**CHAPTER 17**

**TITLE**

Mouse Models of Asthma

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# Summary

The complexity of asthma requires the development of different animal models to study underlying mechanisms. As previously described mice represent a useful tool for the development of human disease model. This chapter will describe different animal models for studying the asthma phenotypes.



**Key words:**Acute, Allergy, Asthma, Chronic, Mouse, Sensitization

# 1. Introduction

Asthma is a heterogeneous disease, in which both inflammation and remodeling of the airway are produced (**1**). The underlying molecular and cellular mechanisms are not entirely understood and animal models represent an alternative to investigate the disease and its progression. Due to the complexity of asthma, a single model is not sufficient to reproduce the disease (**2**). Different protocols using ovalbumin (OVA) as allergen have been described in which the exposure can be acute or chronic (see tables 1 and 2)

Acute models are useful for studying the inflammatory response, whereas chronic models better replicate the clinical features of asthma as well as the remodeling of the airway and bronchial hyperresponsiveness (**9, 10, 11**). Other models usingallergens that cause human disease such as House Dust Mite (HDM) (**12**) pollen (**13**) or fungal spores (**14**) have also been developed. In addition, mouse models to study non-allergic asthma have also been proposed (**15, 16**, **17**). Thus, in this chapter we will describe different models for the study of both allergic and non-allergic asthma.

# 2. Materials

## 2.1. Animals

1. Adult females BALB/c 6-8 weeks old (Charles Rivers Lab)
2. Adult females A/J 6-8 weeks old (Charles Rivers Lab)

## 2.2. Common Material

1. 1X PBS (Phosphate-Buffered Saline) 1.37 mMNaCl, 2.6 mMKCl, 10 mM Na2HPO4, 1.8 mM KH2PO4. Adjust the pH to 7.4 with HCl.
2. Tuberculin syringes (BDBiosciences)
3. Needle 20G short (BDBiosciences)
4. Ketamine (Imalgene 1000®, Merial)
5. Medetominine (Medetor®, Virbac)

## 2.3. Models of Allergic Asthma

## 2.3.1Allergic Models with Ovoalbumin

1. Ovoalbumin (OVA) (Sigma-Aldrich)
2. Aluminium hydroxide (Sigma-Aldrich)

## 2.3.2 Allergen-specific Models

## 2.3.2.1 Pollen

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

## 2.3.2.2 Dust / Mites

## 2.3.2.2.1 House Dust Mite (HDM)-induced Asthma Model

1. House Dust Mite (HDM) Extract (Allergopharma)

## 2.3.2.2.2 House Dust Mite (HDM)-induced Severe Asthma Model

1. House Dust Mite (HDM) Extract (Allergopharma)

2.Freund´s adjuvant

## 2.3.2.3 Molds

1.*Alternariaalternata*(strain18586)

2. Potato dextrose agar plates (Difco)

3. Cell scraper

4. Haemocytometer

5. Czapek’s medium

6. NH4HCO3+polyvinyl polypyrrolidone (Sigma-Aldrich)

7. Glycerol

8. Aluminium hydroxide (Sigma-Aldrich)

# 2.4 Non-Allergic Models

## 2.4.1 Asthma Induced by Tobacco Smoke

1. Plexiglaschamber

2. Nebulizer (Mega Medical, Seoul, Korea)

## 2.4.2 LPS induced Asthma Model

1. *E. coli* LPS

# 3. Methods

## 3.1 Model of Allergic Asthma

## 3.1.1 Allergic Asthma with Ovoalbumin

These models are shown asAcute and Chronic according to the duration of the treatment,

### 3.1.1.1 Acute Model

1. The experiments are performed with mice adult female BALB / c of 6-8 weeks old (see Note 1-2) (Figure 1).
2. Animals must be kept confined meeting the current ethics rules of handling animals for experimentation (see Note 3-4).
3. The number of mice should be calculated according to the experiment. As an example we will describe a protocol for 8 mice. The animals are divided into 2 groups:
    - Group 1: 4 mice that are not administered Intra-peritoneal / Intra-nasal OVA (in place are given PBS 1X) as control group.
    - Group 2: 4 mice administered Intra-peritoneal / Intra-nasal OVA.

4. On day 0 (start of experiment), the animals are injected, by intra-peritoneal route, with 20 µg of OVA emulsified in 0.2ml 1X PBS containing 2 mg of aluminium hydroxide (these doses are administered to each mouse). Controls are injected with 0.2 ml of 1X PBS containing 2 mg of aluminium hydroxide (see Note 5).

5. Animals are kept stabled for one week (7 days) for the reaction of hyper-sensitization to OVA occurs.

6. On day 7 a second dose of OVA is administered to reinforce hyper-sensitization. The intra-peritoneal injection is repeated as in point 4.

7. Housed mice are kept for another 7 days.

8. After 14 days the provocation phase began. A total of four provocations is performed.

9. The first intra-nasal provocation with OVA is performed on day 14. The mouse has to be anesthetized subjected to mild sedation by administering an intra-peritoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg). Wait for the complete sedation of the animal before starting handling (see Notes 6-9).

10. The animal is placed upright with the nose up, and 50 µl of 1X PBS are given to the control group or 50 µl of OVA (0.1% in PBS 1X) to the experimental group. Administer by dropping on the snout (helping with a micropipette), favoring inoculation during the animal's inspiratory process.

11. Keep the animal in that position for 1-2 minutes.

12. Return the animal to the cage, and place it horizontally on one side to favor its ventilation (see Note 10).

13. The procedure described in points 9 to 13 is repeated 3 times with a period of 2 days (48h) between provocations corresponding to day 16 (second provocation), 18 (third provocation) and 20 (fourth provocation).

14. On day 22 proceed to the collection of samples. Animals are sacrificed 2 day after the last provocation. The animal is anesthetized with the same mixture described in paragraph 10 (see Note 11).

15. Proceed to the necropsy with the anesthetized animal. The mouse is placed in *decubitus supino* position fixing it to a surface by the legs.

16. The procedure for obtaining samples:

    - Blood is obtained by cardiac puncture at the ventricular level. This puncture serves as gathering blood and as euthanasia method. Blood provides cells and serum free molecules.

    - BALF (Bronchoalveolar Lavage): Canulate the trachea, inject 1ml of PBS 1X 2 times and recover. BALF is used for obtaining local cells and soluble factors.

    - Tissues: The organs of interest are collected for molecular biology as well as histological studies.

    - Hematopoietic organs can be collected for *in vitro* cell culture.

## 3.1.1.2 Chronic Model

1. The experiments are performed with mice adult female BALB / c of 6-8 weeks old (see Note 1-2) (Figure 2).

2. Animals must be kept confined meeting the current ethics rules of handling animals for experimentation (see Note 3-4).

3. It starts with a population of female BALB / c mice, 8 mice for example. The animals are divided into 2 groups:

    - Group 1: 4 mice that were not administered OVA Intra-peritoneal / Intra-nasal (in place PBS 1X is given) as control group.

    - Group 2: 4 mice administered OVA Intra-peritoneal / Intra-nasal.

4. On day 0 (start of experiment), the animals are injected, by intra-peritoneal route, with 20 µg of OVA emulsified in 0.2ml 1X PBS containing 2 mg of aluminium hydroxide (these doses are administered to each mouse). Controls are injected with 0.2 ml of 1X PBS containing 2 mg of aluminium hydroxide.

5. Animals are kept stabled for one week (7 days) for the reaction of hyper-sensitization to OVA occurs.

6. On day 7, the second OVA dose is administered, to reinforce hyper-sensitization. Repeat point 4.

7. Housed mice are kept for other 7 days.

8. On day 14 the provocation phase is started. In this model provocation frequency changes performing three provocations per week.

9. The first intra-nasal provocation with OVA on day 14 of the experiment is performed after anesthetize the animal. Administer an intraperitoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg) and wait for the complete sedation of the animal before starting to handle the animals.

10. The sedated animal is placed upright with his nose up and 50 µl of 1X PBS are given to the control group or 50 µl of OVA (0.1% in PBS 1X) to the experimental group dropping the mixture to the snout (helping with a micropipette), favoring inoculation during the inspiratory process.

11. Keep the animal in that position for 1-2 minutes.

12. Return the animal to the cage, placing horizontally on one side for favoring ventilation

13. In this chronic model the clinical course should be monitored using a Clinical Score (see Note 13).

14. The procedure described in points 11 to 14 is repeated 3 times per week for 12 weeks (3 months).

15. Record the signs observed in mice during this period.

16. After that time, proceed to the collection of samples. The sacrifice of animals is performed 2 days (48h) after the last provocation

17. The animal is anesthetized with the same mixture described in paragraph 11.

18. With the anesthetized animal, we proceed to the necropsy. The mouse is placed in *decubitus supino* position fixing it to a surface by the legs.

19. The procedure for obtaining samples:

    - Blood is obtained by cardiac puncture at the ventricular level. This puncture serves as gathering blood and as euthanasia method. Blood provides cells and serum free molecules.

    - BALF (Bronchoalveolar Lavage): Canulate the trachea, inject 1ml of PBS 1X 2 times and recover. BALF is used for obtaining local cells and soluble factors.

    - Tissues: The organs of interest are collected for molecular biology as well as histological studies.

    - Hematopoietic organs can be collected for *in vitro* cell culture.

### 3.1.1 Allergen-specific Model

In this section we describe asthma mouse models, in which relevant allergens are used.

## Pollen

1. *Oleaeuropaea* (Olive), responsible for a high percentage of seasonal allergic asthma in the Mediterranean region, (Figure 3) is used as specific allergen.

2. The experiments are performed with mice adult female BALB / c of 6-8 weeks old.

3. Mice are sensitized by subcutaneous injections of crude olive pollen (*O. europaea*) extract.

4. The mice are divided into two groups:

    - Control group: Inoculated with 1X PBS.

    - Experimental group: Inoculated with pollen allergen.

5. Prepare the mixture of 25µg pollen extract with 50 mg of aluminium hydroxide in 200 µl of PBS 1X for each experimental mouse and inoculation.

6. On day 0, subcutaneously inoculates the control group with 200 µl of PBS 1X, and the experimental group with 200 µl of the prepared mixture in point 5.

7. During the stage of hyper-sensitization, make an inoculation per week for the first 3 weeks.

8. From week 3 to 10 the animal is not treated.

9. At week 10, initiate the provocation phase.The first challenge is performed 3 days before slaughter of animals.

10. Mice are given an intraperitoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg) mixture, achieving a smooth sedation.

11. Place the sedated animal, horizontally with the muzzle up, and using a micropipette, intranasalinoculate 30 µl of pollen extract (15 µg of extract of pollen per mouse and provocation) to the experimental group, and 30 µl of PBS 1X to the control group.

12. 24 hours after, repeat steps 11 and 12 for the second provocation.

13. After 48 hours, animals are sacrificed.

14. The animal is anesthetized with the same mixture described in point 11.

15. With the anesthetized animal, proceed to the necropsy, setting the animal in *decubitus supino* position to obtain samples as previously described.

## 3.1.1.2 Dust/Mite

## 3.1.1.2.1 House Dust Mite (HDM)-induced Asthma Model

**Protocol 1**

1. House dust mite (HDM) is responsible for a high percentage of non-seasonal allergic asthma worldwide (Figure 4).

2. The HDM is dissolved in 1X PBS at a concentration of 100 µg of extract in 50 µl of 1X PBS (without adjuvant) per mouse of the experimental group. In control mice 50 µl of 1X PBS are given.

3. The experiments are performed with mice adult female BALB / c of 6-8 weeks old.

4. Day 0, the first administration of HDM extract or PBS 1X, depending on the group, is performed intranasal.

5. Mice are given an intraperitoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg) mixture, achieving a smooth sedation.

6. Repeat steps 4-6 on days 7, 14 and 21 of the experiment.

7. On day 23 (48 hours after the last allergen exposure) proceed to the collection of samples.

8. The animal is anesthetized with the same mixture described in point 5.

9. With the anesthetized animal, proceed to the necropsy,setting the animal in *decubitus supino* position to obtain samples (blood, BALF, tissues and / or hematopoietic organs).

**Protocol 2**

1. In this protocol the allergen is used at a 10 times lower concentration than used in protocol 1 with adjuvant (aluminium hydroxide) (Figure 5).

2. The HDM is dissolved in 1X PBS at a concentration of 10 µg of extract plus 0.5 mg aluminium hydroxide in 50 µl of 1X PBS per mouse in the experimental group. In control mice, 50 µl of 1X PBS with 0.5 mg of aluminium hydroxide are given.

3. The experiments are performed with mice adult female BALB / c of 6-8 weeks old.

4. Start on day 0 the first intraperitonealadministration of HDM extract or PBS 1X (plus adjuvant for both), depending on the group (sensitization phase).

5. Housed mice are maintained for 14 days.

6. On day 14, step 4 is repeated.

7. Housed mice are maintained 7 days more.

8. The HDM is prepared at a concentration of 10 µg in 50 µl of extract 1X PBS without aluminium hydroxide.

9. On day 21, the first provocation via intra-nasal is performed by administering the allergen prepared in section 8. Mice are given an intraperitoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg) mixture, achieving a smooth sedation and allergen deposition is intranasalperformed.

10. Repeat step 9 the days 22 and 23 of the experiment.

11. Day 25 (48 hours after the last allergen exposure), proceeds to the collection as samples.

12. The animal is anesthetized with the same mixture described in point 10.

13. With the anesthetized animal, proceed to the necropsy setting the animal in *decubitus supino* position to obtain samples (blood, BALF, tissues and / or hematopoietic organs).

## 3.1.1.2.2Severe Asthma ModelHDM-induced

1. In this protocol the complete Freund's adjuvant (Figure 6) is used. This adjuvant is compound for suspension of *Mycobacterium* heat inactivated in mineral oil plus dispersant agent (egmanoleate)

2. The HDM is dissolved in 1X PBS at a concentration of 100 µg more Freund's adjuvant extract in 50 µl of PBS 1X per mouse in the experimental group. In control mice, are given 50 µl of 1X PBS plus Freund's adjuvant.

3. The experiments are performed with mice adult female BALB / c of 6-8 weeks old.

4. The first administration of 50µl of HDM extract or PBS 1X (plus adjuvant for both), depending on the group (sensitization phase) is performed on day 0 subcutaneously.

5. Housed mice are maintained for 14 days. The HDM is prepared at a concentration of 100 µg of extract in 50 µl of 1X PBS.

6. On the day 14 a single provocation is performed by administering the allergen prepared in point 5 by via intra-nasal. Mice are given an intraperitoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg) mixture, achieving a smooth sedation.

7. The day 15 (24 hours after the last allergen exposure), proceed to the samples collection. The animal is anesthetized with the same mixture previously described.

8. With the anesthetized animal, proceed to the necropsy setting the animal in *decubitus supino* position to obtain the samples (blood, BALF, tissues and / or hematopoietic organs).

### 3.1.1.3Molds

1. Spores of the fungus *Alternaria alternate* (strain18586) are used as allergen extract.

2. These molds are cultured at 27ºC on potato dextrose agar plates for one week before gently harvesting the spores with a cell scraper.

3. Spores are diluted in PBS 1X, counted with a haemocytometerand resuspended in PBS 1X at a concentration of 2-3x$10^7$ spores/ml.

4. Mold cultures are grown for 3 weeks at 27ºC in flasks containing 250 ml of Czapek's medium.

5. Mold pellicles are harvested and homogenized in 0.4% NH4HCO3 + polyvinyl polypyrrolidone with an ultra-thurax.

6. The homogenates are then agitated for 3 h at 4ºC.

7. Extracts were centrifuged twice 30 minutes at 20 000 g, dialyzed against PBS 1X and stored at -20ºC in 50% glycerol.

8. The experiments are performed with mice adult female BALB / c of 6-8 weeks old.

9. Mice are sensitized by intraperitoneal immunization with 2x$10^6$ spores emulsified in aluminium hydroxide on day 0 and day 7 (Figure 7).

10. Control mice are immunized with PBS 1 X aluminium hydroxide.

11. On day 13, 14 and 15 mice are intranasalchallenged with 2x$10^5$ spores. Mice are given an intraperitoneal dose of Ketamine (75 mg / Kg) + Medetominine (1 mg / kg) mixture, achieving a smooth sedation.

12. Allergen deposition is performed via intra-nasal with 100 µl of the spore solution.

13. The day 17 (48 hours after the last allergen exposure), proceed to the collection of samples.

14. The animal is anesthetized with the same mixture described in point 11.With the anesthetized animal proceed to the necropsy setting the animal in *decubitus supino* position to obtain samples (blood, BALF, tissues and / or hematopoietic organs).

## 3.2 Models of Non Allergic Asthma

### 3.2.1 Asthma Induced by Tobacco Smoke

1. The experiments are performed with mice adult female BALB / c of 6-8 weeks old.

2. A population of 8 mice is divided into 2 groups, one is treated with ovoalbumin (OVA) (sensitized) and the other with 1X PBS (control) (Figure 8).

3. Mice are sensitized with 10 μg OVA adsorbed in 250 μg of aluminium hydroxide 0,2 ml by intraperitoneal injection on days 0, 5, 14, 21, and 28 in all mice except controls that are treated with PBS 1X.

4. One week after the final injection, mice are nebulized with 2% OVA for 7 consecutive days from day 35 to 41, and then are again nebulized with 2% OVA onday 49. Controls are nebulized with PBS 1X.

5. For exposure to smoke, miceare subjected to whole-body mainstream cigarettesmoke exposure produced by a cigarette in a Plexiglaschamber (16 × 25 × 16 cm) with an inlet for pressurized air (air: smoke = 3:1).

6. Mice are daily exposed from day 20 to day 49 (30days), with or without smoke exposure according to the experimental group

7. On the first day(day 20), the smoke of a cigarette is administered for10 minutes, and on the second day the smoke of two cigarettesis administered.

8. The amount of cigarette smokeis daily increased by one cigarette per dayfor five days.The interval between smoke exposures is15 minutes.

9. After the five day, the animals are exposed tofive cigarettes per day from day 25 to day 49.

10. All mice aresacrificed the following day (day 50).

### 3.2.1.2 LPS induced Asthma Model

1. The experiments are performed with mice adult female BALB / c of 6-8 weeks of age.

2. In this model, mice are exposed to multiple intratracheal instillations of *E. coli* LPS (Figure 9).

3. The dose of LPS delivered (5µg/mouse) is equivalent to smoke 25 cigarettes approximately.

4. In contrast with smoking models, the amount of insult added is known.

5. Chronic inflammation is induced by a non-surgical intratracheal instillation of LPS (2 doses per week) for a total of 12 weeks.

## 4. Notes

Note 1: The mice can be bred from established colonies or buy them from specialized laboratories (eg, Charles Rivers Lab). If you buy from an outside laboratory, it is advisable to acquire animals with six weeks of life, because, once received the mice must remain in quarantine for 8 days as an adaptation to the animal house. Thus begin the experiment at week 7.Start all the experiments with animals at the same age.

Note 2: In addition to the BALB / c strain, the C57BL strain can also be used. In our studies we always use the BALB / c strain, obtaining the stronger immune response to the treatment. To see the differences between the two strains see chapter "Application of Mouse Models to the Study of Asthma" in this book.

Note 3: The animals are kept in standards housing conditions, in cages (no more than 6 mice per cage) placed on racks thermostatic to 37ªC and protected with filter caps. Animals are maintained under a 12-hour light-dark cycle, with *ad libitum* access to water and standard laboratory food. Install the vivarium with aspect of reality, in laminar flow hood to maintain sterility.

Note 4: All experimental procedures must be conformed to international standards of animal welfare and be approved by the Animal Experimentation Ethics Committee of the work institution.

Note 5: Aluminium hydroxide acts as adjuvant, allowing a slower and prolonged release of antigen (OVA in this case).

Note 6: It has to be achievedenough level of sedation to properly work with animals, but not overdo it to facilitate their post-sedation recovery.

Note 7: Due to the small size of mice the inoculated volumes of the anesthetic mixture must be low. To inoculate a larger volume and thus work more safely, you should dilute the anesthetic mixture ½ in PBS 1X.

Note 8: The anesthetic mixture is inoculated in the lower right part of the animal. For this maneuver, the animal should beupside-down, with his head down to avoid visceral perforation during the process. In addition,short needles are employed for this step.

Note 9: Once the anesthetic mixture is inoculated, the animal is placed in the cage until sedation. Monitor respiratory rate and effort, color of mucous membranes at regular intervals (no longer than 15 minute intervals). Assess level of anesthesia by loss of pedal reflex (toe pinch) or loss of pinna reflex (ear flap pinch).

Note 10: The ideal is that the animal recovers in a period not exceeding one hour. Place rodent in warm, dry, clean,quietenvironmentawayfromotheranimals, cover orreplacebedding material withtoweling material. Rodents must be continuouslymonitoreduntilmaintainingupprightposture and walkingnormally before returntothe animal housing room.

Note 11: We can put more volume of dilutedanesthetic mixture or use undiluted to ensure a deeper level of sedation at this step.

Note 12: To follow lung function in mice, the respiratory frequency can be determined by counting the number of breaths per minute, both pre- and post-provocation.

Note 13: The Clinic Score establishes a scale of 0-5 as a function of the signs observed:

- Score 0: No symptoms.
- Score 1: Scratching head and snout.

- Score 2: Periocular-peribucal edema, piloerection, decreased activity and increased to a respiratory rate.
- Score 3: Breath sounds, increased work of breathing, cyanosis and tail.
- Score 4: Absence of activity in the absence to the stimulus, tremors or seizures.
- Score 5: Dead.

Note 14: In the chronic model, at an intermediate stage, it may be necessary to perform an *in vivo* sampling. Blood can be obtained without anesthesia we can from the dorsal part of the rear leg (Pedal vein) or saphenous, and with anesthesia from the tail or the carotid (**18**).

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**Table 1. Different treatment strategies in acute OVA mouse models.** Description of the differences in both sensitization and challenge stages.

| Sensitization | Challenge | References |
|---|---|---|
| OVA/Alum (IP) 0 & 14 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 |

All mice female strain BALB/c

OVA: Ovoalbumin, Alum: Aluminium hidroxide, IP: Intraperitoneal, IN: Intranasal

**Table 2. Different treatment strategies in chronic OVA mouse models.** Description of the differences in both sensitization and challenge stages.

| Sensitization | 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 & 73-75 days | 7 |
| OVA/Alum (IP) 0 & 14 days | OVA IN 12 weeks, 3 days/week | 8 |

All mice female strain BALB/c

OVA: Ovoalbumin, Alum: Aluminium hidroxide, IP: Intraperitoneal, IN: Intranasal

**FIGURE LEGENDS**

**Figure 1. General Acute Mouse Model.**IP: Intra-peritoneal, IN: Intra-nasal, PBS: Phosphate Saline Buffer, OVA: Ovoalbumin, Alum: Aluminium hydroxide

**Figure 2. General Chronic Mouse Model.**IP: Intra-peritoneal, IN: Intra-nasal, PBS: Phosphate Saline Buffer, OVA: Ovoalbumin, Alum: Aluminium hydroxide

**Figure 3. Allergen-specific Model: Pollen.** SC: Sub-cutaneus, IN: Intra-nasal, PBS: Phosphate Saline Buffer

**Figure 4. Allergen-specific Model: House Dust Mite (HDM)-induced Asthma Model Protocol 1.** IN: Intra-nasal, PBS: Phosphate Saline Buffer, HDM: House Dust Mite

**Figure 5. Allergen-specific Model: House Dust Mite (HDM)-induced Asthma Model Protocol 2.** IP: Intra-peritoneal, IN: Intra-nasal, PBS: Phosphate Saline Buffer, HDM: House Dust Mite, Alum: Aluminium hydroxide

**Figure 6. Allergen-specific Model: House Dust Mite (HDM)-induced Severe Asthma Model.** SC: Sub-cutaneo, IN: Intra-nasal, HDM: House Dust Mite, CFA: Complete Freund´s Adjuvant, PBS: Phosphate Buffer Saline

**Figure 7. Allergen-specific Model: Molds.** IP: Intra-peritoneal, IN: Intra-nasal, PBS: Phosphate Saline Buffer, Alum: Aluminium hydroxide

**Figure 8. Asthma Induced by Tobacco Smoke: IP: Intra-peritoneal, N: Nebulization, OVA: Ovoalbumin, Alum: Aluminium hydroxide, PBS: Phosphate Buffer Saline**

**Figure 9. LPS induced Asthma Model.** LPS: Lipo-polisacarid, IT: Intra-traqueal, PBS: Phosphate Buffer Saline