

General and Specific Mouse Models for Asthma Research

Fernando Marqués-García and Elena Marcos-Vadillo

Abstract

To study the complexity of human asthma disease, the development of different animal models is needed. Among all different laboratory animals, mice represent a useful tool for the development of asthma. This chapter will describe protocols for designing different animal models applied to the studying of asthma phenotypes.

Key words Acute, Allergen, Chronic, Mouse, Intrinsic, Occupational

1 Introduction

Human asthma is a heterogeneous and complex disease, in which both inflammation and remodeling of the airway structure take place [1]. The molecular and cellular mechanisms that trigger the disease are not entirely understood; therefore, animal models represent an alternative to investigate the mechanisms and progression of the disease. Due to the complexity of the disease, a single model is not sufficient to reproduce all changes [2]. Mouse species is widely used for generating such models. Different models have been generated in which the exposure can be acute or chronic, *see* Tables 1 and 2 for examples induced by ovalbumin (OVA).

The acute models are useful for studying the inflammatory response, whereas chronic models better replicate the clinical features of asthma as well as remodeling of the airway and bronchial hyperresponsiveness [9–11]. OVA is rarely involved in human allergic asthma processes, there are multiple models developed based on allergens causing human disease such as House Dust Mite (HDM) [12] pollen [13], fungal spores [14] etc. Also it has been worked in the generation of mouse models to study occupational asthma [15, 16] and intrinsic asthma [17]. In this chapter, different models for the study of asthma will be described.

Table 1
Different treatment strategies in acute OVA mouse models for strain BALB/c

Sensitisation	Challenge	References
OVA/Alum (IP) 0 & 12 days	OVA aerosol 18–23 days	[3]
OVA/Alum (IP) 0 & 14 days	OVA aerosol 28–30 days	[4]
OVA/Alum (IP) 0 & 14 days	OVA IN 14,25,26 & 27 days	[5]

OVA ovalbumin, Alum aluminium hydroxide, IP intraperitoneal, IN intranasal

Table 2
Different treatment strategies in chronic OVA mouse models

Sensitisation	Challenge	References
OVA/Alum (IP) -7 & -21 days	OVA 6/8 weeks, 3 days/week	[6]
OVA/Alum (IP) 0 & 14 days	OVA IN 14, 27, 28, 47, 61 & 73 days	[7]
OVA/Alum (IP) 0 & 14 days	OVA IN 12 weeks, 3 days/week	[8]

Description of the differences in both stages in female strain BALB/c
OVA ovalbumin, alum aluminium hydroxide, IP intraperitoneal, IN intranasal

2 Materials

2.1 Animals

1. 6–8 Weeks old female BALB/c mice (Charles Rivers Lab).
2. 6–8 Weeks old female A/J old (Charles Rivers Lab).

2.2 Common Material

1. 1× PBS (Phosphate-Buffered saline): 1.37 mM NaCl, 2.6 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄. Adjust the pH to 7.4 with HCl.
2. Tuberculin syringes (BD Biosciences).
3. Needle 20G short (BD Biosciences).
4. Ketamine chlorhydrate (Imalgen, Merial).
5. Medetomidine hydrochloride (Medetor, Virbac).
6. 100 µl micropipette and appropriate tips (Thermo-Fischer).

2.3 OVA Asthma Model

1. Ovalbumin (Sigma-Aldrich).
2. Aluminum hydroxide (Sigma-Aldrich).

2.4 Allergen-Specific Asthma Models

2.4.1 Pollen

1. Crude olive pollen (*Olea europaea*) extract (ALK-Abello Laboratories).
2. Aluminum hydroxide (Sigma-Aldrich).

2.4.2 Dust/Mites

1. House Dust Mite (HDM) Extract (Allergopharma).
2. Freund's adjuvant: 1 ml contains 1 mg of *Mycobacterium tuberculosis* (H37Ra, ATCC 25177), heat killed and dried, 0.85 ml paraffin oil and 0.15 ml mannide monooleate (Sigma-Aldrich).

2.4.3 Molds

1. *Alternaria alternata* (strain 18586).
2. Potato dextrose agar plates: Per liter, 200 g of infusion from potatoes, 20 g dextrose, and 15 g agar pH 5.6 (Difco).
3. Cell scraper.
4. Hemocytometer.
5. Czapek's medium: Per litre, 30 g sucrose, 2 g NaNO₃, 1 g K₂HPO₄, 0.5 g MgSO₄, 0.5 KCl, 0.01 FeSO₄, 15 g agar pH 7.3 (Himedia Labs).
6. NH₄HCO₃ + polyvinyl polypyrrolidone (Sigma-Aldrich).
7. Glycerol.
8. Aluminum hydroxide (Sigma-Aldrich).

2.5 Tobacco-Induced Asthma Models

1. Plexiglas chamber.
2. Nebulizer (Mega Medical).
3. Tobacco cigarettes.
4. *E. coli* LPS (Sigma-Aldrich).

3 Methods

3.1 Ovalbumin Asthma Model

Models based on the use of OVA are grouped into two types according to the duration of the treatment, as described below:

- Acute Model.
- Chronic Model.

3.1.1 Acute Model

The experiments are performed with 6–8 weeks old female BALB/c mice (*see* **Notes 1** and **2**) (Fig. 1). Animals must be kept confined and handled to meet the current ethics rules of handling animals for experimentation (*see* **Notes 3** and **4**). Start from a population of, for example, eight mice. The animals are divided into two groups:

- Group 1: Four mice that are not treated with OVA Intraperitoneal/Intranasal (in place 1× PBS is given).
- Group 2: Four mice are treated with OVA Intraperitoneal/Intranasal.

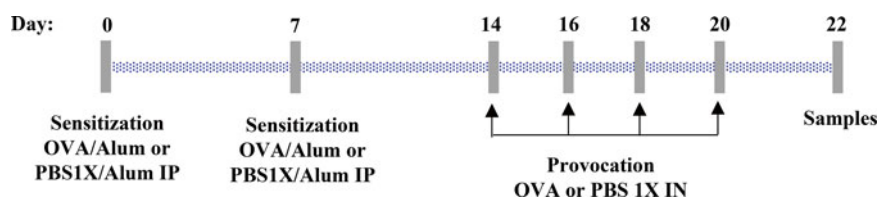


Fig. 1 Acute mouse model. *IP* intraperitoneal, *IN* intranasal, *PBS* phosphate-buffered saline, *OVA* ovalbumin, *Alum* aluminum hydroxide

1. On day 0 (start of experiment), inject the animals intraperitoneally with 20 μ g of OVA emulsified in 0.2 ml 1 $\times$ PBS containing 2 mg of aluminum hydroxide (these doses are administered to each mouse). Inject the controls with 0.2 ml of 1 $\times$ PBS containing 2 mg of aluminum hydroxide (*see Note 5*).
2. Keep the animals stabled for 1 week (7 days) for the hyper-sensitization reaction to occur.
3. On day 7, administer the second dose of OVA to reinforce hyper-sensitization by repeating **step 1**.
4. Keep the mice housed for another 7 days.
5. After 14 days of hyper-sensitization, organize the provocative steps, four provocations in total.
6. Perform the first intranasal provocation with OVA.
7. Anesthetize the animal with mild sedation. Administer an intraperitoneal dose of Ketamine (75 mg/kg) + Medetomidine (1 mg/kg). Wait for the complete sedation of the animal before handling (*see Notes 6–9*).
8. Stand upright the sedated animal with his nose up and give 50 μ l of OVA (0.1% in 1 $\times$ PBS) for the experimental group or 50 μ l of 1 $\times$ PBS for the control group. Administrate the dose dropping drop wise to the snout (helping with a micropipette), favoring inoculation during the inspiratory process of the animal.
9. Keep the animal in that position for 1–2 min.
10. Return the animal to the cage, put horizontally on its side for favoring ventilation (*see Note 10*).
11. Repeat the procedure described in **steps 7–10**, three times with a frequency of 2 days (48 h) between one provocation and the next. This protocol would correspond to day 16 (second provocation), 18 (third provocation), and 20 (fourth provocation) (*see Note 11*).
12. On day 22 of the experiment, proceed to the collection of the samples.
13. 2 Days (48 h) after the last provocation, perform the necropsy of the animals.

14. Anesthetize the animal with the same mixture described in paragraph 7 (*see* **Note 12**).
15. With the anesthetized animal, proceed to the necropsy. The mouse is placed in *decubitus supino* position fixing the legs to a surface.
16. For obtaining samples:
 - Blood is obtained by cardiac puncture at the ventricular level. This puncture serves as gathering blood process and as euthanasia method. Cellular and soluble factors present in serum can be analyzed.
 - BALF (Bronchoalveolar Lavage): Cannulate the trachea and inject 1 ml of 1× PBS, two times. BALF is used for studying cellular and soluble factors.
 - Tissues: Collect the organs of interest. They can be used for molecular biology as well as histological studies.
 - Hematopoietic organs: Can be employed for in vitro cell culture.

3.1.2 Chronic Model

The experiments are performed with 6–8 weeks old female BALB/c mice (*see* **Notes 1** and **2**) (Fig. 2). Animals must be kept confined and handled to meet the current ethics rules of handling animals for experimentation (*see* **Notes 3** and **4**). Start for example with 8 mice. The animals are divided into two groups:

- Group 1: Four mice that are not treated with OVA Intraperitoneal/Intranasal (in place 1× PBS is given).
 - Group 2: Four mice are treated with OVA Intraperitoneal/Intranasal.
1. On day 0 (start of experiment), inject the animal intraperitoneally with 20 µg of OVA emulsified in 0.2 ml 1× PBS containing 2 mg of aluminum hydroxide (these doses are administered to each mouse). Inject the controls with 0.2 ml of 1× PBS containing 2 mg of aluminum hydroxide (*see* **Note 5**).
 2. Keep the animal animals stabled for 1 week (7 days) for the OVA hyper-sensitization to occur.

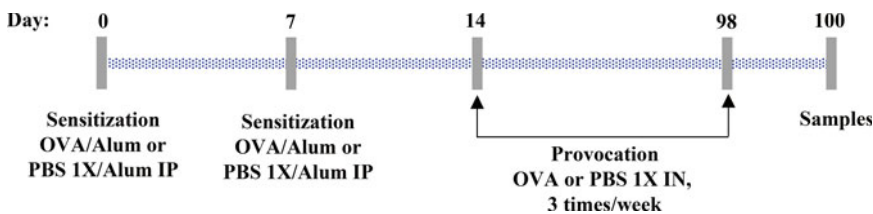


Fig. 2 Chronic mouse model. *IP* intraperitoneal, *IN* intranasal, *PBS* phosphate-buffered saline, *OVA* ovalbumin, *Alum* aluminum hydroxide

3. On day 7, administer the second dose to reinforce hyper-sensitization by repeating the **step 1**.
4. Keep the mice housed for another 7 days.
5. After 14 days of hyper-sensitization, organize the steps of provocation. In this model, the frequency is three provocations per week.
6. Perform the first intranasal provocation with OVA on day 14 of the experiment. Anesthetize the animal by administering an intraperitoneal dose of Ketamine (75 mg/kg) + Medetomidine (1 mg/kg). Wait for the complete sedation of the animal before you start handling (*see* **Notes 6–9**).
7. Stand upright the sedated animal with his nose up and give 50 μ l of 1 $\times$ PBS to the control group or 50 μ l of OVA (0.1 % in 1 $\times$ PBS) to the experimental group. Administrate dropping drop wise to the snout (helping with a micropipette), favoring inoculation during the inspiratory process animal.
8. Keep the animal in that position for 1–2 min.
9. Return the animal to the cage, putting horizontally on its side for favoring ventilation (*see* **Note 10**).
10. In this chronic model, monitor the clinical course of the mice. Use a classification or Clinical Score that assigns a numerical value to the clinical signs developed for mouse (*see* **Note 13**).
11. Repeat the procedure described in **steps 6–9**, three times per week for 12 weeks (3 months).
12. Observe and record the signs observed in mice during this period. After that time, proceed to the collection of samples.
13. Proceed to the necropsy of the animals 2 days (48 h) after the last provocation.
14. Anesthetize the animal with the same mixture described in paragraph 6 (*see* **Note 12**).
15. Place it in *decubitus supino* fixing it to a surface by the legs.
16. For obtaining the samples:
 - Blood is obtained by cardiac puncture at the ventricular level. This puncture serves as gathering blood and euthanasia method. Cellular as well as soluble factors present in serum can be analyzed.
 - BALF (Broncho alveolar Lavage): Cannulate the trachea and inject 1 ml of 1 $\times$ PBS 2 times. BALF is used for cellular and soluble factors studies.
 - Tissues: Collect the organs of interest for molecular biology as well as histological studies.
 - Collect the hematopoietic organs for in vitro cell culture.

3.2 Specific Allergen Asthma Model

In this section, we focus on describing asthma mouse models, where relevant allergens in humans are used to trigger allergic asthma. Models for the following allergens are described:

- Pollen.
- Dust/Mite.
- Molds.

3.2.1 Pollen

Use *Olea europaea* (Olive) as pollen allergen that is responsible for a high percentage of seasonal allergic asthma in the Mediterranean region (Fig. 3). The experiments are performed with 6–8 weeks old female BALB/c mice.

The mice are divided into two groups:

- Control: Inoculated with 1× PBS.
- Experimental: Inoculated with pollen allergen.
 1. Prepare the mixture of 25 µg pollen extract with 50 mg of aluminum hydroxide in 200 µl of 1× PBS for each mouse and inoculation.
 2. On day 0, inoculate subcutaneously the control group with 200 µl of 1× PBS and the experimental group with 200 µl of the mixture prepared in **step 1**.
 3. During the stage of hyper-sensitization, inoculate once a week for the first 3 weeks.
 4. From week 3 to week 10 do not treat the animals.
 5. At week 10, perform the elicitation phase after 72 h (3 days) and 48 h (2 days) before slaughter. Perform the first challenge 3 days before slaughter of animals.
 6. Inject an intraperitoneal dose of ketamine (75 mg/kg) + Medetomidine (1 mg/kg) mixture to the animal.
 7. Place the sedated animal horizontally with the muzzle up and inoculate (using a micropipette) 30 µl of pollen extract (15 µg of extract of pollen per mouse and provocation) to the experimental group and 30 µl of 1× PBS to the control group.
 8. Repeat **steps 6** and **7** for the second provocation, 2 days before the necropsy of the mice.

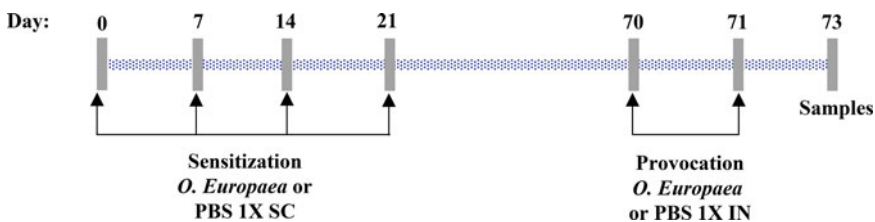


Fig. 3 Specific allergen asthma model: Polen. SC sub-cutaneous, IN Intranasal, PBS phosphate-buffered saline

9. At 48 h, proceed to the necropsy of the animals for the collection of samples.
10. Anesthetize the animal with the same mixture described in **step 6**.
11. With the anesthetized animal, proceed to the necropsy, setting the animal in decubitus supino position.
12. Obtain the samples (blood, BALF, tissues, and/or hematopoietic organs).

3.2.2 House Dust Mite (HDM)-Induced Asthma Model

Protocol 1

Use extract of house dust mite (HDM) as allergen. It is responsible for a high percentage of non-seasonal allergic asthma worldwide (Fig. 4). The experiments are performed with 6–8 weeks old female BALB/c mice.

1. Dissolve the HDM in 1× PBS at a concentration of 100 µg of extract in 50 µl of 1× PBS (without adjuvant) per mouse of the experimental group. Give 50 µl of 1× PBS to the controls.
2. Start the experiment (day 0), perform the first administration of HDM extract or PBS, depending on the group. Give the animals an intraperitoneal dose of ketamine (75 mg/kg) + Medetomidine (1 mg/kg) mixture. Perform the allergen deposition intranasal.
3. Repeat the days 7, 14, and 21 of the experiment.
4. On day 23 (48 h after the last allergen exposure) proceed to the collection of samples.
5. Anesthetize the animal with the same mixture described in **step 2**.
6. With the anesthetized animal, perform the necropsy, setting the animal in *decubitus supino* position to obtain samples (blood, BALF, tissues, and/or hematopoietic organs).

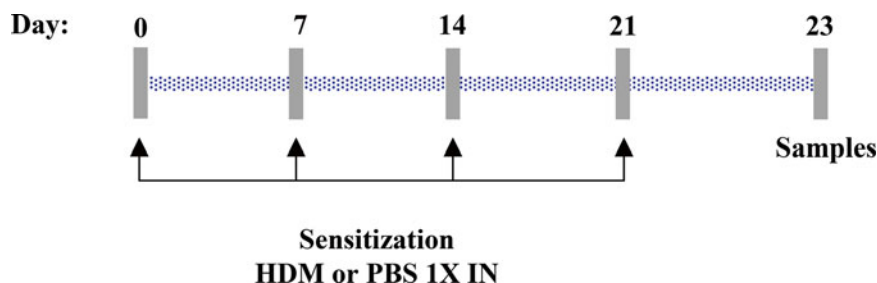


Fig. 4 Specific allergen asthma model: house dust mite (HDM)-induced asthma model protocol 1. *IN* intranasal, *PBS* phosphate-buffered saline, *HDM* house dust mite

Protocol 2

1. In this protocol, the allergen extract of house dust mite (HDM) at a concentration ten times lower than that used in protocol 1 (Fig. 5) and an adjuvant (aluminum hydroxide) are used.
2. Dissolve the HDM in 1× PBS at a concentration of 10 µg of extract plus 0.5 mg aluminum hydroxide in 50 µl of 1× PBS per mouse in the experimental group. Give 50 µl of 1× PBS with 0.5 mg of aluminum hydroxide to the control mice.
3. Prepare the 6–8 weeks old female BALB/c mice.
4. Start the experiment (day 0) with the first administration of HDM extract or 1× PBS (plus adjuvant for both), depending on the group. In this case, it is administrated by intraperitoneal pathway.
5. Maintain the mice housed for 14 days.
6. On day 14, repeat the **step 4**.
7. Maintain the mice housed for 7 days more.
8. The HDM is prepared at a concentration of 10 µg in 50 µl of extract in 1× PBS without aluminum hydroxide.
9. On day 21, perform the first provocation by administering the allergen prepared in section 8, intranasal.
10. Inject an intraperitoneal dose of ketamine (75 mg/kg) + Medetomidine (1 mg/kg) mixture.
11. The allergen deposition is intranasal.
12. Repeat **steps 10** and **11** on days 22 and 23 of the experiment.
13. On day 25 (48 h after the last allergen exposure), proceed to the collection as samples.
14. Anesthetize the animal with the same mixture described in **step 10**.
15. With the anesthetized animal, proceed to the necropsy setting the animal in *decubitus supino* position for obtaining samples (blood, BALF, tissues, and/or hematopoietic organs).

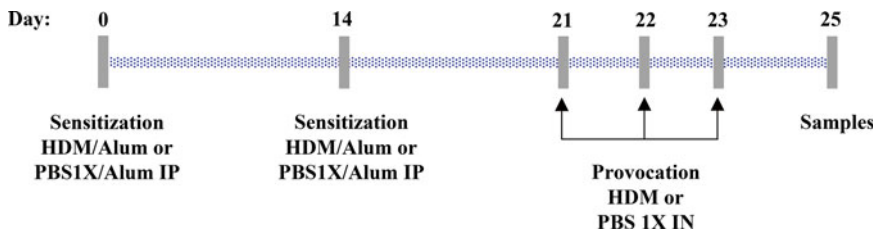


Fig. 5 Specific allergen asthma model: house dust mite (HDM)-induced asthma model protocol 2. *IP* intraperitoneal, *IN* intranasal, *PBS* phosphate-buffered saline, *HDM* house dust mite, *Alum* aluminum hydroxide

3.2.3 House Dust Mite (HDM)-Induced Severe Asthma Model

1. In this protocol, the complete Freund's adjuvant (Fig. 6) is used along with an allergen extract of house dust mite (HDM).
2. The HDM is dissolved in 1× PBS at a concentration of 100 µg of more adjuvant extract in 50 µl of PBS 1× per mouse in the experimental group. Give 50 µl of 1× PBS plus adjuvant to the control mice.
3. The experiments are performed with 6–8 weeks old female BALB/c mice.
4. Perform the first administration of HDM extract or PBS 1× (plus adjuvant for both), depending on the group (sensitization phase) on day 0. In this case, administer by sub-cutaneous pathway.
5. Maintain the mice housed for 14 days.
6. Prepare the HDM at a concentration of 100 µg of extract in 50 µl of 1× PBS.
7. On day 14, perform a single provocation by administering the allergen prepared by via intranasal.
8. Inject an intraperitoneal dose of ketamine (75 mg/kg) + Medetomidine (1 mg/kg) mixture.
9. Perform the allergen deposition intranasal.
10. On day 15 (24 h after the last allergen exposure), proceed to the collection of samples.
11. The animal is anesthetized with the same mixture described in **step 8**.
12. With the anesthetized animal, proceed to the necropsy setting the animal in *decubitus supino* position for obtaining samples (blood, BALF, tissues, and/or hematopoietic organs).

3.2.4 Molds

1. Use extract of spores of the fungus *Alternaria alternata* (strain 18586) as allergen.
2. These molds are cultured at 27 °C on potato dextrose agar plates for 1 week, before gently harvesting the spores with a cell scraper.
3. Dilute the spores in 1× PBS for counting with a hemocytometer and resuspend in 1× PBS at a concentration of $2-3 \times 10^7$ spores/ml.



Fig. 6 Specific allergen asthma model: house dust mite (HDM)-induced severe asthma model. SC sub-cutaneo, IN intranasal, HDM house dust mite, CFA complete Freund's adjuvant, PBS phosphate-buffered saline

4. Grow the mold cultures for 3 weeks at 27 °C in flasks containing 250 ml of Czapek's medium.
5. Harvest the mold pellicles and homogenize in 0.4% NH_4HCO_3 + polyvinyl pyrrolidone with an ultra-thurax.
6. Agitate the homogenates for 3 h at 4 °C.
7. Centrifuge the extracts twice, 30 min at $20,000\times g$, dialyze against 1× PBS, and store at -20 °C in 50% glycerol.
8. Perform the experiments with 6–8 weeks old female BALB/c mice.
9. To induce the allergic lung inflammation, sensitize by intraperitoneal immunization with 2×10^6 spores emulsified in aluminum hydroxide on day 0 and day 7 (Fig. 7).
10. In control mice, use 1× PBS aluminum hydroxide.
11. On day 13, 14, and 15, challenge the mice intranasal with 2×10^5 spores.
12. Give an intraperitoneal dose of ketamine (75 mg/kg) + Medetomidine (1 mg/kg) mixture.
13. Perform the allergen deposition intranasal with 100 μl of the spore solution.
14. On day 17 (48 h after the last allergen exposure), proceed to the collection of the samples.
15. Anesthetize the animal with the same mixture described in step 12.
16. With the anesthetized animal, proceed to the necropsy, setting the animal in *decubitus supino* position for obtaining the samples (blood, BALF, tissues, and/or hematopoietic organs).

3.3 Tobacco-Induced Asthma Model

1. Perform the experiments with 6–8 weeks old female BALB/c mice.
2. Divide a population of 8 mice into 2 groups, one treat with ovalbumin (OVA) (sensitized) and the other with 1× PBS (control) (Fig. 8).
3. Sensitize mice with 10 μg OVA adsorbed in 250 μg of aluminum hydroxide 0.2 ml by intraperitoneal injection on day 0, 5,

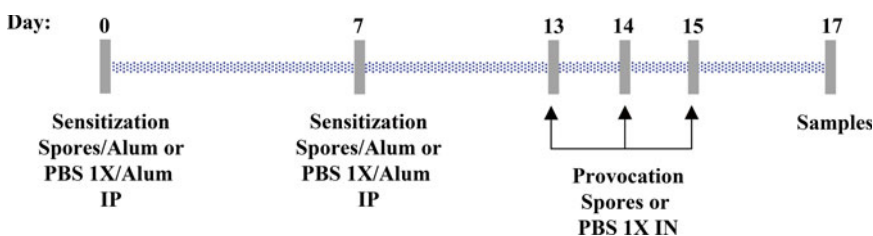


Fig. 7 Specific allergen asthma model: molds. *IP* intraperitoneal, *IN* intranasal, *PBS* phosphate-buffered saline, *Alum* aluminum hydroxide

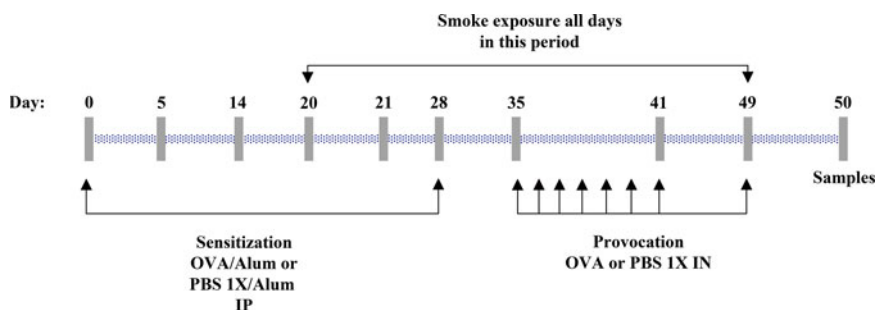


Fig. 8 Tobacco-induced asthma model. *IP* intraperitoneal, *N* Nebulization, *OVA* ovalbumin, *Alum* aluminum hydroxide, *PBS* phosphate-buffered saline

- 14, 21, and 28 in all mice except the controls that are treated with 1× PBS.
4. One week after the final injection, nebulize the mice with 2 % OVA for 7 consecutive days from day 35 to 41, and then again nebulize with 2 % OVA on day 49.
5. Nebulize the control mice with 1× PBS.
6. Divide each group in two subgroups. Expose two mice to the tobacco smoke and the other two mice not (1× PBS).
7. Expose the mice to tobacco smoke produced by a cigarette in a Plexiglas chamber (16×25×16 cm) with an inlet for pressurized air (air: smoke = 3:1).
8. Expose mice from the day 20 to day 49 (30 days), with or without smoke exposure at same times.
9. On the first day (day 20), administer the smoke of a cigarette for 10 min, and on the second day the smoke of two cigarettes.
10. Increase gradually the amount of cigarette smoke daily, one cigarette per day for 5 days.
11. The interval between smoke exposures is 15 min.
12. After the fifth day, expose the animals to five cigarettes per day from day 25 to day 49.
13. Proceed to the necropsy of the mice the following day (day 50).

Chronic asthma models can also be developed using LPS. The experiments can be performed as well with 6–8 weeks old female BALB/c mice. In these models, the mice are exposed to multiple intratracheal instillations of *E. coli* LPS with a delivered dose of (5 µg/mouse) equivalent to smoking approximately 25 cigarettes. In contrast to smoking models, the amount of insult added is known. The chronic inflammation is induced by giving mice a non-surgical intratracheal instillation of LPS (2 doses per week) for a total of 12 weeks.

Other interesting model is the intrinsic asthma model. The hyperreactivity is demonstrated to have a marked bronchoconstriction induced by methacholine. A widely used for the study of intrinsic asthma model is the mouse strain A/J. This strain presents bronchial hyperreactivity without treatment, handling and independently to the allergen employed.

4 Notes

1. Mice can be bred from established colonies or bought in laboratories specialized in breeding animals for research (e.g., Charles Rivers Lab). If you buy from an outside laboratory, it is advisable to acquire animals with 6 weeks of life, because, once received must remain in quarantine for 8 days as an adaptation to the recipient animal house. Thus begin experiments with them at 7 weeks. Start with mice of the same age in all experiments. Calculate the number of animals according to the specific requirements of your experiment.
2. In addition to the BALB/c strain, also can use the C57BL strain. In our studies, the BALB/c strain provides a better and stronger response to the treatment. To see the differences between the two strains at the level of experimentation in asthma, *see* Chapter 15 of this book.
3. The animals are kept in standard housing conditions. Keep them under sterile conditions, in cages (no more than 6 mice per cage) placed on racks thermostatic to 37 °C and protected with filter caps. The animals are maintained under a 12-h light-dark cycle, with *ad libitum* access to water and standard laboratory food. The animal handling is done in laminar flow hood to maintain sterility.
4. All experimental procedures must conform to international standards of animal welfare and approved by the Animal Experimentation Ethics Committee of the work institution.
5. The aluminum hydroxide acts as adjuvant allowing a slower and prolonged release of antigen (OVA in this case).
6. It has to achieve enough level of sedation to work properly with animals, but not overdo it to avoid staying too long sedated and thus facilitate their post-sedation recovery.
7. Due to the small size of mice, the volumes of the inoculated anesthetic mixture should be low. To inoculate a larger volume and thus work more safely, you should dilute the anesthetic mixture ½ in 1× PBS.
8. The anesthetic mixture is inoculated in the lower right part of the animal. For this maneuver, put the head of the animal

down to avoid visceral perforation during the process. Also, employ short needles for this step.

9. Once the anesthetic mixture is inoculated, the animal is placed in the cage until sedation. The level of animal consciousness can be assessed by auscultation of the palmar and ocular reflex.
10. For controlling the sedation level, the animal should recover in a period not exceeding 1 h. This time is more than enough to perform the maneuvers at that point.
11. To follow lung disease in mice, it can determine the respiratory frequency by counting the number of breaths per min, both pre- and post-provocation.
12. Increase the volume of dilute anesthetic mixture or use undiluted, to ensure a deeper level of sedation, if desired in this step.
13. The Clinical Score establish a scale of 0–5 as a function of the signs observed as described below:
 - Score 0: No symptoms.
 - Score 1: Scratching head and snout.
 - Score 2: Periocular-peribuccal edema, piloerection, decreased activity, and increased to a respiratory rate.
 - Score 3: Breath sounds, increased work of breathing, cyanosis peribuccal and tail.
 - Score 4: Absence of activity in response to stimulation, tremors, or seizures.
 - Score 5: Dead.
14. Due to its long duration, the chronic model may be necessary to analyze an *in vivo* sample taken at an intermediate point. The sample will be mainly blood that can be obtained from several extraction points. Without anesthesia, we can get it from the dorsal part of the rear leg, pedea or saphenous, with anesthesia from the tail vein or carotid [18].

Acknowledgments

This work was supported by grants of the Junta de Castilla y León ref. GRS1047/A/14 and GRS1189/A/15.

References

1. Kim HY, DeKruyff RH, Umetsu DT (2010) The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat Immunol* 7:577–584
2. Nials AT, Uddin S (2008) Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis Model Mech* 1:213–220
3. McMillan SJ, Xanthou G, Lloyd CM et al (2005) Therapeutic administration of budesonide ameliorates allergen-induced airway remodelling. *Clin Exp Allergy* 35:388–396
4. Tomkinson A, Duez C, Cieslewicz G et al (2001) A murine IL-4 receptor antagonist that

- inhibits IL-4- and IL-13-induced responses prevents antigen-induced airway hyperresponsiveness. *J Immunol* 166:5792–5800
5. Henderson WR, Lewis DB, Albert RK et al (1996) The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J Exp Med* 148:1483–1494
6. Temelkovski J, Hogan SP, Shepherd DP et al (1998) An improved murine model of asthma. *Thorax* 53:849–856
7. Henderson WR, Tang L, Chu SJ et al (2002) A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 164:108–116
8. Fraga-Iriso R, Núñez-Naveira L, Brienza NS et al (2009) Desarrollo de un modelo murino de inflamación y remodelación de vías respiratorias en asma experimental. *Arch Bronconeumol* 45(9):422–428
9. Zosky GR, Sly PD (2007) Animal models of asthma. *Clin Exp Allergy* 37:973–988
10. Kumar RK, Herbert C, Foster PS et al (2008) The classical ovalbumin challenge model of asthma in mice. *Curr Drug Targets* 9:485–494
11. Lloyd CM (2007) Building better mouse models of asthma. *Curr Allergy Asthma Rep* 7:231–236
12. Cates EC, Fattouh R, Wattie J et al (2004) Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J Immunol* 173:6384–6392
13. Conejero L, Higaki Y, Baeza ML et al (2007) Pollen-induced airway inflammation, hyperresponsiveness and apoptosis in a murine model of allergy. *Clin Exp Allergy* 37:331–338
14. Havaux X, Zeine A, Dits A et al (2004) A new mouse model of lung allergy induced by the spores of *Alternaria alternata* and *Cladosporium herbarum* molds. *Clin Exp Immunol* 139: 179–188
15. Maes T, Provoost S, Lanckacker EA et al (2010) Mouse models to unravel the role of inhaled pollutants on allergic sensitization and airway inflammation. *Respir Res* 11:7
16. Kim DY, Kwon EY, Hong GU et al (2011) Cigarette smoke exacerbates mouse allergic asthma through Smad proteins expressed in mast cells. *Respir Res* 12:49
17. Hadeiba H, Corry DB, Locksley RM (2000) Baseline airway hyperactivity in A/J mice is not mediated by cells of the adaptive immune system. *J Immunol* 164(9):4933–4940
18. Hoff J (2000) Methods of blood collection in the mouse. *Lab Anim* 29:10