



Chapter 23

Mouse Models of Asthma

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Abstract

Mouse models are critical for delineating the mechanisms that underlie asthma pathogenesis and developing new treatments. In this chapter we describe four different asthma models that offer unique benefits and allow investigators to answer distinct research questions. We also describe key surgical procedures that are necessary for assessing experimental asthma.

Key words Mouse model, Experimental asthma, Allergen, Immunization, Lung, Bronchoalveolar lavage

1 Introduction

Asthma is a common, chronic, and heterogeneous disease of the airways that is characterized by airway hyperresponsiveness (AHR), airflow obstruction, and airway inflammation and remodeling. In the majority of asthma endotypes, the inflammation has a master regulatory role that drives the other features of asthma. Based on the nature of the inflammation, asthma is classified into eosinophilic (most common), neutrophilic, mixed granulocytic, or pauci-inflammatory [1]. Eosinophilic asthma is frequently a result of exaggerated type 2 immune responses to airborne allergens. The type 2 responses are driven by adaptive and innate lymphocytes. Most studies have focused on the importance of CD4 T cells and, more recently, innate lymphoid type 2 cells (ILC2s) [2]. CD4 T cells promote the emergence of allergen-specific IgE which is a classical indicator of the allergic response.

In addition to cells of the immune system, airway structural cells are activated and contribute to the pathogenesis of asthma [3]. Activation of airway structural cells is manifested by goblet cell hyperplasia and mucus overproduction, subepithelial fibrosis, smooth muscle hypertrophy, and an increased number of blood vessels. These structural changes are collectively called “airway remodeling” [4].

Mouse models are instrumental in mechanistic studies of asthma. Existing mouse models recapitulate many features of human asthma. Over the years, several models have been developed. The unifying feature of all these models is the use of an environmental trigger (usually an allergen) to elicit an asthma-like reaction in the lung. In this chapter we describe four models. These include the classic ovalbumin sensitization model and models based on exposures to house dust mite extract, *Alternaria alternata* extract, and a combination of dust mite, ragweed, and aspergillus extracts. Each one has unique advantages and disadvantages, as described below.

The classic ovalbumin (OVA)-induced model involves sensitization with OVA in the presence of alum as an adjuvant, followed by airway challenge with OVA to elicit features of asthma [5–7]. These features are then typically studied 24–72 h after the challenge. Multiple variants of this model have been described. In this chapter we describe a variant with two subcutaneous sensitizations followed by five consecutive challenges through the intranasal route [7]. The OVA-induced model generates robust adaptive type 2 immune response (including emergence of OVA-specific IgE), eosinophilic inflammation of airways, goblet cell hyperplasia, and AHR. The key advantage of this model is its compatibility with OVA-related reagents and mouse strains. The OT2 and DO11.10 mouse strains are transgenic for OVA peptide-specific T-cell receptors (TCRs) that pair with the CD4 receptor. The OT2 and DO11.10 strains are extremely useful in studies of CD4 T-cell responses. The added bonus is that the OT2 and DO11.10 strains were developed on C57BL/6 and BALB/c backgrounds, respectively. C57BL/6 and BALB/c represent the two most commonly used genetic backgrounds in mouse models of experimental asthma. The disadvantages of the OVA-induced model include the use of an artificial antigen, use of an adjuvant, and an immunization route that are not directly relevant to human asthma. The OVA-induced model is an acute model; features of asthma resolve within 1–2 weeks after cessation of the challenge procedure.

To overcome some of the problems of the OVA-induced model, the house dust mite (HDM) extract-induced model has been developed [8, 9]. The HDM extract-induced model utilizes a human asthma-relevant allergen and immunization method (administration of the allergen directly onto airway mucosal surfaces, no artificial adjuvant). The natural immunization is possible due to intrinsic adjuvant-like properties of HDM, including its protease activity. In general, the HDM extract-induced model reiterates most of the asthma features that are characteristic of the OVA-induced model. However, the generation of allergen-specific IgE is not as robust as in the OVA-induced model [10]. Similarly to the OVA-induced model, features of asthma in the HDM extract-induced model resolve over a very short period of time.

In recent years, two new valuable mouse asthma models have been developed. These models address two important aspects of asthma—involvement of innate lymphoid cells and persistence of asthma in the absence of an environmental trigger. The first model involves three to four consecutive intranasal challenges with an extract of the fungus *Alternaria alternata* [11]. The features of asthma are studied shortly after the last challenge, typically at 24 h. Understanding of the responses to *Alternaria* is relevant to human pathology since exposure to this fungus is associated with severe asthma [12]. The *Alternaria* extract-induced model generates strong responses of the innate immune system, including responses of innate lymphoid type 2 cells (ILC2s), which induce airway eosinophilia and AHR [11, 13]. The role of T cells and B cells in this model is uncertain at this moment and requires further investigation. The second model involves intranasal administration of three allergens—extracts of dust mite, ragweed, and *Aspergillus* (DRA) without adjuvant—twice a week for 6 weeks [7, 14]. In the DRA extract-induced model, high-level AHR persists for at least 6 months after cessation of the allergen exposure. Airway inflammation and remodeling peak 1–2 months postexposure, but remain significantly increased at 6 months. The persistence of asthma is independent of T cells. Instead, it relies on ILC2s. T cells contribute to the severity (magnitude) of asthma features. This is the first model, to our knowledge, that truly reflects the chronic aspect of human asthma, i.e., the long-term persistence of airway pathology in the absence of an environmental trigger.

Protocols for each of the four animal models of asthma are provided below.

2 Materials

2.1 OVA-Induced Model of Acute Asthma

1. Mixture of OVA and alum: Dissolve OVA grade V (*see Note 1*) to a final concentration of 1.2 mg/mL in sterile phosphate-buffered saline (PBS) to prepare OVA solution # 1. For immunization, you will need 50 μ L (60 μ g) of solution # 1 per mouse per day. The solution can be distributed into single-use aliquots and frozen at -80°C . Use the aliquots within 1 year of preparation. To prepare the immunizing mixture, add an equal volume of Imject Alum (Pierce) dropwise to OVA solution #1. Imject Alum is an aqueous suspension of aluminum hydroxide (40 mg/mL) and magnesium hydroxide (40 mg/mL) (*see Note 2*). You will need 50 μ L (2 mg of aluminum hydroxide and 2 mg of magnesium hydroxide) of Imject Alum per mouse per day. Work under sterile conditions. Mix (vortex mixer) the OVA-alum suspension for 30 min. Mixing allows the Imject Alum to adsorb OVA. Use immediately after preparation.

2. OVA solution # 2: Dissolve OVA grade V (*see Note 1*) to a final concentration of 4 mg/mL in sterile PBS. OVA solution # 2 is used for challenge by intranasal instillation. You will need 15 μ L (60 μ g) of this solution per mouse per challenge day. The solution can be distributed into single-use aliquots and frozen at -80°C . Use the aliquots within 1 year of preparation. Work under sterile conditions.
3. 70% (v/v) ethanol.
4. Isoflurane.
5. Isoflurane vaporizer connected to an anesthesia induction chamber.
6. 1 mL syringes.
7. 25 gauge needles.
8. 6-week-old C57BL/6 or BALB/C mice (*see Note 3*).

2.2 HDM Extract-Induced Model of Acute Asthma

1. House dust mite (HDM) solution # 1: Dissolve the HDM powder (lyophilized extract of *Dermatophagoides pteronyssinus*, Greer Laboratories) to a final concentration of 0.67 mg/mL (*see Notes 1, 4, and 5*) in sterile PBS. Prepare single-use aliquots, and freeze at -20°C . Use the aliquots within 1 year of preparation. Each aliquot should be thawed only once. Do not refreeze. HDM solution # 1 is used for challenge by intranasal instillation. You will need 15 μ L (10 μ g) of solution # 1 per mouse per challenge day. HDM solution # 1 also serves as a stock solution for preparation of HDM solution # 2.
2. HDM solution # 2: Use HDM solution # 1 to prepare solution # 2 at a final concentration of 20 μ g/mL. Use sterile PBS for dilution. HDM solution # 2 is used for immunization by intratracheal instillation. You will need 50 μ L (1 μ g) of solution # 2 per mouse.
3. Isoflurane.
4. Isoflurane vaporizer connected to an anesthesia induction chamber.
5. A stand for intratracheal instillation: An angled (60° incline) platform with a rubber band stretched between two bolts.
6. Forceps.
7. 6–8-week-old C57BL/6 or BALB/C mice (*see Note 3*).

2.3 Alternaria Extract-Induced Model of ILC2-Mediated Asthma

1. *Alternaria* solution: Dissolve the lyophilized *Alternaria alternata* extract (Greer Laboratories) to a final concentration of 0.33 mg/mL in sterile PBS (*see Notes 1, 4, and 5*). You will need 15 μ L (5 μ g) of this solution per mouse per challenge day. The solution should be distributed into single-use aliquots and frozen at -20°C . Use the aliquots within 1 year of preparation. Each aliquot can be thawed only once. Do not refreeze.

2. Isoflurane.
3. Isoflurane vaporizer connected to an anesthesia induction chamber.
4. 6–8-week-old C57BL/6 or BALB/C mice (*see Note 3*).

2.4 DRA Extract-Induced Model of Chronic Asthma

1. DRA solution: Dissolve lyophilized extracts of HDM (*Dermatophagoides pteronyssinus*), ragweed (*Ambrosia artemisiifolia*), and *Aspergillus fumigatus* (all extracts from Greer Laboratories) in sterile PBS to a final concentration of 0.33 mg/mL, 1 mg/mL, and 0.33 mg/mL, respectively (*see Notes 1, 4, and 5*). You will need 15 μ L of this solution (5 μ g of HDM, 15 μ g of ragweed, and 5 μ g of *Aspergillus*) per mouse per challenge day. The solution should be distributed into single-use aliquots and frozen in -20°C . Use the aliquots within 1 year of preparation. Each aliquot can be thawed only once. Do not refreeze.
2. Isoflurane.
3. Isoflurane vaporizer connected to an anesthesia induction chamber.
4. 6–8-week-old C57BL/6 or BALB/C mice (*see Note 3*).

2.5 Tracheotomy and Bronchoalveolar Lavage (BAL)

1. PBS-EDTA solution: Prepare sterile PBS containing 0.5 mM EDTA.
2. Mixture of xylazine at 30 mg/kg mouse weight and ketamine at 500 mg/kg mouse weight.
3. 70% (v/v) ethanol.
4. Intratracheal cannula: 18 or 21 gauge needle with polyethylene tubing around the metal part of the needle. The tubing protects the trachea from laceration by the sharp end of the needle.
5. 25 gauge needles.
6. 1 mL syringe.
7. Scissors and forceps.
8. 15 mL conical tubes.
9. Refrigerated centrifuge with a swinging bucket rotor.
10. Upright microscope.
11. Hemacytometer.
12. C57BL/6 or BALB/c mice (*see Note 3*).

2.6 Lung Harvest

1. Sterile PBS.
2. Mixture of xylazine at 30 mg/kg mouse weight and ketamine at 500 mg/kg mouse weight.
3. 70% (v/v) ethanol.
4. 25 gauge needles.

5. 1 and 10 mL syringes.
6. Scissors and forceps.
7. 60 mm plastic dishes.
8. C57BL/6 or BALB/c mice (*see* **Note 3**).

3 Methods

3.1 OVA-Induced Model of Acute Asthma (Fig. 1a)

3.1.1 Immunization

Day 0

1. Disinfect the dorsal skin of the mouse neck with 70% ethanol.
2. Grasp the dorsal skin of the neck using your thumb and your forefinger. The skin in the neck area is loose and can be gently lifted between your fingers.
3. Insert a 25 gauge needle under the lifted skin and subcutaneously inject 100 μ L of the OVA-alum mixture.
4. Release the mouse (*see* **Note 6**).

Day 7

Repeat the immunization procedure as described in **steps 1–4** for Day 0.

3.1.2 Challenge

Day 14

1. Place the mouse in an anesthesia induction chamber connected to an isoflurane vaporizer. Switch on the vaporizer. Anesthetize the mouse with 4–5% isoflurane in oxygen or air. Anesthesia is considered adequate when an animal stays still quietly, loses a toe pinch reflex, and its respiratory rate has decreased and is noticeably deeper.
2. Remove the mouse from the anesthesia induction chamber, and immediately (*see* **Note 7**) proceed with intranasal instillation.
3. Intranasal instillation: Hold the mouse vertically head upward and rear legs downward. Slowly administer 15 μ L of OVA solution # 2 into one nostril using a sterile pipet tip. Maintain a vertical position of the mouse with the head upward for 1 min to permit the OVA solution to distribute downward into the lung.
4. Place the mouse into its cage. Switch off the isoflurane vaporizer.
5. Monitor the mouse during recovery from anesthesia.

Days 15, 16, 17, and 18

Repeat the challenge procedure on each day as described in **steps 1–5** for Day 14.

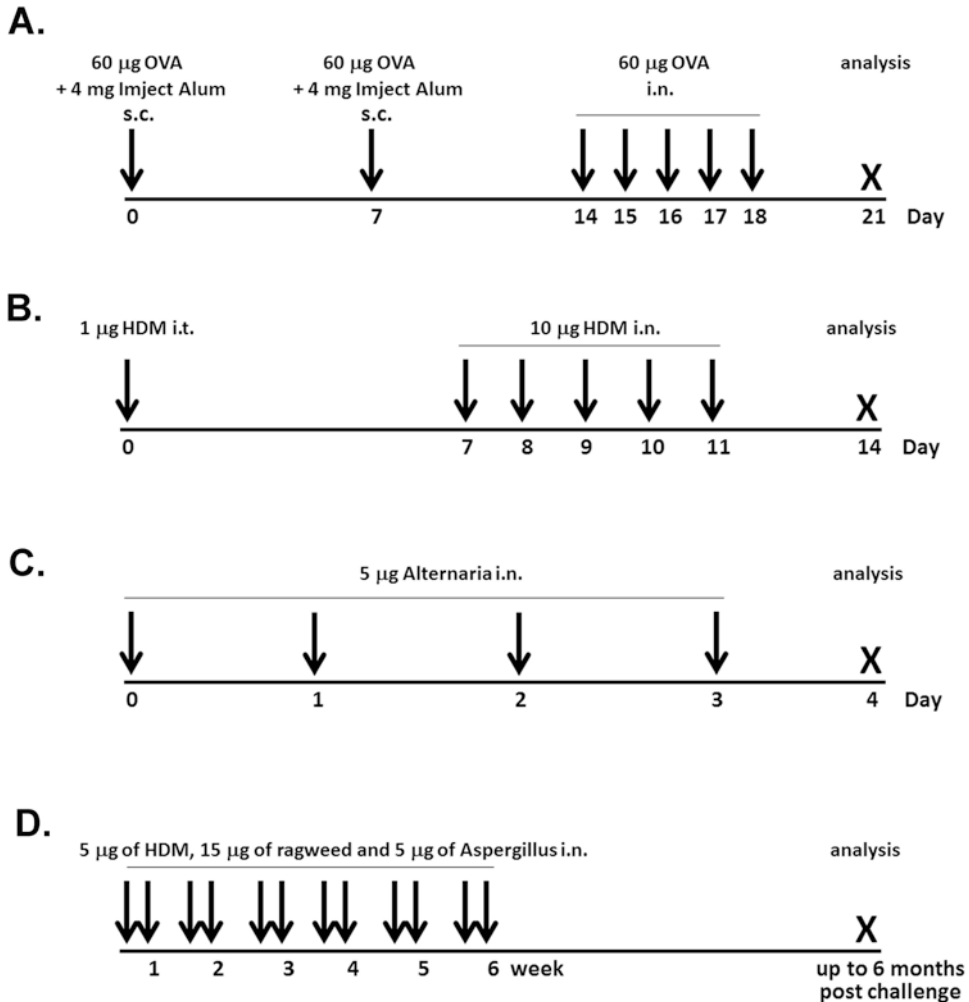


Fig. 1 Four models of experimental asthma. (a) Ovalbumin (OVA)-induced model of acute asthma, (b) house dust mite (HDM) extract-induced model of acute asthma, (c) *Alternaria alternata* extract-induced model of ILC2-mediated asthma, (d) dust mite–ragweed–*Aspergillus* (DRA) extract-induced model of chronic asthma. *i.n.* intra-nasal route, *i.t.* intratracheal route, *s.c.* subcutaneous route; see the text for detailed description of models

3.1.3 Analysis

Day 21

Measure features of asthma (see **Note 8**).

3.2 HDM Extract-Induced Model of Acute Asthma (Fig. 1b)

3.2.1 Immunization

Day 0

1. Anesthetize a mouse with isoflurane as described in Subheading 3.1.2.
2. Remove the mouse from the anesthesia chamber, and immediately (see **Note 7**) proceed with the intratracheal instillation procedure.
3. Intratracheal instillation: Gently suspend the mouse by its cranial incisors on a rubber band attached to the angled instil-

lation stand. Use forceps to pull the tongue forward and then downward. A full extension of the tongue is needed to prevent the animal from swallowing. Place the pipet tip containing 50 μ L of HDM solution # 2 over the tongue. Inject the solution toward the back of the throat. Keep the tongue fully extended, and monitor mouse's breathing to ensure that the fluid is aspirated into the lungs. The common practice is to maintain the tongue extended for five breaths after the liquid disappears from the throat. Next, release the tongue.

4. Place the mouse into its cage. Switch off the isoflurane vaporizer.
5. Monitor the mouse during recovery from anesthesia.

3.2.2 Challenge

Day 7

1. Anesthetize the mouse with isoflurane as described in the Subheading 3.1.2.
2. Perform the intranasal instillation procedure as described in the Subheading 3.1.2 using 15 μ L of HDM solution # 1 per mouse.

Days 8, 9, 10, and 11

Repeat the challenge procedure on each day as described in steps 1 and 2 for Day 7

3.2.3 Analysis

Day 14

Measure features of asthma (*see Note 8*).

3.3 *Alternaria* Extract-Induced Model of ILC2-Mediated Asthma (Fig. 1c)

3.3.1 Challenge

Day 0

1. Anesthetize the mouse with isoflurane as described in the Subheading 3.1.2.
2. Perform intranasal instillation as described in the Subheading 3.1.2 using 15 μ L of the *Alternaria* solution per mouse.

Days 1, 2, and 3

Repeat the challenge procedure on each day as described in steps 1 and 2 for Day 0.

3.3.2 Analysis

Day 4

Measure features of asthma (*see Note 8*).

3.4 DRA Extract- Induced Model of Chronic Asthma (Fig. 1d)

3.4.1 Immunization and Challenge

Day 0 (Week 1)

1. Anesthetize the mouse with isoflurane as described in the Subheading 3.1.2.
2. Perform intranasal instillation as described in the Subheading 3.1.2 using 15 μ L of the DRA solution per mouse.

Day 3 (Week 1)

Repeat the intranasal instillation procedure as described in **steps 1 and 2** for Day 0.

Weeks 2, 3, 4, 5, and 6

Repeat the intranasal instillation procedure twice a week as described in **steps 1 and 2** for Day 0 of week 1.

3.4.2 Analysis

Up to 6 Months Post-challenge

Measure features of asthma (*see* **Note 8**).

3.5 Tracheotomy and Bronchoalveolar Lavage (BAL)

1. Euthanize the mouse through intraperitoneal injection of xylazine (30 mg/kg mouse weight) and ketamine (500 mg/kg mouse weight). Use a 25 gauge needle and 1 mL syringe for injection (*see* **Note 9**).
2. Disinfect the ventral skin of the neck with 70% ethanol.
3. Cut the ventral skin of the neck in the midline. Carefully separate the salivary glands and the sternohyoid muscle laterally to expose the trachea.
4. Make a small incision in the trachea. The incision should be made between the first and the second cartilage ring proximal to the larynx.
5. Insert the cannula into the trachea. The cannula should be attached to the 1 mL syringe containing the PBS-EDTA solution. Inject 1 mL of this solution into the lungs. Massage the chest for a few seconds. Aspirate and collect the BAL fluid into a 15 mL conical tube.
6. Centrifuge this first milliliter of the BAL fluid for 5 min, $300 \times g$, 4 °C. Collect the supernatant, and freeze it at –80 °C for future measurements (*see* **Notes 10 and 11**). Keep the BAL cell pellet on ice.
7. Repeat the inject-aspirate procedure twice to collect an additional 2 mL of the BAL fluid.
8. Pool these two lavages, and add them to the cell pellet obtained from the first lavage. Centrifuge 5 min, $300 \times g$, 4 °C to obtain the final cell pellet. Discard the supernatant.
9. Perform cell counts using a hemacytometer.
10. Cells can be cytopsin and stained with the Diff-Quick stain for morphometric analysis and differential counting. Alternatively, cells can be stained with fluorescently labeled antibodies and analyzed by flow cytometry.

3.6 Lung Harvest

1. Euthanize the mouse as described in the Subheading [3.5](#).
2. Disinfect the ventral side of the mouse (the neck, chest, and abdomen) with 70% ethanol.

3. Make a midline incision of the ventral skin, separate it aside, and remove it. Lift the xiphoid process of the sternum with forceps. Cut the diaphragm away from the ribs. Cut the entire sternum in the midline from its caudal part to the cranial part (*see Note 12*). Open the rib cage, and, using scissors, remove its ventral and lateral parts to expose the lung and the heart.
4. Make a small incision in the left ventricle of the heart. Insert a 25 gauge needle into the right ventricle, and slowly perfuse the pulmonary circulation with 5–10 mL of PBS to remove blood. Continue the perfusion until the lungs are expanded and white and the fluid flowing out of the left ventricle is completely clear.
5. Remove the lungs from the chest cavity. Make sure to separate the lungs from mediastinal organs and tissues including the heart, major blood vessels, thymus, lymph nodes, esophagus, and trachea.
6. Place the lungs in a dish containing PBS on ice.
7. The lungs can be then processed for histology, RNA or protein analysis, and flow cytometry (*see Note 13*).

4 Notes

1. It is advisable to measure the endotoxin content of an allergen. A common approach is to use the limulus amebocyte lysate (LAL) assay.
2. Aluminum hydroxide and magnesium hydroxide are not soluble in water. As a result, Imject Alum is a suspension of particles that sediment to the bottom of the bottle over time. Therefore, shake the bottle of Imject Alum thoroughly before each use.
3. BALB/c and C57BL/6 strains differ in the magnitude of pulmonary responses [15]. BALB/c mice develop stronger AHR. C57BL6/mice develop more robust eosinophilia in the BAL fluid (BALF).
4. Allergen extracts are complex mixtures of protein and nonprotein molecules. Allergen batches (lots) differ in composition, including variations in the protein content. It is advisable to use the same lot for all mice in a given project. If another lot is needed to continue the project, make sure to buy a lot with similar protein content.
5. Concentration of an allergen should reflect its protein content.

6. Gently mix the OVA-alum mixture between injections/mice. This will prevent the alum from depositing at the bottom of a tube and ensure a homogenous distribution of the suspension.
7. The application of an experimental agent should be done immediately after removal of a mouse from the anesthesia induction chamber. The isoflurane anesthesia is very short-lasting, and mice recover from anesthesia within a few minutes.
8. The key features of experimental asthma include elevated airway eosinophils and type 2 cytokines, peribronchial inflammation and goblet cell hyperplasia, emergence of allergen-specific IgE, and airway hyperresponsiveness (AHR) to methacholine (*see* Chapter 19). The *Alternaria* extract-induced model is used to analyze responses of ILC2s.
9. Other methods of euthanasia can also be used, including an overdose of pentobarbital (300 mg/kg mouse weight) or CO₂ asphyxiation.
10. The first milliliter of the BAL supernatant has the highest concentration of proteins (e.g., cytokines). It is important not to dilute it with additional lavages.
11. BAL supernatant can be used for measurements of inflammatory mediators (e.g., cytokines and chemokines), growth factors, enzymatic activities (e.g., lactate dehydrogenase), metabolites (e.g., ATP, uric acid), and mucus.
12. Keeping the scissor tips horizontal will ensure that the heart, large blood vessels, and the lung are not punctured.
13. Histological stains (*see* Chapter 20) are used for morphometric measurements of peribronchial inflammation (hematoxylin and eosin—H&E), goblet cell hyperplasia (periodic acid—Schiff—PAS), and deposition of extracellular matrix (trichrome stain). The lung tissue sections can also be stained for individual proteins using specific antibodies (immunohistochemistry and immunofluorescence). The lung can be lysed; proteins and RNAs can be then measured by western blot, ELISA, real-time PCR, or RNA-seq. Finally, the lung can be digested with collagenase, and the cell suspension can be analyzed by flow cytometry.

Acknowledgments

This work was supported by the National Institutes of Health grant R01HL122995 to M.M.G.

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