



Chapter 7

Eosinophils in Asthma Models to House Dust Mite for Drug Development

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Abstract

Murine models of asthma are developed to better understand the mechanisms of asthma including eosinophil recruitment in the airways with the aim of evaluating new therapeutic strategies. They are intended to model the typical features of human disease, in particular airway inflammation, hyperresponsiveness (AHR), and remodeling. The phenotype of inflammatory cells recovered from the bronchoalveolar lavage fluid (BAL) is studied with innovative flow cytometry techniques while airway obstruction is measured using the forced oscillation technique, and airway responsiveness approached by barometric plethysmography in awake and unconstrained animals. We here describe models of asthma of house dust mite (HDM) as a clinically relevant allergen: a short study design (8 days) model of hypereosinophilic asthma and a chronic (31 days) asthma model, both suitable to evaluate the potential of new drug candidates to prevent allergic asthma.

Key words Allergic asthma, Mouse model, House dust mite (HDM), Ovalbumin (OVA)

1 Introduction

Asthma is defined as a chronic eosinophilic inflammatory disorder of the airways, clinically characterized by intermittent, reversible airway obstruction and airway hyperresponsiveness (AHR) [1], where repeated exposure to allergen leads to chronic inflammation of the airways [2]. A large eosinophilic infiltrate is associated with airway remodeling including subepithelial fibrosis, goblet cell hyperplasia, airway smooth muscle thickening, and increased vascularization. A large amount of knowledge on the mechanisms of allergic asthma results from animal studies. Among the animal models, the mouse is the most widely used species nowadays. Although not naturally prone to asthma, mice mount asthma-like artificial symptoms with airway inflammation and hyperresponsiveness likely similar to those in human. However, differences persist in the pathophysiology of human and murine asthma. AHR in mice is transient after allergen challenge, whereas it is present

continuously in humans even in the absence of symptoms [3]. Chronic models are more difficult to develop in mice in the absence of environmental cofactors such as pollution or lung infection. Thus, allergic asthma models in the mouse do not replicate human disease, but support most existing paradigms and are useful to inform the development of new anti-asthma strategies [4].

The most commonly used allergens in mouse models of asthma are house dust mite extracts (HDM) and ovalbumin derived from chicken egg (OVA) [5, 6]. HDM exposure is the most common in the environment, inducing allergic responses in 85% of asthma patients [1]. It is thus defined as a realistic, clinically relevant allergen.

We here describe the optimized procedures to sensitize and challenge mice i) in a short design model suitable for two allergens: HDM (this chapter) or OVA [5] and ii) in a chronic model of low doses of HDM without adjuvant. The short models generate marked airway inflammation, remodeling, and hyperresponsiveness measured by the forced oscillation technique (Flexivent[®]) [7] and are well adapted to drug candidate testing in early stages of development. The chronic model to HDM is particularly well adapted to study curative anti-asthma activity of drug candidates.

2 Materials

Solutions of HDM extract are prepared, aliquoted, and frozen ready-to-use as a standardization to increase reproducibility between experiments. All solutions are prepared using sterile and pyrogen-free commercial saline (NaCl 0.9%, B. Braun, Boulogne, France), and all reagents are kept on ice.

1. HDM solutions: Prepare a sterile 800 µg/mL solution of HDM extract (total protein weight) as follows: reconstitute the HDM lyophilisate (Stallergènes-Greer, Der p1) in the required volume of cold sterile saline directly in the supplier tube, and vortex. If required, depending on the manufacturer information about endotoxin levels in the HDM lyophilisate, adjust the endotoxin concentration using lipopolysaccharide endotoxin (LPS, *E. coli*, O55:B5) to a final concentration of 80 ng/mL in the HDM solution (100 ng LPS/mg HDM, *see Note 1*). Use a concentrated LPS solution of 1.6 mg/mL to add only few microliters of LPS in the HDM-reconstituted solution.
2. Aliquot HDM stock solution as (i) 0.55 mL into 1.5 mL and (ii) 25 µL in 0.5 mL Eppendorf[®] protein LoBind microtubes. Freeze immediately and store aliquoted at –80 °C.

3. Aluminum hydroxide powder: Right at the time the aluminum hydroxide powder [$\text{Al}(\text{OH})_3$, alum, Sigma-Aldrich, cat. no. 239186] arrives at the lab (*see Note 2*), weigh aliquots of 80 mg in 5 mL Eppendorf[®] tubes. Be careful to store alum aliquots protected from light at room temperature.
4. In parallel, prepare aliquots of ready-to-use sterile saline for control experiments. Transfer 1 mL sterile saline into 1.5 mL microtubes and 4 mL saline into sterile 5 mL tubes (Eppendorf[®], 0030122321). Immediately store the aliquots at -80°C .
5. HDM and alum conjugates: Conjugation of HDM to alum is a critical step to guarantee the reproducibility of sensitization. Before use, bring an aliquot of 800 $\mu\text{g}/\text{mL}$ of HDM stock solution and an aliquot of 4 mL sterile saline to room temperature. Take an aliquot of alum (80 mg), and add 0.5 mL HDM stock solution and 3.5 mL sterile saline. This suspension should be prepared fresh every day.
6. Gently homogenize the HDM-alum suspension for 4 h at 4°C on a rotator mixer to allow adsorption of HDM on alum (alum is not soluble and this will be presented as a suspension).
7. Anesthetic solution for sensitization and challenge: In a 5 mL Eppendorf[®] tube, add 0.5 mL of a 100 g/L commercial solution of ketamine (Merial, Imalgene[®]), 0.17 mL of a 20 g/L commercial solution of xylazine (Bayer, Rompun[®] 2%), and 3.33 mL of sterile saline. The prepared solution contains 12.5 mg/mL ketamine base and 0.83 mg/mL xylazine base from hydrochloride. This preparation must be prepared extemporaneously.
8. Anesthetic solution for bronchoalveolar lavage: In a 15 mL Eppendorf[®] tube, add 3 mL of a 100 g/L commercial solution of ketamine (Merial, Imalgene[®]), 1 mL of a 20 g/L commercial solution of xylazine (Bayer, Rompun[®] 2%), and 4 mL of sterile saline. The prepared solution contains 37.5 mg/mL ketamine base and 24.9 mg/mL xylazine base from hydrochloride. This preparation must be prepared extemporaneously.
9. Solutions for bronchoalveolar lavage procedure: PBS-EDTA 3 mM: in a sterile 1-L DURAN[®] glass bottle (Schott, 21,801,545), add 970 mg of EDTA (ethylenediaminetetraacetic acid disodium salt dihydrate, Sigma-Aldrich, E1644) and 1 L of endotoxin-free PBS (phosphate-buffered saline). This preparation may be stored protected from light at 4°C for 1 week.

3 Methods

This procedure describes sensitization and challenge of mice to HDM. In the short design asthma model (8 days), mice are sensitized intraperitoneally (i.p.), and then challenged by intranasal (i.n.) administration at the indicated times (Fig. 1a). In the chronic asthma model, mice are both sensitized and challenged to HDM by i.n. administration only (Fig. 1b, c).

Control mice are sensitized with the HDM-alum suspension in the 8-day model or challenged with solvent (saline) alone in the 31-day model.

The 8-day HDM asthma model requires alum hydroxide as an adjuvant to drive and boost the inflammatory response, and rapidly develop reproducible and robust allergic asthma features characterized by eosinophilic infiltration in the bronchoalveolar lavage, mucus hyperplasia, and airway hyperresponsiveness measured in response to methacholine (50 mg/mL) (Fig. 2a, b, c). It is suitable to test drug candidates as shown for dexamethasone (Fig. 2d).

The 31-day asthma model is generated without alum with 5 i.n. administrations of HDM required for sensitization, followed by 2 i.n. challenges 7 days apart allowing maturation of asthma features in the airways (inflammation, remodeling, and AHR). At this point, the asthma model is set (Fig. 1b) and can be monitored every day for several days (Fig. 3). A third stage composed of three additional i.n. challenges one day apart can be added to mimic allergen exacerbation increasing the inflammatory response (Fig. 4).

Assessment of asthma features: AHR is measured by the forced oscillation technique [7, 8]; inflammatory cell recruitment in particular eosinophils is assessed in the collected bronchoalveolar lavage [5, 9] by flow cytometry [10].

3.1 Short Design Model (8 Days): Perform HDM Sensitization with Adjuvant

1. Before use in animals, bring the HDM-alum suspension solution to room temperature. Inject 100 μ L of the anesthetic solution per mouse of 25 g, i.e., 4 mL/kg. The administered dose is 50 mg/kg ketamine and 3.3 mg/kg xylazine.
2. Hold the mouse (*see Note 3*) in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers.
3. Use 1 mL syringes and 25-G needles to inject the allergen-alum suspension, and administer i.p. 4 mL/kg, i.e., 100 μ L per mouse of 25 g. Gently homogenize the suspension between each injection (*see Note 4*). Each mouse receives 10 μ g HDM adsorbed on 2 mg alum in saline.

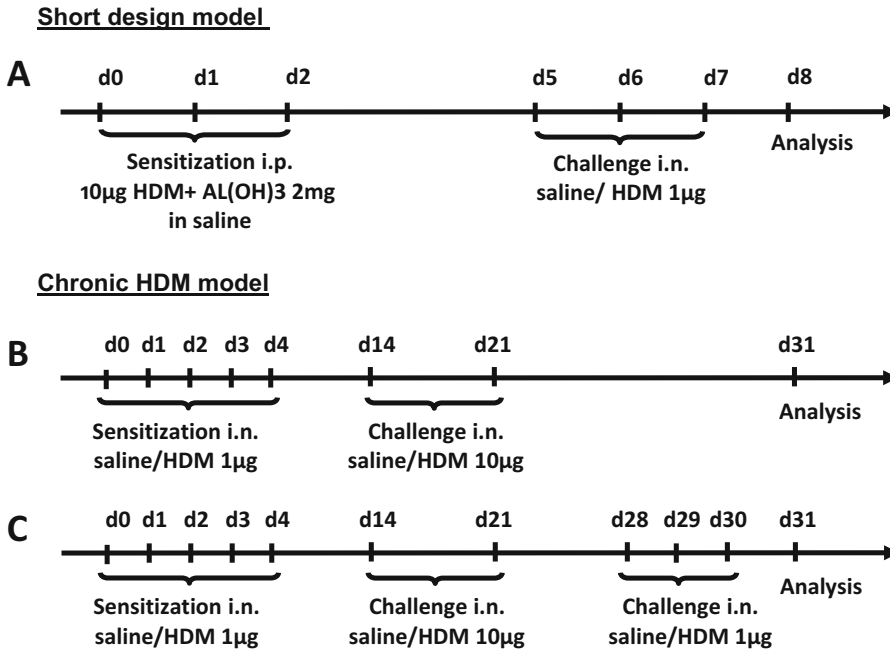


Fig. 1 Allergic asthma models to house dust mite (HDM) in mice. **(a)** The short design model of hyper eosinophilia (8 days) is composed of a sensitization step with intraperitoneal (i.p.) administrations of HDM-alum suspension on days 0, 1, and 2, followed by intranasal (i.n.) challenges with HDM or saline for control mice on days 5, 6, and 7. **(b)** The chronic asthma model (31 days) is composed of a sensitization step with daily i.n. challenges of HDM on days 0 to 4, followed by i.n. challenges with HDM or saline for control mice on days 14 and 21. **(c)** The chronic asthma model (31 days) may be complemented by a third step of i.n. challenges to HDM on days 28, 29, and 30. Assessments of airway inflammatory cell recruitment, remodeling, AHR, and HDM-specific IgE production are performed at indicated time points

3.2 Short Design Model (8 Days): HDM Challenge

1. Take a 25 µL aliquot of 800 µg/mL HDM stock solution and add immediately 975 µL of endotoxin-free saline; vortex for 10 s at maximal speed. Final concentration of HDM is 40 µg/mL.
2. Mice have to be anesthetized for i.n. administration. Anesthetize mice as follows: hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers and use 1 mL syringes and 25-G needles to inject i.p. 4 mL/kg of the anesthetic solution (*see Note 5*).
3. Place the mouse in a cage and wait until vibrissae do not move anymore.
4. Hold the mouse in your hand in a vertical position with its head up and its rear legs down. Administer, drop by drop, 12.5 µL HDM solution in each nostril, or of saline alone for controls, by using sterile tips and a 20 µL precision pipette (*see Note 6*). Each mouse receives 1 µg HDM.

8-day asthma model

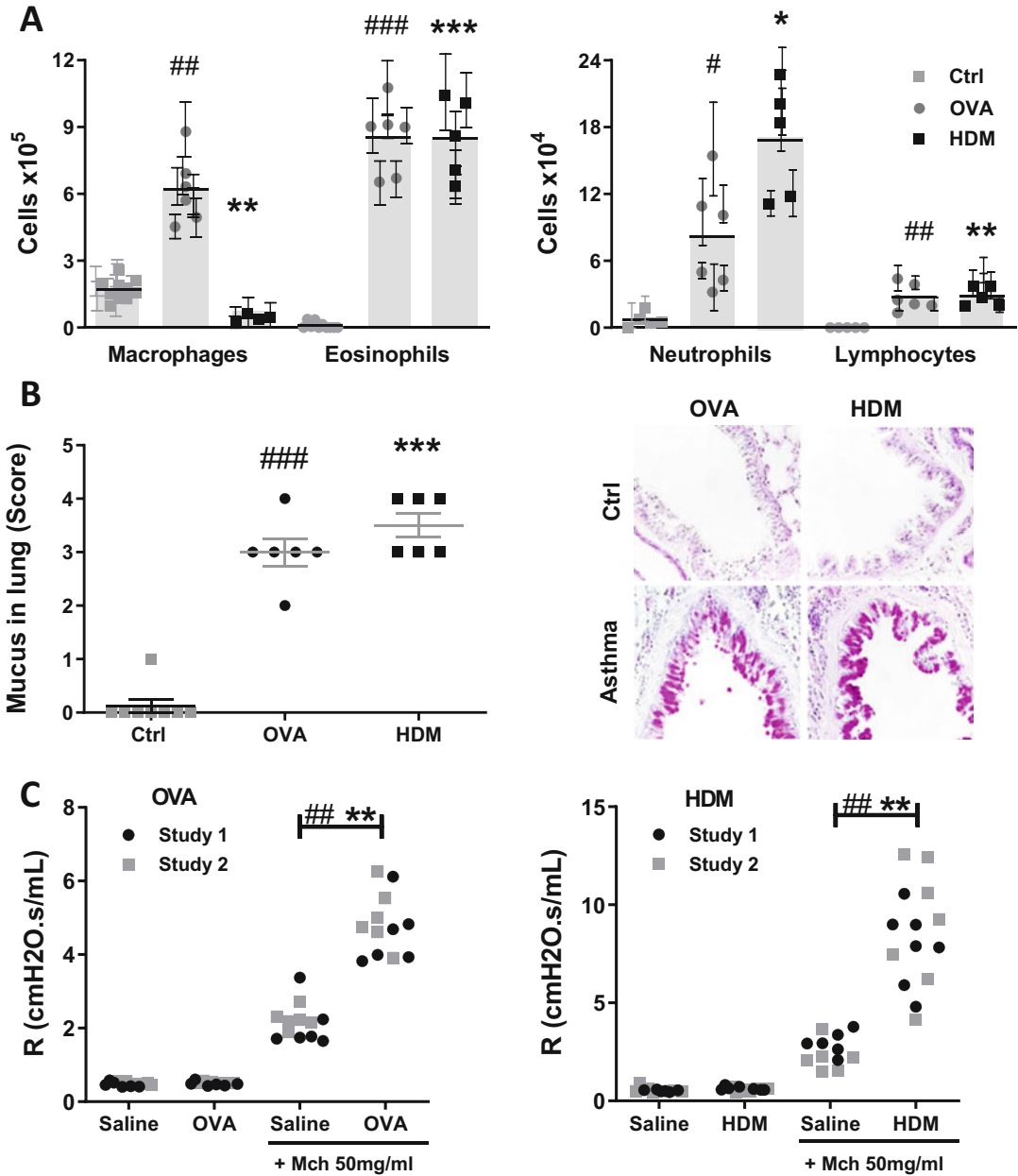


Fig. 2 Comparison of asthma features (inflammatory cell recruitment, mucus hyperplasia, and airway resistance) between HDM and OVA allergens in the short design asthma model. **(a)** Inflammatory cells in BAL on day 8 in mice sensitized and challenged with saline (gray square), HDM (black squares), and OVA (gray dots). Each dot represents the mean value from $n = 6$ independent experiments including 6 mice/study group. Blocks are means and bars are SEM values. **(b)** Excessive mucus production highlighted by periodic acid Schiff (PAS) staining (right) and scored (left). **(c)** Airway resistance (R) expressed as cm H₂O.s.mL⁻¹ in response to nebulized methacholine (50 mg/mL) was assessed by the Flexivent[®] technique (Scireq, Canada through EMKA Technologies, Paris, France). Individual values obtained in a first study (S1, black symbols) and in a second study (S2, gray symbols) are represented ($n = 6$ mice/group).

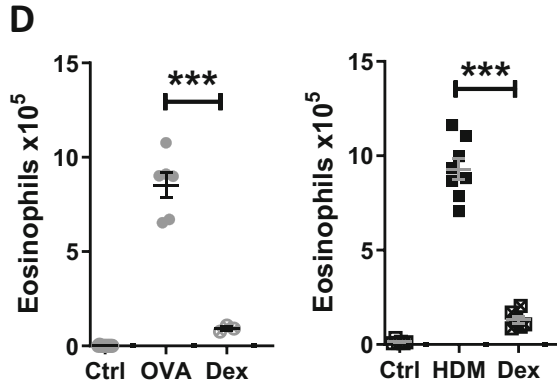


Fig. 2 (continued) (d) Comparative effect of glucocorticoid: Balb/c mice were sensitized and challenged with HDM (black symbols) or OVA (gray symbols), and treated i.p. with dexamethasone (1 mg/kg) 1 h before each HDM or OVA challenge. Eosinophils are shown. Dots and squares are individual values, lines are means, and error bars show SEM values ($n = 6$ per group). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ between control and HDM group and # $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$ between control and OVA group in the 8-day models

- Keep the mouse in your hand in a vertical position for at least 1 min so that the solution can be distributed in the airways, and check that the mouse breathes normally (*see Note 7*).
- Next, place the mouse in a horizontal decubitus on a heating blanket until the mouse is completely awake.

3.3 Chronic Asthma Model (31 Days): Perform HDM Sensitization

- Take a 25 μ L aliquot of 800 μ g/mL HDM stock solution and add immediately 975 μ L of endotoxin-free saline; vortex for 10 s at maximal speed. Final concentration in HDM is 40 μ g/mL.
- Mice have to be anesthetized for i.n. administration. Before use in animals, bring the solution to room temperature. Inject 100 μ L of the anesthetic solution per mouse of 25 g, i.e., 4 mL/kg. The administered dose is 50 mg/kg ketamine and 3.3 mg/kg xylazine. Anesthetize mice as follows: Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers and use 1 mL syringes and 25-G needles to inject i.p. 4 mL/kg of the anesthetic solution (*see Note 5*).
- Place the mouse in a cage and wait until vibrissae do not move anymore.
- Hold the mouse in your hand in a vertical position with its head up and its rear legs down. Administer, drop by drop, 12.5 μ L HDM solution in each nostril or of saline alone for controls, by using sterile tips and a 20 μ L precision pipette (*see Note 6*). Each mouse receives 1 μ g HDM.

31-day asthma model

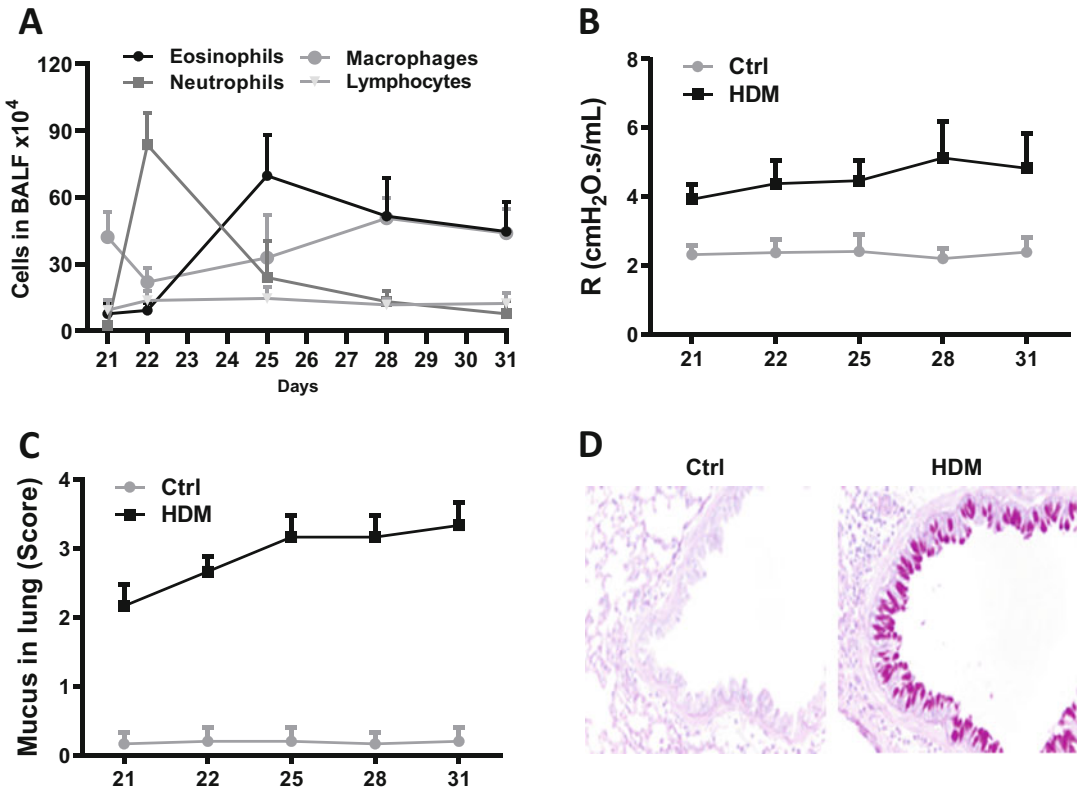


Fig. 3 Chronicity of asthma features in the chronic 31-day asthma model without exacerbation stage. (a) Evolution of the inflammatory cell recruitment in BAL from the last challenge (on day 21) in mice sensitized and challenged to HDM. Each dot represents the mean value from $n = 8$ mice and bars are SEM values. (b) Airway resistance (R) expressed as cm H₂O.s.mL⁻¹ in response to nebulized methacholine (50 mg/mL) was assessed by the Flexivent[®] technique (Scireq, Canada through EMKA Technologies, Paris, France) before and over time from the last challenge (day 21) in mice sensitized and challenged with saline (gray) or HDM (black). Dots are means and bars are SEM values ($n = 8$). Excessive mucus production highlighted with periodic acid Schiff (PAS) staining (right, d) and scored (left, c) before and over time from the last challenge (day 21) in mice sensitized and challenged with saline (gray) or HDM (black). Dots are means and bars are SEM values ($n = 8$)

5. Keep the mouse in your hand in a vertical position for at least 1 min so that the solution can be distributed in the airways, and check that the mouse breathes normally (*see Note 7*).
6. Next, place the mouse in a horizontal decubitus on a heating blanket until the mouse is completely awake.

3.4 Chronic Asthma Model (31 Days): HDM Asthma Maturation

1. Take a 550 μ L aliquot of 800 μ g/mL HDM stock solution and add immediately 550 μ L of endotoxin-free saline; vortex for 10 s at maximal speed. Final concentration of HDM is 400 μ g/mL.

Rechallenged chronic asthma model

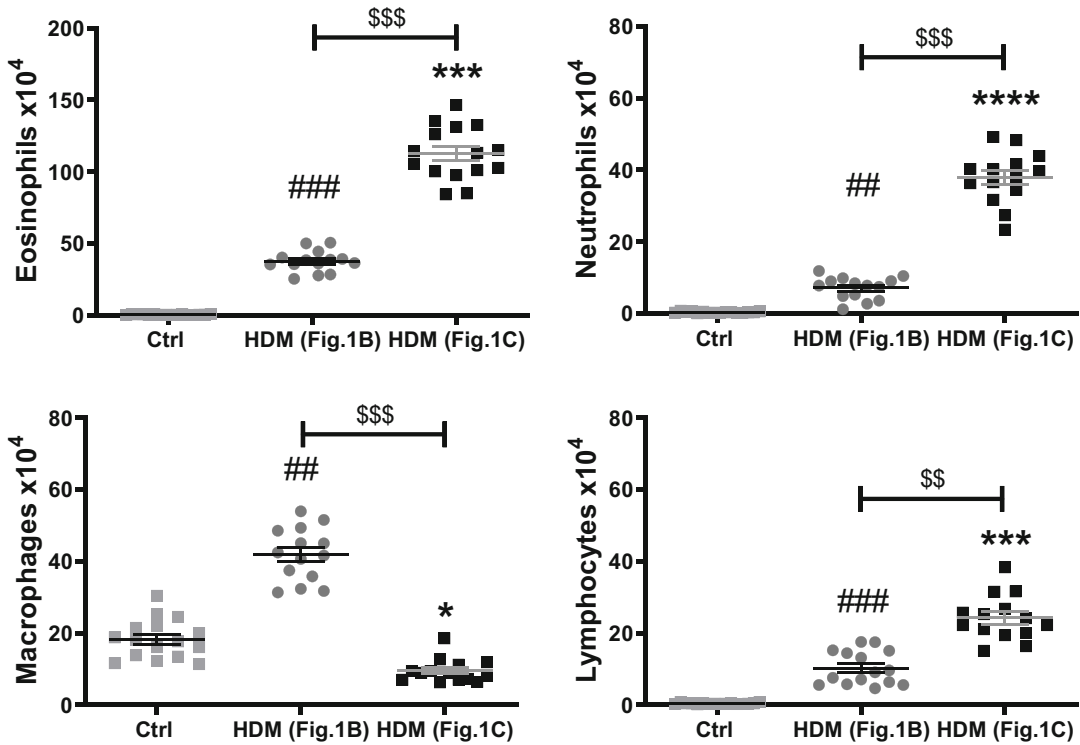


Fig. 4 Effect of the allergen exacerbation stage on inflammatory cell recruitment in the 31-day chronic asthma model. Inflammatory cell recruitment in BAL performed on day 31 in mice sensitized and challenged with saline (gray squares) or HDM without exacerbation stage (as in Fig. 1b) or with HDM exacerbation stage (as in Fig. 1c). Dots and squares are individual values, horizontal bars are means, and error bars show SEM values ($n = 15$ per group). ## $p \leq 0.01$, ### $p \leq 0.001$ between control and HDM group (as in Fig. 1b), * $p \leq 0.05$, *** $p \leq 0.001$ between control and exacerbated HDM group (as in Fig. 1c); \$\$\$ $p \leq 0.01$, \$\$\$ $p \leq 0.001$ between HDM group (as in Fig. 1b) group and exacerbated HDM group (as in Fig. 1c)

2. Mice have to be anesthetized for i.n. administration. Anesthetize mice as follows: Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers and use 1 mL syringes and 25-G needles to inject 4 mL/kg i.p. of the anesthetic solution (see Note 5).
3. Place the mouse in a cage and wait until vibrissae do not move anymore.
4. Hold the mouse in your hand in a vertical position with its head up and its rear legs down. Administer, drop by drop, 12.5 μ L HDM solution in each nostril or of saline alone for controls, by using sterile tips and a 20 μ L precision pipette (see Note 6). Each mouse receives 10 μ g HDM.

5. Keep the mouse in your hand in a vertical position for at least 1 min so that the solution can be distributed in the airways, and check that the mouse breathes normally (*see Note 7*).
6. Next, place the mouse in a horizontal decubitus on a heating blanket until the mouse is completely awoken.

3.5 Chronic Asthma Model (31 Days): HDM Exacerbation (Fig. 1c)

1. Take a 25 μL aliquot of 800 $\mu\text{g}/\text{mL}$ HDM stock solution and add immediately 975 μL of endotoxin-free saline; vortex for 10 s at maximal speed. Final concentration in HDM is 40 $\mu\text{g}/\text{mL}$.
2. Mice have to be anesthetized for i.n. administration. Anesthetize mice as follows: Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers and use 1 mL syringes and 25-G needles to inject 4 mL/kg i.p. of the anesthetic solution (*see Note 5*).
3. Place the mouse in a cage and wait until vibrissae do not move anymore.
4. Hold the mouse in your hand in a vertical position with its head up and its rear legs down. Administer, drop by drop, 12.5 μL HDM solution in each nostril or of saline alone for controls, by using sterile tips and a 20 μL precision pipette (*see Note 6*). Each mouse receives 1 μg HDM.
5. Keep the mouse in your hand in a vertical position for at least 1 min so that the solution can be distributed in the airways, and check that the mouse breathes normally (*see Note 7*).
6. Next, place the mouse in a horizontal decubitus on a heating blanket until the mouse is completely awoken.

3.6 Bronchoalveolar Lavage Procedure

1. Mice have to be anesthetized for BAL procedure as follows: Before use in animals, bring the solution to room temperature. Inject 200 μL of the anesthetic solution per mouse of 25 g, i.e., 8 mL/kg. The administered dose is 300 mg/kg ketamine and 20 mg/kg xylazine. Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers and use 1 mL syringes and 25-G needles to inject i.p. 8 mL/kg of the anesthetic solution (*see Subheading 2, item 6*).
2. Place the animal in a dorsal decubitus position until the mouse is deeply anesthetized.
3. Using scissors, make a small incision in the neck skin. Separate salivary glands, incise the sternohyoid muscle to expose the trachea, and place a cotton thread (black haberdashery cotton thread no. 40) under the trachea.

4. Make a small semi-incision of the trachea to allow a 21-G lavage tube to pass into the trachea (*see Note 8*). Stabilize the tube and needle by attaching them with the cotton thread.
5. Load a 1 mL syringe (Terumo, BS-01T) with 0.5 mL sterile PBS-EDTA, place it in the 21-G lavage tube, and inject 0.5 mL PBS-EDTA into the lung. Massage the chest for 10 s, re-aspirate saline of this first lavage, and keep the recovered lavage fluid into a 5 mL tube placed on ice.
6. Repeat the procedure ten times per animal (*see Note 9*).
7. Pool lavages recovered and centrifuge them for 5 min at $300 \times g$ at 4 °C to pellet cells. Discard the supernatant and add 1 mL of PBS-EDTA to the cell pellet. Homogenize (1–2 s on a vortex) prior to analyzing the cell population (*see Note 10*).

4 Notes

1. LPS content in HDM extracts differs in each batch. We note that LPS is required for HDM sensitization in mice to increase asthma features, in particular the inflammatory response (Fig. 5).
2. Be very careful while choosing the aluminum hydroxide reagent. The quality of aluminum hydroxide influences the number of eosinophils recovered in BAL in the 8-day model. We recommend to use aluminum hydroxide from Sigma-Aldrich (cat. no. 239186).
3. These protocols are developed to be used with 9-week-old male or female Balb/c mice (8-day model) or only female Balb/c mice (31-day model) to study airway inflammatory cell recruitment, remodeling, AHR, and OVA-specific IgE production [9, 11]. C57/BL6 mice may be used in the 8-day model to study eosinophilic airway inflammation, but there will not be any increase in AHR.
4. Gently homogenize the suspension by 3–4 repeated reversals of the tube/syringe between each use so that the components do not drop at the bottom of the tube/syringe.
5. Anesthesia is key to a successful i.n. administration. Too light anesthesia reduces the reproducibility of the sensitization, and then of inflammatory cell recruitment; on the other hand, too strong anesthesia enhances respiratory failure during and after i.n. administrations. It may be necessary to adapt the dose of anesthetic to warrant the best quality of anesthesia, and to increase the dose over time.

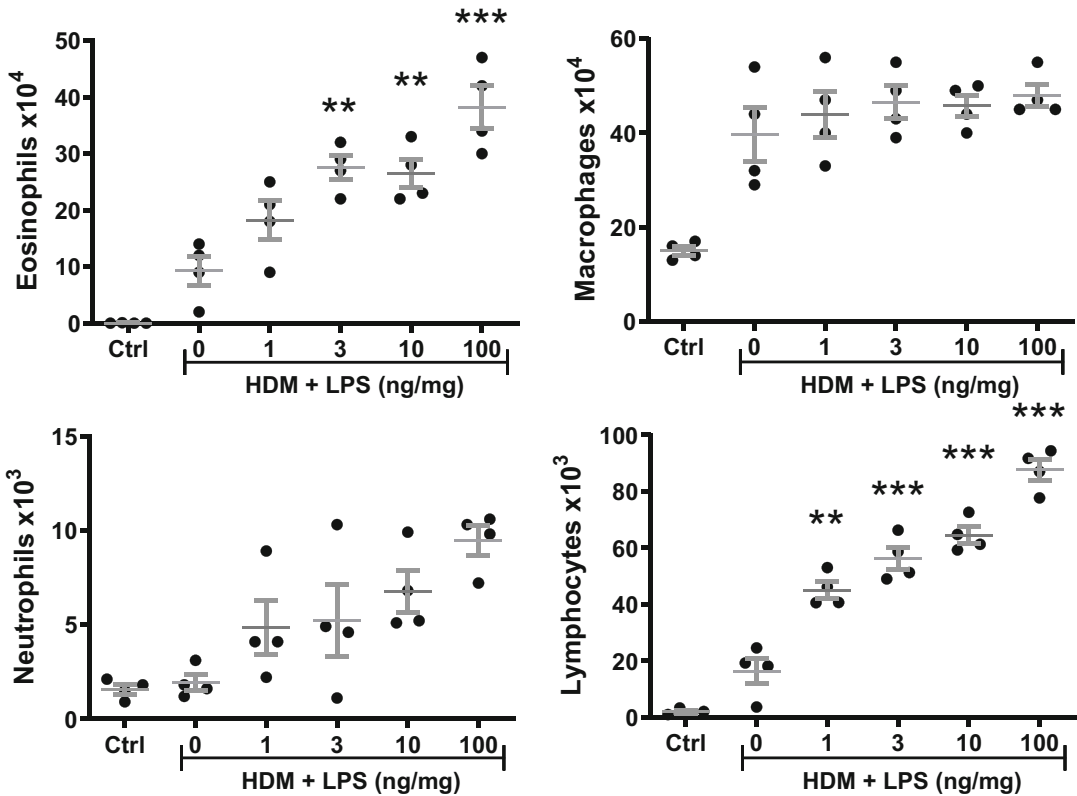


Fig. 5 Effect of LPS concentration in HDM extract on the inflammatory cell recruitment in the 31-day chronic asthma model. Inflammatory cell recruitment in BAL recovered on day 31 in mice sensitized and challenged with saline (gray square) or HDM extract added with increasing doses of LPS (*E. coli* 055:B5, ng/mg of total protein extract). According to the manufacturer's information, HDM extract used for this study contained undetectable amounts of endotoxin. Dots are individual values, lines are means, and error bars show SEM values ($n = 4$ per group). ** $p \leq 0.01$, *** $p \leq 0.001$ between HDM without LPS and HDM with LPS group

6. Solution has to be administered i.n. drop by drop, slowly and very carefully to maximize the distribution of HDM into the airways. Intranasal challenge is a simple technique, easy to perform, but it requires expertise to obtain reproducible results.
7. If the mouse does not breathe normally, perform a thorax massage by pressing the rib cage several times, quickly but carefully. Please take care to identify each mouse that stops breathing, because this may affect AHR and eosinophilic inflammation of the airways.
8. While semi-incising the trachea, take care not to cut through the trachea. For tracheotomy, use a 21-G lavage tubing, carefully placed over a 21-G needle or a single-use Terumo surshield® IV catheter (Terumo, 1SR*SFA2025A).

9. Supernatant from the first two lavages may be pooled and separated from the eight following lavages for cytokine and/or mucus analysis, since higher concentrations are present in the two first lavages. In this case, centrifuge the initial two first lavages for 5 min at $300 \times g$ at 4 °C, then freeze the supernatant for further analysis, and add the cell pellet to the eight following lavages.
10. We previously described the staining and analysis of leukocytes in BAL using manual counting [6, 9] or by flow cytometry [10].

References

1. Global initiative for asthma (GINA) (2019) Pocket guide for asthma management and prevention. <http://www.ginasthma.org/>
2. Berend N, Salome CM, King GG (2008) Mechanisms of airway hyperresponsiveness in asthma. *Respirology* 13:624–631
3. Bates JH, Rincon M, Irvin CG (2009) Animal models of asthma. *Am J Physiol Lung Cell Mol Physiol* 297(3):L401–L410
4. Epstein MM (2006) Are mouse models of allergic asthma useful for testing novel therapeutics? *Exp Toxicol Pathol* 57(Suppl 2):41–44
5. Daubeuf F, Frossard N (2014) Eosinophils and the ovalbumin mouse model of asthma. *Methods Mol Biol* 1178:283–293
6. Daubeuf F, Frossard N (2013) Acute asthma models to ovalbumin in the mouse. *Curr Protoc Mouse Biol* 3:31–37
7. Daubeuf F, Reber L, Frossard N (2013) Measurement of airway responsiveness in the anesthetized mouse. <http://www.bio-protocol.org/wenzhang.aspx?id=645>
8. Daubeuf F, Reber L, Frossard N (2013) Measurement of airway responsiveness on vigil and unrestrained mouse. <http://www.bio-protocol.org/wenzhang.aspx?id=328>
9. Daubeuf F, Frossard N (2012) Performing bronchoalveolar lavage in the mouse. *Curr Protoc Mouse Biol* 2:167–175
10. Daubeuf F, Becker J, Aguilar-Pimentel JA, Ebel C, Hrabě de Angelis M, Hérault Y, Frossard N (2017) A fast, easy, and customizable eight-color flow cytometric method for analysis of the cellular content of bronchoalveolar lavage fluid in the mouse. *Curr Protoc Mouse Biol* 7(2):88–99
11. Reber LL, Daubeuf F, Plantinga M, De Cauwer L, Gerlo S, Waelput W, Van Calenbergh S, Tavernier J, Haegeman G, Lambrecht BN, Frossard N, de Bosscher K (2012) A fully dissociated glucocorticoid receptor modulator reduces airway hyperresponsiveness and inflammation in a murine model of asthma. *J Immunol* 188:3478–3487