

Establishment and characterization of an experimental mouse model of allergic rhinitis

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Abstract Allergic rhinitis (AR) is a common worldwide disease. Animal studies on AR were adopted in various investigations. However, animal studies simply aimed at establishing an animal model for AR have been seldom seen. The purpose of this study was to introduce an easy-to-establish experimental mouse model of AR. To develop a mouse model of AR, 38 Balb/c mice were randomly assigned to two groups. Mice in the study group were sensitized by intraperitoneal (IP) injection of ovalbumin (OVA) on day 1 and 6, followed by continuous inhalation (IH) of OVA solution for 1 week (day 8–14) using a newly designed inhalation box. The control group mice received sensitization of IP normal saline and IH sterilized distilled water instead of OVA. Before and after sensitization, the frequencies of nasal symptoms (sneezing, nasal rubbing) were recorded and the serum levels of total immunoglobulin E (IgE) were evaluated using ELISA. Finally, the murine nasal mucosal tissues were stained by Giemsa solution to estimate the degree of mast cell infiltration. After sensitization by IP and IH OVA, the study group showed significant phenotypic changes including increased

sneezing and rubbing. Pathological and cytological findings also confirmed significant elevated serum total IgE titer and local mast cell infiltration in the study group statistically. We successfully developed a workable experimental animal model for AR that was more easily sensitized using our new-designed inhalation box, with less stress and more precisely to be observed.

Keywords Allergic rhinitis · Animal model · Ovalbumin · Inhalation

Introduction

Allergic rhinitis (AR) has worldwide distribution with the prevalence increasing year by year. Patients suffering from AR present with different degrees of rhinorrhea, sneezing, nasal itching, nasal obstruction, or eye symptoms. Immunologically, hypersensitivity type I mechanism mediates AR, which is mainly triggered by a T-helper 2 (Th2) dominant immune response with immunoglobulin E (IgE)-linking allergen, a large antigen. The downstream effector cells such as mast cells and eosinophils also play important roles [1]. Currently, immunotherapy remains the therapeutic modality for cure; however, symptomatic control through medication such as antihistamines or inhaled steroids is the mainstream clinical therapy.

Animal studies have been adopted in various investigations to study AR. Among them, mice are the most common animals used [2–7], followed by guinea pig [8–12]. Operating antigen, which is also called “allergen”, is important to sensitize mice and render them “allergic”. Ovalbumin (OVA) and pollen [9–13] are the universal reagents for experimental allergen use. After selecting the proper allergen, the most important step is to apply it into

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the mouse body to sensitize and elicit symptoms of AR. Two major steps are widely accepted and used: general sensitization and local sensitization. General sensitization is usually accomplished through intraperitoneal (IP) or subcutaneous (SC) injection of allergen such as OVA [2–8, 14, 15] to generate a typical IgE-mediated systemic immune response. Once the systemic IgE-mediated response is established, continuous exposure of the same allergen to a specific site such as skin or airway may lead to the development of Th2-dominant local atopic reactions such as skin or nasal symptoms. This step is defined as local sensitization, which is mediated mainly by the homing effect. For airway allergy model such as AR or asthma, intranasal instillation (IN) of the allergen through the animals' nostrils was the most common method for local sensitization in previous animal studies [2–11, 13, 14, 16].

There are numerous publications describing different methods for rendering mice allergic to specific antigens using the above two steps. However, there is no standardized method that can be followed universally. For example, the time point and duration for performing IP or IN varied from each other, not to mention the dosage of allergen they utilized [2–16]. Besides, most methods used to sensitize the airway for assessment of hyperresponsiveness to antigen presentation were intended to establish an animal model of asthma and seldom for a model of AR. Moreover, because different methods were generated by different researchers, it is difficult to compare the efficiency and therapeutic effects of these methods. Thus, a standardized, widely accepted animal model for AR is desired.

As mentioned above, mice, such as balb/c [2–7, 17–20], B10.S [13], and c57BL/6 [21], are the most common animals used in studies of AR. In addition to mice, guinea pigs [8–12] or brown Norway rats [15, 22] have also been used. Mice provide an easy means of assessing allergic symptoms such as sneezing or nasal rubbing (represents the motion due to itching), while rats may be a better choice if nasal obstruction is the target response for measure. In other words, if observation for active motion such as sneezing and nasal rubbing is considered important, mice, especially balb/c, might be very good candidates.

It is very important to choose an ideal allergen. OVA is the most common reagent used in published literatures. It is the main protein found in egg white, making up 60–65 % of the total protein [23]. The OVA protein of chicken is made up of 385 amino acids, and its relative molecular mass is 45 kDa [24]. OVA can be injected IP [2, 4–7, 14, 17–21] or SC [8, 15, 22] for general sensitization. Among those studies, the IP OVA dosage ranged from 1 to 20 µg per balb/c mouse, with a predominant dose of 20 µg [4, 7, 17–19]. For local sensitization, OVA was almost given via IN at various concentrations [2–8, 14, 21]. In addition to

OVA, pollen [9–13] or even fungus (*Aspergillus fumigatus*) [16] can be an alternative choice of sensitizing allergen. Nabe et al. applied one kind of Japanese cedar pollen (*Cryptomeria japonica*) for both general and local sensitization and elicited satisfactory responses [9–13]. However, this kind of pollen is endemic and not representative of worldwide pollen exposure.

We performed IN for local sensitization in our preliminary study; however, we found that it was not satisfactory to maintain consistent data. In spite of precise and cautious manipulation, instilled fluid during IN may be more or less inevitably lost. Sometimes, there was struggling and the mice became stressful. To improve the above conditions, anesthesia can be used, but aspiration of the fluid from nose to trachea may occur. Devices designed to expose the mice into a fixed chamber with a central inhalational delivery system reduce such situations. In contrast to IN, this kind of device delivers the allergen to the mice through inhalation (IH) and seems to be more comfortable and natural. Thus, the concept “IH” for allergen delivery might be an alternative method. Our study aim was to develop a simple, effective, less stressful and more comfortable system for allergen delivery in the AR animal model.

Materials and methods

Animals

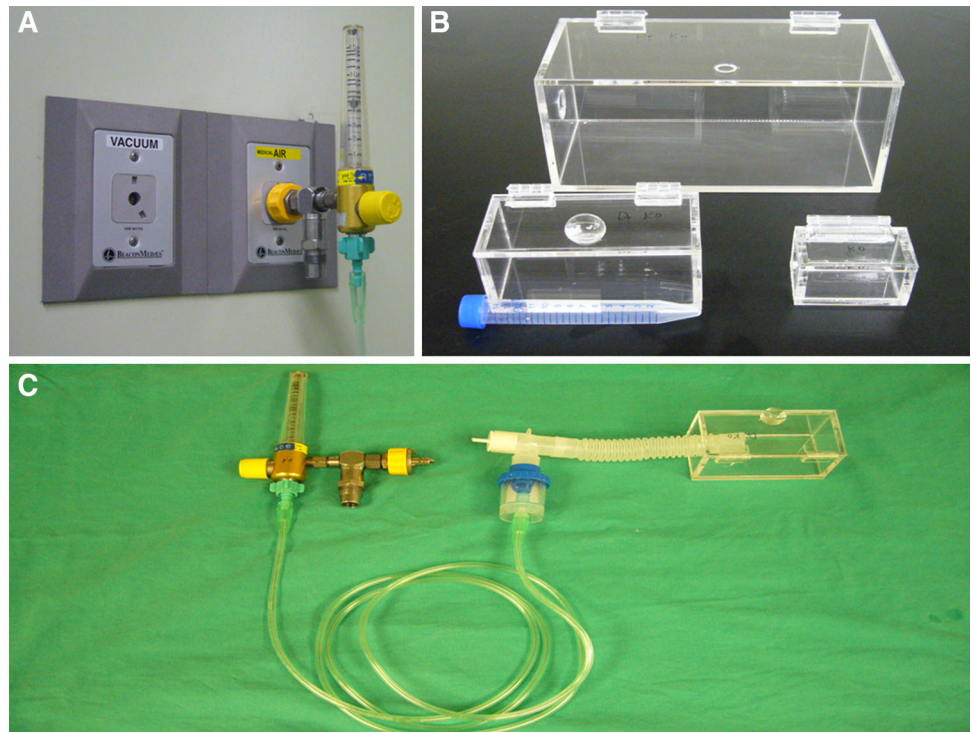
4-week-old Balb/cByJNarl mice were obtained from the National Laboratory Animal Center (Taipei, Taiwan) and housed in an air-conditioned room maintained at 22–24 °C with 50–60 % humidity. Food and water were available ad libitum. Mice were reared in the facility and used for the study at 6–8 weeks of age. All animal experiments conducted in this study were approved by the animal research ethics board of the Chang Gung Memorial Hospital–Kaohsiung Medical Center.

Provocation

The purpose of provocation was to find out a powerful reagent that could facilitate our observation of animal behavior because allergic symptoms may not always spontaneously happen. In other words, this reagent can induce an immediate response of sneezing or nasal rubbing after challenge. From the degree of provocation, we can easily differentiate the allergic mice from non-allergic ones. In our provocation test, 15 randomly selected balb/c mice were included and tested under different conditions to directly provoke nasal phenotypic symptoms such as sneezing and nasal rubbing motions. Instead of traditional

Fig. 1 Demonstration of the procedure and instruments employed in the animal model under development.

a Flowmetry was used to provide stable and desirable air flow. **b** Different sizes of the inhalation (IH) box: large, medium, and small. The length of the medium one was almost equal to a centrifuge tube. **c** The IH device was completed by connecting the flowmetry to a small-volume nebulizer, which was linked to the IH box via a plastic tube



IN administration, we preferred IH with consistent air flow for allergen delivery (Fig. 1a). A new acrylic inhalation box (IH box) was designed to provide a more comfortable environment for mice in the event of air flow exposure. At first, we designed three sizes of IH box: small, medium, and large (Fig. 1b). In the pilot study, the smallest one which could house only one mouse caused claustrophobia, whereas the largest one was not satisfactory for symptom observation because mice usually ran around and it also consumed much more OVA than the smallest one. The medium one (11 × 6 × 6 cm) could house one mouse each time with an appropriate space, preventing mouse from running around or causing claustrophobia. It seemed to provide the best efficiency for observation with nearly no side effect. Thus, we used the medium-sized IH box for further experiments.

The ability of sterilized distilled water (dH₂O) and histamine solution (His) to elicit nasal symptoms was compared. His was made using histamine (Sigma, St. Louis, Missouri, USA) dissolved in dH₂O at a concentration of 1 mg/mL. Ten microliter of dH₂O or His was, respectively, loaded into a small-volume nebulizer, which was linked to the IH box and flowmetry with a delivery flow rate of 5 L/min (Fig. 1c) for 5 min. During provocation, mouse behavior was observed and the frequencies of sneezing and nasal rubbing were recorded for 5 and 10 min. The results were compared and the stronger reagent was used as the provocation reagent for further studies.

Sensitization for AR model

Thirty-eight balb/c mice were randomly categorized into the study and control group, 19 for each. For general sensitization, 20 µg OVA (Sigma, St. Louis, Missouri, USA) plus 2 mg aluminum hydroxide gel (Imject[®] alum, Pierce, Rockford, USA) with a total volume of 0.1 mL was injected IP in the study group on day 1 and day 6. Normal saline (NS) was injected IP in the control group, respectively, on day 1 and day 6.

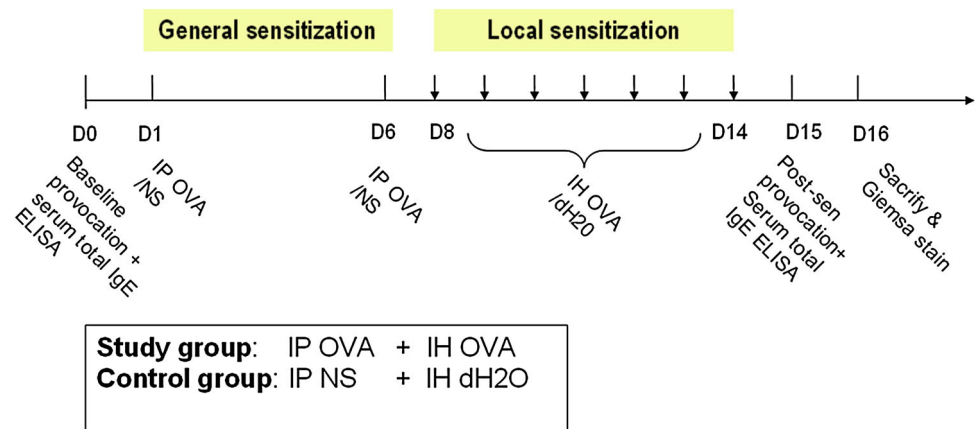
From day 8 to day 14, local sensitization was achieved by exposing the mice to IH reagents inside the IH box for 5 min daily. In the study group, 10 mL OVA solution (Sigma, grade V, St. Louis, Missouri, USA) at a concentration of 25 mg/mL was used as IH reagent and loaded into the small-volume nebulizer. Likewise, dH₂O was administered to the control mice under the same condition. With a consistent flow rate of 5 L/min, the sensitizing reagents (OVA solution or dH₂O) were aerosolized into the IH box for inhalation. The flowchart is provided in Fig. 2.

Measurement of phenotypes

Before general sensitization (day 0) and after total local sensitization (day 15), AR symptoms were evaluated by counting the frequencies of sneezing and nasal rubbing events for 5 and 10 min during and after provocation challenge by 1 mg/mL histamine solution. Blood samples of

Fig. 2 Flowchart of the study.

IP intraperitoneal, *IH* inhalation, *OVA* ovalbumin, *NS* normal saline, *dH₂O* sterilized distilled water, *post-sen* post-sensitization



about 0.5 mL were obtained by retro-orbital puncture also before and after sensitization, to compare the serum titer of total IgE by ELISA (anti-mouse IgE ELISA, Immunology Consultants Laboratory, Newberg, USA). The ELISA procedure was followed by the guideline of Immunology Consultants Laboratory. Finally, the mice were killed by IP injection of an overdose of 6 % chloral hydrate (Sigma, Deisenhofen, Germany). The murine nasal mucosa were excised and fixed in 10 % neutral-buffered formaldehyde for 72 h at 20 °C and then embedded in paraffin. The paraffin-embedded specimens were sectioned into 3 µm thickness on a microtome, mounted on slides and deparaffinized. After that, specimens were stained with 10 % Giemsa solution (Merck, Darmstadt, Germany) to detect the extent of mast cell infiltration. Mast cells in the nasal mucosal sections were counted and recorded under high-power light microscopy. Six different sections of each mouse were calculated. The average number of mast cells per high-power field (n/hpf) in each mouse was quantified and used for data analysis.

Statistic analysis

Student's *t* test was used to compare the frequencies of sneezing and rubbing between dH₂O and His in the provocation effect. It was also used to compare the presentations of nasal symptoms, serum total IgE titer, and average n/hpf before and after sensitization between the two experimental groups. A critical *p* value less than .05 was considered to be significant.

Results

The results of provocation are depicted in Table 1. Fifteen randomly selected mice completed the provocation test. These results demonstrated the average values of frequencies observed during 5 or 10 min with standard error. Compared with dH₂O, His showed a higher efficacy to induce more

Table 1 Comparison of the provocation effect of dH₂O and histamine

	dH ₂ O (<i>n</i> = 15)	His (<i>n</i> = 15)	<i>p</i> value
Symptoms observation period			
S 5 min	0.4 ± 0.16	1.73 ± 0.67	.07
R 5 min	12.87 ± 1.75	17.87 ± 1.31	.03*
S 10 min	0.93 ± 0.38	4.00 ± 1.28	.035*
R 10 min	22.4 ± 2.28	28.73 ± 1.05	.02*

The values in each group presented as average ± SE

dH₂O sterilized distilled water, His histamine solution, S the frequency of sneezing, R the frequency of rubbing

* *p* < .05

sneezing either in 5 min (1.73 for His compared to 0.40 for dH₂O, *p* = .07) or 10 min (4.00 for His compared to 0.93 for dH₂O, *p* = .035). Similarly, His also provoked a significant higher nasal rubbing, both in 5 min (17.87 for His compared to 12.87 for dH₂O, *p* = .03) and in 10 min (28.73 for His compared to 22.4 for dH₂O, *p* = .02). Thus, His was chosen as the provocation reagent for further use.

Thirty-eight mice, with 19 in each group, completed the experimental study for sensitization. To compare the phenotypic changes between the study and control groups, frequencies of nasal symptoms were evaluated before and after sensitization (Table 2). These results demonstrated the average values with standard deviation. Before sensitization, there was no difference in sneezing or rubbing on observing for 5 or 10 min. After sensitization, both the symptom scores for sneezing at 5 min (*p* = .002) and 10 min (*p* = .002), and rubbing at 5 min (*p* = .001) and 10 min (*p* < .001) markedly increased in the study group.

After sensitization, the average serum total IgE titer was also higher in the study group (1.08 ng/mL) than in the control group (0.52 ng/mL), with a critical *p* value < .001. The average mast cell amount counted per hpf in the study group was 5.74, higher than 2.19 of the control group (*p* < .001) (Fig. 3).

Discussion

Our results on provocation showed that histamine was a more powerful trigger to induce behavioral allergy symptoms such as sneezing than dH₂O. Satisfactory reagents for

Table 2 Comparison of the sensitization effect between the two groups

	Control (<i>n</i> = 19)	Study (<i>n</i> = 19)	<i>p</i> value
Symptoms			
Pre S 5 min	0.37 ± 0.68	0.47 ± 0.84	.675
Pre R 5 min	13.84 ± 7.34	10.84 ± 6.00	.176
Pre S 10 min	0.84 ± 1.07	1.89 ± 2.98	.161
Pre R 10 min	24.47 ± 8.31	21.68 ± 8.01	.299
Post S 5 min	0.63 ± 1.07	5.16 ± 5.42	.002*
Post R 5 min	13.74 ± 8.10	23.21 ± 8.73	.001*
Post S 10 min	0.95 ± 1.39	8.95 ± 9.41	.002*
Post R 10 min	23.32 ± 10.94	40.68 ± 14.61	<.001**
Total IgE titer (ng/ml)			
Pre	0.28 ± 0.11	0.32 ± 0.16	.463
Post	0.52 ± 0.25	1.08 ± 0.27	<.001**
Average mast cells count (n/hpf)	2.19 ± 1.35	5.74 ± 0.86	<.001**

The values in each group presented as average ± standard deviation
Control = intraperitoneal (IP) injection of normal saline (NS) + inhalation (IH) of sterilized distilled water (dH₂O)

Study = IP injection of ovalbumin (OVA) + IH OVA

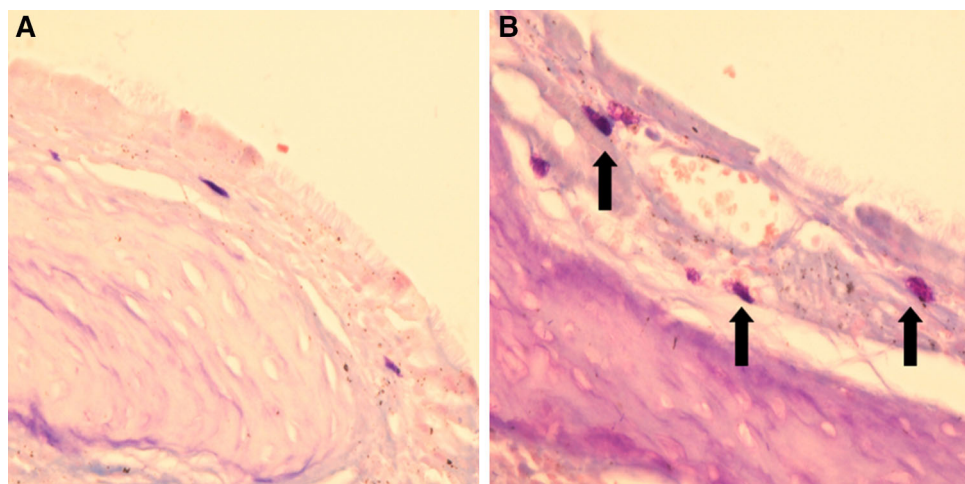
n/hpf = number of mast cells counted per high-power field, and a total of six different high-power fields were recorded per mouse. Each n/hpf represented the average mast cells counted in each mouse, and the average n/hpf in each group was used for comparison

Pre pre-sensitization, Post post-sensitization, S the frequency of sneezing, R the frequency of rubbing

* *p* < .05

** *p* < .001

Fig. 3 Mast cell infiltration was detected in murine nasal mucosas under light microscopy after Giemsa stain. The number of mast cells per high-power field (n/hpf) was calculated for six different sections in each mouse. **a** There were only few mast cells seen in the control group. **b** A significantly increased amount of mast cells was observed in the study group. Arrow: mast cell. Original magnification, ×400



provocation should not cause a long-term effect or have any influence on the targeting immune response. Histamine [5, 6, 13] and leukotriene D4 (LTD4) [9–11] have been reported as being used for the purpose of provocation in previous studies. Histamine is produced and released by mast cells or basophils, which can cause vasodilation, smooth muscle contraction, pain, and itching sensation in the nasal mucosa via activation of the histamine receptor. Compared to LTD4, histamine is cost-effective with minimal side effects. However, histamine in solid form is not suitable for IH. Therefore, histamine solution provides a better choice under nebulization. Nevertheless, precautions should be always taken to avoid inhalation injury resulting from accidental environmental exposure or overdose.

For allergen delivery, IN has been the most common method used for either provocation or local sensitization. It is simple without the need of any specific instrument. It can be performed with [14–16, 22] or without anesthesia [4, 5]. Although it is simple, precise and cautious manipulation is very important to avoid struggling of mice, aspiration, or instilled fluid loss from the nostrils. Besides, it is also hard to differentiate the true cause of behavioral symptoms: do the motions of sneezing or rubbing truly result from the allergen sensitization or just induced by the feeling of discomfort from the instillation itself?

In contrast to IN, IH seems to be another alternative modality. To improve the efficiency of allergen delivery, nebulization was utilized in some studies [17, 19, 22]. However, those studies focused on asthma but not AR, and the details of the device were not described. As mentioned above, some commercial devices can keep the mice in a fixed chamber with a central inhalational delivery system. This kind of device provides a comfortable environment and is close to our ideal settings. According to the concept of “IH”, we simplified and designed an acrylic IH box. With a small-volume nebulizer, IH box allows the administration of a sensitizing reagent more naturally.

Besides, anesthesia is not needed so that it reduces the possibility of aspiration and also accidental reagent fluid loss.

To evaluate the phenotypes of AR in animal models, several methods have been reported including symptom observation [2, 5–7, 9–11, 13], serum total IgE titer [4, 8, 15], specific anti-OVA IgE or IgG1 titer [4, 8–11, 13, 15], eosinophils infiltration by HE stain under light microscopy [2, 6, 13, 14, 16], nasal resistance or volume detection [4, 8–11], and nasal lavage [4, 8, 12, 20]. According to our results, mice sensitized by IP and IH OVA presented with significantly higher frequencies of sneezing and nasal rubbing, elevated serum total IgE titer, and increased mast cell infiltration. In other words, we successfully produced an animal model using OVA sensitization by increasing the murine phenotypic symptoms, pathologic serum IgE titer, and cytologic mast cells presentation. However, there are two issues about methodology. First, we decided to evaluate the titer of serum total IgE [4, 8, 15], but not specific anti-OVA IgE (or IgG1) in both groups, because it was impossible to detect any titer of specific anti-OVA IgE (or IgG1) in control mice if they were sensitized only using NS and dH₂O. Although we could compare the change after sensitization in each group, it would be unfair and unreasonable if we compared the titer of anti-OVA IgE in the study mice to the titer of total IgE in the control mice. Besides, in future studies, estimating the therapeutic effect of different reagents, using the same baseline (total IgE titer, but not each specific IgE titer) for comparison sounds reasonable. Second, HE stain is commonly used in allergy model to detect eosinophils. However, mast cell is also a key cell in allergy presentation [5]. Giemsa stain is a good tool to detect mast cell infiltration. It is widely used to detect infection of parasite, malaria [25], *Helicobacter pylori* [26], and atopic response [27]. Both mast cell and eosinophils are the key cells in allergy pathophysiology. However, we found that the mast cell can be more easily identified by its violet granules under Giemsa staining (Fig. 3). Thus, we provided an alternative method to evaluate the local presentation of mast cells that are important tissue reactors in allergic diseases.

Conclusions

To summarize our study, balb/c mice sensitized with IP and IH OVA showed increased frequencies of AR symptoms (sneezing, nasal rubbing), serum total IgE titer, and mast cell infiltration. OVA is a widely accepted and easily available allergen in animal study. The IH box might be a useful tool for AR animal models as it provides a simple, more convenient and natural environment for inhalational exposures. Our method is also easily reproducible and cost-

effective. In the future, we hope elucidation of pathophysiology following this animal model may lead to emergence of candidate drugs to treat AR.

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Conflict of interest The authors declare that they have no conflict of interest.

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