgical scissors, forceps, laboratory tape, and suture thread.
6. 70 % ethanol.

2.2 Jugular Vein Cannulation or Aerosolized Delivery of Methylcholine

1. 30G1/2" needles.
2. Polyethylene tubing, 0.28 mm internal diameter (ID) (Becton Dickinson PE10).
 - (a) Create catheter by cutting the tubing into lengths approximately 40 cm. Cut a 30G needle away from its plastic fitting (approx. 1 cm), and carefully insert the blunt end into the catheter.
 - (b) In the other end, insert a Hamilton syringe (50–100 µL capacity) charged with PBS.

- (c) Slowly expel the PBS until it reaches the bevel of the needle, record volume, and trim the tubing until its total volume is 30 μL .
3. Hamilton 50 μL syringe, model 1705RN without needle (*see* **Note 3**).
4. Removable needles for Hamilton syringe, 30G-2".
5. Acetyl- β -methylcholine chloride (MCh): prep volume and diluent.
6. Aeroneb attachment to flexiVent (for aerosolized drug delivery, *see* **Note 4**).

3 Methods

3.1 Surgical Preparation, Anesthesia, and Paralyzation

1. Before anesthetizing the first mouse, make sure that the flexiVent instrument is calibrated and ready for ventilation (*see* Subheading 3.2).
2. Anesthetize mice with 2.5 % avertin, i.p. or an alternative method (500 mg/kg, or 200 μL /10 g body weight).
3. Secure mouse with its ventral side up to a styrofoam dissection board using laboratory tape (*see* **Note 5**).
4. Using an elastic band drawn across the dissection board, secure the head by placing elastic band underneath the upper incisors.
5. Make sure to calibrate the flexiVent machine with the catheter before inserting into the mouse (*see* **Note 6**).
6. Using scissors and forceps, remove skin and tissue above the neck area, exposing the trachea.
7. Make a pinhole cut in the trachea just inferior of the larynx and thyroid, carefully insert the 18G catheter into the trachea, and secure using suture thread.
8. Connect the mouse to the flexiVent machine and begin default ventilation (Subheading 3.2, step 5).

3.2 flexiVent Instrument Setup, Calibration, and Operation

1. Open up the flexiVent application on the desktop.
2. Select module 1 (FV-M1, 1.7 mL) and then start a new experiment from a template.
3. For jugular vein administration of MCh, select "Mouse EKG v5.2.1," create a new experiment folder, and perform the system calibrations for airway pressure, cylinder pressure, and the EKG (*see* **Note 7**).
4. After calibration, select the TLC (total lung capacity) and SnapShot-150 perturbation signals, connect the 18G catheter to the machine, and perform the dynamic tube calibration.

Ensure that the resulting values are within the acceptable limits (*see Note 8*).

5. Disconnect the catheter from the machine, insert it into the trachea of surgically prepared mouse, and then connect the catheter and mouse back to machine to begin default ventilation.
6. Immediately after connecting the mouse to the ventilator, inject 100 μL of pancuronium bromide (0.5–1.0 mg/kg, 100 μL i.p. of 200 $\mu\text{g}/\text{mL}$ for a 20 g mouse) intraperitoneally (or intramuscularly) to prevent efforts against the ventilation of the machine (*see Note 9*).
7. Allow 5 min for the muscle relaxant to take effect before beginning data acquisition. Connect the three EKG leads to the right forepaw and right and left hind paw.
8. While waiting for the pancuronium to take effect, prepare the mouse for insertion of the jugular vein catheter.
 - (a) Surgically expose the external jugular vein and carefully dissect the connective tissue surrounding it.
 - (b) Ensure that the catheter is filled with solution (either PBS/saline or MCh), and then insert into the external jugular vein.
 - (c) Secure the tubing to dissection board using laboratory tape.
9. Acquire snapshot readings at baseline, then administer 30 μL of PBS/saline via the jugular vein catheter, and immediately begin acquiring snapshot readings to monitor any increase in resistance. Perform a deep-lung inflation (TLC manoeuvre) before each PBS or MCh drug delivery to return respiratory system to baseline [7] (*see Notes 10 and 11*).
10. Once the resistance readings have returned to baseline, continue with the MCh doses (30 μL volume, 30–2,000 $\mu\text{g}/\text{kg}$). Immediately after administration of each MCh dose, begin taking snapshot acquisitions every 5 s until the peak of the response curve has been reached. Then, decrease the acquisition rate and monitor airway resistance until it again returns to baseline.
11. Repeat **step 10** with the successively higher MCh doses (*see Notes 12 and 13*).
12. After completion of the final MCh dose, humanely euthanize the mouse via institutional guidelines. Lung tissue and other biological samples can be collected and used for other analyses. We typically collect bronchoalveolar lavage fluid (BALF), peripheral blood by cardiac puncture, and lung tissue for gene expression, flow cytometric, and ELISA assays.
13. Results can be plotted as either raw resistance values or as a % increase over baseline.

4 Notes

1. The degree of airway hyper-responsiveness depends greatly on the mouse strain and the disease model. In models of OVA/alum-induced allergic asthma, C57Bl/6J mice develop milder responses than other strains. For example, Balb/c or 129/Sv strains typically provide more robust AHR readouts [13, 14].
2. It is important to carefully sand down the edges of the tracheal catheter; any remaining edge will cut the tracheal wall when inserted.
3. Alternatively a small volume insulin syringe (i.e., 3/10 cc with a 29G needle) can be used in place of a Hamilton syringe.
4. We find that the jugular vein administration route for MCh gives a more robust response than the nebulized delivery method. To use the nebulizer, connect the Aeroneb attachment to the flexiVent machine and use the “Mouse AN-EKG” template.
5. To reduce stress and injury, use masking or laboratory tape to secure mouse limbs rather than pinning down with needles. Mice can also be placed on heating pads or warmed with heat lamps.
6. It is important to calibrate the machine with the same catheter used for that mouse. Any difference in length between different catheters will affect the readouts.
7. If using the Aeroneb attachment for nebulized drug delivery, select the “Mouse AN-EKG” file.
8. The snapshot perturbation performs a single frequency forced oscillation manoeuvre that obtains the resistance and elastance measurements of the entire respiratory system (i.e., airways and chest wall). The Quick Prime-3 or Prime-8 perturbations perform a series of frequency oscillations and are able to obtain segregate resistance from the central airways and lung tissue. *NB:* To perform the quick prime and prime-8 manoeuvres, make sure that they are selected when performing the calibrations.
9. Do not administer pancuronium bromide until the animal is connected to the ventilator; otherwise the paralytic will impair their respiration.
10. Administration of MCh will cause a spike in the heart rate observed from the EKG leads. Make sure that the heart rhythm returns to baseline before performing the subsequent MCh challenge.
11. Delivery of MCh via the jugular vein provides a rapid increase in airway resistance. Therefore perform snapshot analyses (data

acquisition takes approximately 1.2 s per manoeuvre) immediately after delivery of MCh. The Quickprime-3 and Prime-8 manoeuvres take much longer to acquire (3 s and 8 s, respectively), so it is advised not to use these in conjunction with intravenous delivery of MCh. Nebulized delivery of MCh provides a broader response curve and is better suited for the Quickprime-3 or Prime-8 perturbations.

12. If collecting multiple doses you will have to readminister the injectable anesthetic to maintain an appropriate surgical plane (a single dose of avertin will last for approximately 15–20 min).
13. There are pre-programmed command scripts that will run various perturbations automatically, negating the need to manually perform snapshot or Quickprime manipulations. There are several default scripts pre-loaded onto the software (flexiWare), which can be modified to fit any experimental requirements.

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