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Development and validation of a Guinea pig model for concurrent allergic rhinitis and asthma using recombinant Der f 2

Feng Xu[1], Li Hu[2], Jianzhong Wang[1] and Lineng Zhang[3*]

Abstract

Background This study aimed to develop a guinea pig model of concurrent allergic rhinitis and asthma.

Methods Thirty-three guinea pigs were randomly divided into the control group and the experimental group. Guinea pigs in the experimental group were sensitized by intraperitoneal injection of recombinant wild-type *Dermatophagoides farinae* group 2 allergen (wt Der f 2) plus $Al(OH)_3$ on day 1 and 8, followed by inhalation of an aerosol of wt Der f 2 on day 16 and 23. The sensitized guinea pigs were challenged with intranasal instillation of wt Der f 2 plus $Al(OH)_3$ gel on day 19 and 26 for nasal symptoms scoring, and on day 30 for the active cutaneous anaphylaxis (ACA) test. Control group guinea pigs received normal saline (N.S.) plus $Al(OH)_3$ in parallel. Cutaneous provocation tests were performed to exclude nonsensitized guinea pigs, and nasal symptom assessments were conducted to exclude non-allergic guinea pigs from the study. The allergic airway model was finally validated using the ACA test, Evans blue dye quantification with wt Der f2, histopathology evaluation, and immunohistochemistry analysis of MUC5AC expression in both nasal mucosa and lung tissue.

Results Two guinea pigs with negative cutaneous reactions and three with less than 5 points of the nasal symptom assessment were excluded from the experimental group. The ACA test showed enhanced allergic reactions in the experimental group, and the quantification of extravasated Evans blue dye demonstrated significantly higher absorbance in the wt Der f 2 spots compared to mu Der f 2. Histologic analyses illustrated pathologic features typical of allergic rhinitis and asthma. MUC5AC levels in the nasal mucosa and lung samples were significantly higher in the experimental group than in the control group.

Conclusion We successfully established a guinea pig model of concurrent allergic rhinitis and asthma using a combination of sensitization, challenge, and validation methodologies with the allergen Der f 2, suitable for pathophysiological studies.

Clinical trial number Not applicable.

Keywords Allergic rhinitis, Asthma, Guinea pig, Intraperitoneal administration, Inhalation, Der f 2

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Introduction

As an airway inflammatory disorder, allergic rhinitis (AR) is characterized by symptoms such as rhinorrhea, sneezing, itching and nasal obstruction. The global prevalence of AR has been estimated to range from 10–40% [1, 2]. AR can be categorized into seasonal and perennial forms, with perennial allergic rhinitis (PAR) being induced by allergens like animal dander, house dust mites, and molds. Epidemiological evidence and clinical observations have shown that AR can progress to asthma, and a significant proportion of asthma patients also suffer from concomitant AR [3, 4]. Both AR and asthma are inflammatory diseases sharing similar pathophysiological mechanisms, which led to the introduction of the 'one airway, one disease' concept in 1997 [5]. House dust mites are among the most common allergen sources for both PAR and asthma, with *Dermatophagoides farinae* (Der f) being a widely distributed American house dust mite. Along with *Dermatophagoides pteronyssinus* group 2 (Der p 2), Der f group 2 (Der f 2) is one of the primary allergens. This 129-amino acid protein, with conserved structural domains, plays a key role in inducing allergic inflammation.

Although numerous experimental models have been developed to elucidate the pathophysiology and pharmacology of AR and asthma individually, few studies have focused on establishing an animal model of concurrent AR and asthma. Moreover, there is a lack of standardized methodologies for generating suitable allergic animal models; variations exist in animal species, allergens, delivery routes, doses, time points and durations. The use of different methodologies to generate AR and asthma models complicates the comparison of their efficiency and treatment effects [6]. Therefore, a standardized animal model for concurrent AR and asthma is urgently needed.

Regarding species, guinea pigs more closely mimic human responses and are therefore a preferred model for studying AR and asthma [7, 8]. Selecting suitable allergens to sensitize animals is crucial for rendering them allergic. House dust mite (HDM) allergens, particularly those from *Dermatophagoides farinae* (Der f) and *Dermatophagoides pteronyssinus* (Der p), are the principal factors that induce AR and asthma [9, 10], making them ideal for experimental model construction. However, the allergen content in different mite extracts can vary significantly among suppliers, and these extracts may contain non-specific antigenic materials that can cause nonallergic inflammation. Therefore, using standardized allergens is essential to ensure experimental reproducibility. Among HDM allergens, recombinant wild-type (wt) Der f 2 has been reported to exhibit similar IgE-binding activity and cutaneous reactivity to the natural allergens [11].

After determining the suitable allergen, the next key step is to deliver the allergen into the animal to stimulate the symptoms of AR or asthma. This study aimed to develop an effective and less stressful method for allergen delivery in the allergic airways of guinea pigs, which are highly responsive to Der f 2. The allergen was administered via intraperitoneal (IP) injection, aerosol inhalation (IH), or intranasal instillation (II). Guinea pigs that were insensitive to Der f 2, as determined by a cutaneous provocation test [12] and subsequent nasal symptom assessment [13] were excluded to minimize variability in animal responses [13, 14].

Materials and methods

Animals

Thirty-three male, specific pathogen-free Hartley guinea pigs, aged four weeks, were provided by the Shanghai Laboratory Animal Center (Chinese Academy of Sciences, China). The animals were housed in a facility maintained at 24 ± 5 °C with 55 ± 5% relative humidity and controlled lighting (12 h light cycle from 6:00 to 18:00). They had free access to a commercial diet and water. No treatments were administered to the animals for at least one week after arrival to allow acclimatization. The guinea pigs were randomly assigned to either the control group (normal saline (N.S.) treatment, $n = 13$) or the experiment group (Der f 2 treatment, $n = 20$). The Animal Ethics Committee of Fudan University Zhongshan Hospital approved all experimental protocols and procedures. All operations were performed under anesthesia while efforts were made to minimize the suffering of the animals.

Antigens

Wild-type (wt) Der f 2 and the mu Der f 2 variant, which involves mutating two pairs of cysteines to serine (C8/119S and C73/78S), were expressed and purified according to the methods described by Takai et al. [15]. Next, the refolding and purification of these proteins were performed using a Sephacryl S-100 HR urea gradient to ensure protein solubility. Finally, the IgE-binding activity of wt Der f2 and mu Der f 2 was assessed by cutaneous reactions and ELISA [11].

Skin prick test

A skin prick test (SPT) was conducted on the volar side of the forearm of a single human volunteer using recombinant wt and mu Der f 2 at concentrations of 20, 200, and 800 ng/mL. The results were compared with those of histamine solution (positive control) and normal saline (N.S.) solution (negative control). Specifically, the forearm skin was pricked gently with a microlancet (Allergopharma Prick Test Lancets) applied to a drop of SPT solution [16]. The wheal-and-flare reactions were traced

with a ballpoint pen, and the maximum wheal diameters were measured 20 min post-test using a caliper. The informed consent to participate was obtained from the human volunteer in the study.

Indirect ELISA

Microtiter plates were coated with 60 ng/mL of wt Der f 2 or mu Der f 2 in coating buffer (15 mM sodium carbonate and 35 mM sodium bicarbonate, pH 9.5) for 16 h at 4 °C. The plates were then rinsed twice with PBS-Tween 20 (PBS containing 0.02% (w/v) Tween 20). Unoccupied sites were blocked by adding 300 µL of 1% (w/v) bovine serum albumin (BSA) per well, and the plates were incubated at 4 °C overnight. After blocking, the plates were rinsed four times with PBS-Tween 20. Sera from allergic patients (100 µL per well) were added in duplicate, and the plates were incubated for 2 h at 37 °C, followed by four washes with PBS-Tween 20. The detection antibody (HRP-labeled anti-human IgE, diluted 1:3000 in PBS; KPL, Gaithersburg, MD) was added to each well, and the plates were incubated for 2 h at 37 °C, followed by four washes. One-component TMB substrate (KPL, Gaithersburg, MD) was added to the wells, and the plates were incubated for 15 min at 37 °C. The reaction was stopped by adding 2 M H_2SO_4, and the absorbance at 450 nm was measured using a microplate reader.

The use of sIgE serum samples from clinical patients in the indirect ELISA experiments of this study was approved by the Ethics Committee of Fudan University Zhongshan Hospital (approval number: B2023-236R).

Sensitization and challenge protocols

For systemic sensitization, guinea pigs in the experimental group received an intraperitoneal (IP) injection of 200 µg of recombinant wt Der f 2 plus 2.5 mg of aluminum hydroxide ($Al(OH)_3$) gel in a total volume of 0.5 mL on days 1 and 8. Control guinea pigs received an IP injection of normal saline (N.S.) plus 2.5 mg of $Al(OH)_3$ gel in the same volume.

For local sensitization, sensitized guinea pigs were housed in a clear acrylic chamber and exposed to an aerosol of wt Der f 2 (4 mL of 1 mg/mL allergen in saline) for 30 min on days 16 and 23. The aerosol was generated using an ultrasonic nebulizer and delivered into the chamber with an air pump at a flow rate of 300 mL/min. Control guinea pigs were exposed to an aerosol of N.S. under the same conditions.

For intensive challenge, sensitized guinea pigs received bilateral intranasal instillations of wt Der f 2 (20 µL) adsorbed onto $Al(OH)_3$ gel (20 µg protein in 0.5 mg $Al(OH)_3$ gel) on days 19, 26, and 30 [17]. To hinder rapid antigen removal through ciliary movement, the surface of the nasal mucosa was anesthetized by inhalation of 4% lidocaine hydrochloride solution for 2 min prior to each sensitization session. N.S. plus $Al(OH)_3$ gel was administered by intranasal instillation to the guinea pigs in the control group. A flowchart of the sensitization and challenge protocol is provided in Fig. 1.

Cutaneous provocation test

On the day before the cutaneous provocation test, the dorsal skin of the guinea pigs was shaved under ketamine anesthesia (50 mg/kg, intramuscular). On day 15, the guinea pigs were subjected to an intradermal injection of wt Der f 2 (0.1 mL of 200 µg/mL) into the dorsal skin. Histamine dihydrochloride (10 mg/mL) and normal saline (N.S.) were used as the positive and negative controls, respectively. Thirty minutes after each intradermal injection, the wheal-and-flare reactions were evaluated. The largest diameter and its corresponding perpendicular diameter were measured with a digital Vernier caliper, and the wheal size was calculated as the average of these two measurements. A wheal diameter at least 3 mm larger than that of the negative control was considered a positive response [18], indicating successful sensitization. Guinea pigs that did not show a positive cutaneous reaction were excluded from further analysis.

Nasal symptoms assessment

On days 19 and 26, nasal symptoms were independently evaluated by two observers who were blinded to group assignment. After a 10-minute adaptation period to allow the animals to acclimate to the environment following the intranasal instillation challenge, the guinea pigs were observed for 10 min for the frequency of sneezing, nasal rubbing, and rhinorrhea. Symptoms were rated on a scale from 0 to 3 (Table 1) [19]. The allergic rhinitis model was considered effective if the total score exceeded 5 points.

Active cutaneous anaphylaxis test

One day before the experiment, the dorsal skin of the guinea pigs was shaved. On the day of the test, 0.5 mL of 10 mg/mL Evans blue dye was injected via the axillary vein. The guinea pigs were then challenged with an intradermal injection of 0.1 mL of 200 µg/mL wt Der f 2 or mu Der f 2 into the dorsal skin. Histamine (10 mg/mL) and normal saline (N.S.) were used as the positive and negative controls, respectively. Thirty minutes after each intradermal injection, the wheal-and-flare response was evaluated by measuring the largest and perpendicular diameters with a digital Vernier caliper. The response was scored based on the average of these two measurements.

Under ketamine anesthesia (50 mg/kg, intramuscular), the skin patches showing a response due to dye extravasation were resected and placed in a mixture of 5 mg/mL sodium sulfate and acetone (3:7, v/v) overnight to extract the dye. The amount of dye in each skin patch was

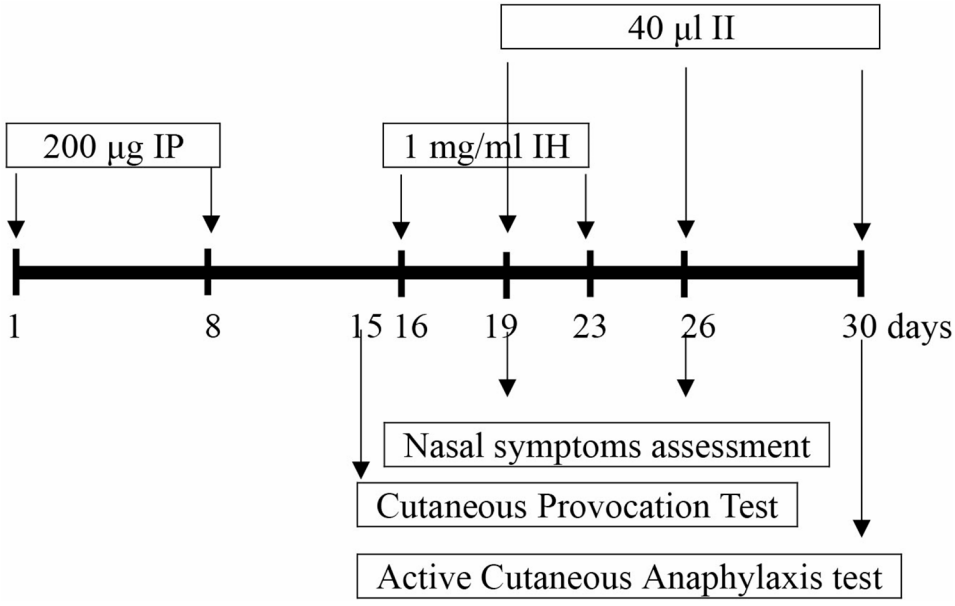

Fig. 1 Schematic illustration of the experimental procedure for creating the allergic airway guinea pig model. Guinea pigs were sensitized via intraperitoneal (IP) injection of recombinant wild-type Der f 2 (wt Der f 2) on days 1 and 8, and by inhalation (IH) of wt Der f 2 on days 16 and 23. They were then bilaterally challenged with intranasal instillation on days 19, 26, and 30. On day 15, a cutaneous provocation test (CPT) was conducted to confirm successful systemic sensitization. Two guinea pigs that failed the CPT (negative for extravasation) were excluded from the experimental group. On days 19 and 26, three additional guinea pigs that scored less than 5 points on the nasal symptom assessment were excluded from the study. On day 30, an active cutaneous anaphylaxis test (ACAT) was performed 2 h after the final challenge to analyze and quantify the allergic response.

Table 1 Scoring system for allergic rhinitis (AR) nasal symptoms

Nasal symptoms	0	1	2	3
Number of nasal itching motion(scratches, time/minute)	None	2	4–6	> 6
Number of sneezes(time/minute)	None	2	4–6	> 6
Nasal flow	None	In one nostril	In both nostrils	Out-flowing

Three guinea pigs scoring less than 5 points on the nasal symptom assessment on days 19 and 26 were excluded from the study.

quantified by measuring the absorbance at 620 nm using a spectrophotometer [20].

Histological assessment

At the end of the experiment, the guinea pigs were sacrificed under ketamine anesthesia (50 mg/kg, intramuscular). An en bloc resection of the maxilla and nose was performed by surgical dissection, and the nasal and lung tissues were immediately fixed in 10% formaldehyde. For histopathological assessment, paraffin-embedded tissue samples were sectioned at 5 μm thickness, deparaffinized using xylene, and washed with ethanol. The sections were then stained with hematoxylin and eosin and examined under a light microscope. The tissues were evaluated for inflammatory cell infiltration, tissue damage, and other histopathological changes by a pathologist who was blinded to group assignment [13].

Immunohistochemistry

The paraffin-embedded nasal mucosa and lung tissues from the experimental and control groups were sectioned at 5 μm thickness and incubated with mouse anti-MUC5AC antibody (Abcam, 45M1, 1:100 dilution). Detection was performed using the Boster ABC (avidin-biotin complex) Kit (Boster Biological Technology, Wuhan, China). Five microscopic fields per section at 200× magnification were photographed. Images were analyzed using Image-Pro Plus 12.0, and the integrated optical density (IOD) of the positive regions was measured. The mean optical density (MOD) was then calculated. Two representative sections from each guinea pig were selected based on the presence of clear tissue structures for examination.

Statistical analysis

Data analyses were conducted using SPSS version 15.0 (Windows). Data are expressed as the mean ± standard deviation (SD). One-way ANOVA was used to assess differences among groups. Post-hoc pairwise comparisons were performed using the LSD test, assuming equal variances. A p-value < 0.05 was considered statistically significant.

Results

Recombinant wt Der f 2 protein maintained cutaneous reactions and IgE binding capacity

To establish a consistent guinea pig model of allergic rhinitis (AR) and asthma, we first engineered and purified recombinant wild-type (wt) Der f 2 and mutant (mu) Der f 2 proteins in our laboratory. To evaluate the allergenicity of these proteins, we assessed their ability to elicit cutaneous reactions in a participant who was previously identified as sensitive to the house dust mite Der f using both the skin prick test (SPT) and the UniCAP allergen detection system. The participant exhibited a strong cutaneous reaction to wt Der f 2, while the response to mu Der f 2 in the SPT was significantly weaker (Fig. 2a).

Next, we determined the IgE reactivity of recombinant wt Der f 2 and mu Der f 2 (C8/119, C73/78S mutations) using an in vitro ELISA assay. The results showed that mu Der f 2 had markedly lower IgE reactivity compared to wt Der f 2 in sera from six patients with a positive SPT to Der f 2 (Fig. 2b). These findings are consistent with previous reports on the cutaneous reactions and IgE binding capacity of recombinant wt Der f 2 [11, 21]. Therefore, wt Der f 2 can be used for sensitization and challenge in guinea pigs, while mu Der f 2 serves as a negative control in animal models or as a potential therapeutic agent for immunotherapy [9].

Identification of sensitized Guinea pigs using the cutaneous provocation test

To identify sensitized guinea pigs, we performed a cutaneous provocation test (CPT) on day 15, as described in the test procedures. Each guinea pig received an intradermal injection of wt Der f 2 on the dorsal surface, which induced regional extravasation. Figure 3 shows the wheal-and-flare reactions (characterized by localized swelling and redness) to wt Der f 2 in sensitized guinea pigs.

The CPT was used to determine whether guinea pigs had been successfully sensitized. Out of the 20 guinea pigs in the experimental group, two were CPT-negative, similar to what was observed in the control group (Fig. 3a), indicating that these two animals were not successfully sensitized and were therefore excluded from further analysis. The remaining 18 guinea pigs were CPT-positive, showing remarkable erythema and edema at the injection site (Fig. 3b). These 18 sensitized guinea pigs were suitable for subsequent nasal system assessments.

Evaluation of allergic rhinitis in Guinea pigs using nasal symptoms assessment

To improve the reproducibility of the experimental model, we evaluated nasal symptoms in guinea pigs that had shown sensitivity in the cutaneous provocation test. The assessment was conducted 10 min after intranasal instillation of the allergen wt Der f 2. Guinea pigs with allergic rhinitis were identified using a nasal symptoms assessment (including sneezing, nose-scratching, and nasal discharge), and those without significant allergic symptoms were excluded from the experimental group. Specifically, 3 guinea pigs that scored fewer than 5 points on the nasal symptoms assessment on days 19 and 26 were excluded from the study, while 15 guinea pigs were included in the experimental group.

In a separate control experiment, 3 additional non-sensitized guinea pigs were challenged with bilateral intranasal instillation of wt Der f 2 (20 μL) adsorbed onto $Al(OH)_3$ gel. No significant symptoms (such as nose-scratching or sneezing) were observed in these control animals, confirming the specificity of the allergic response in the sensitized group (data not shown).

Validation of allergic airway models using active cutaneous anaphylaxis and Evans blue dye quantification

To validate the allergic airway models, we used the active cutaneous anaphylaxis (ACA) test and quantified Evans blue dye extravasation. The ACA test demonstrated that wt Der f 2 induced local inflammation, as evidenced by Evans blue dye extravasation (a marker of vascular permeability) in all sensitized and challenged guinea pigs. The mean diameter of the inflammatory reaction 30 min after the last challenge was significantly larger in wt Der f 2-treated guinea pigs (10.7 ± 2.3 mm) compared to control animals (1.1 ± 0.5 mm) (Fig. 4a).

In contrast, mu Der f 2 injection sites exhibited a significant reduction in cutaneous anaphylaxis, with a mean reaction diameter of 4.0 ± 0.4 mm, representing a 62.6% decrease in the dye extravasation area compared to wt Der f 2 (Fig. 4a). Quantification of extravasated Evans blue dye via absorbance measurements revealed a similar trend. Specifically, the absorbance of wt Der f 2 spots (0.816 ± 0.072) was significantly higher than that of mu Der f 2 spots (0.178 ± 0.033) (Fig. 4b), corresponding to a 78.2% reduction in the mu Der f 2 spots compared to the wt Der f 2 spots.

These results, including the inflammatory reaction diameter and Evans blue dye quantification, provide reliable and direct parameters for evaluating the efficacy of the allergic airway animal model.

Histopathological analysis of nasal mucosa and lung tissues

Histologic analyses revealed pathologic features characteristic of allergic rhinitis and asthma in both the nasal mucosa and lung tissues.

Nasal mucosa

Histologic examination of the nasal mucosa in recombinant Der f 2-treated animals showed pathologic

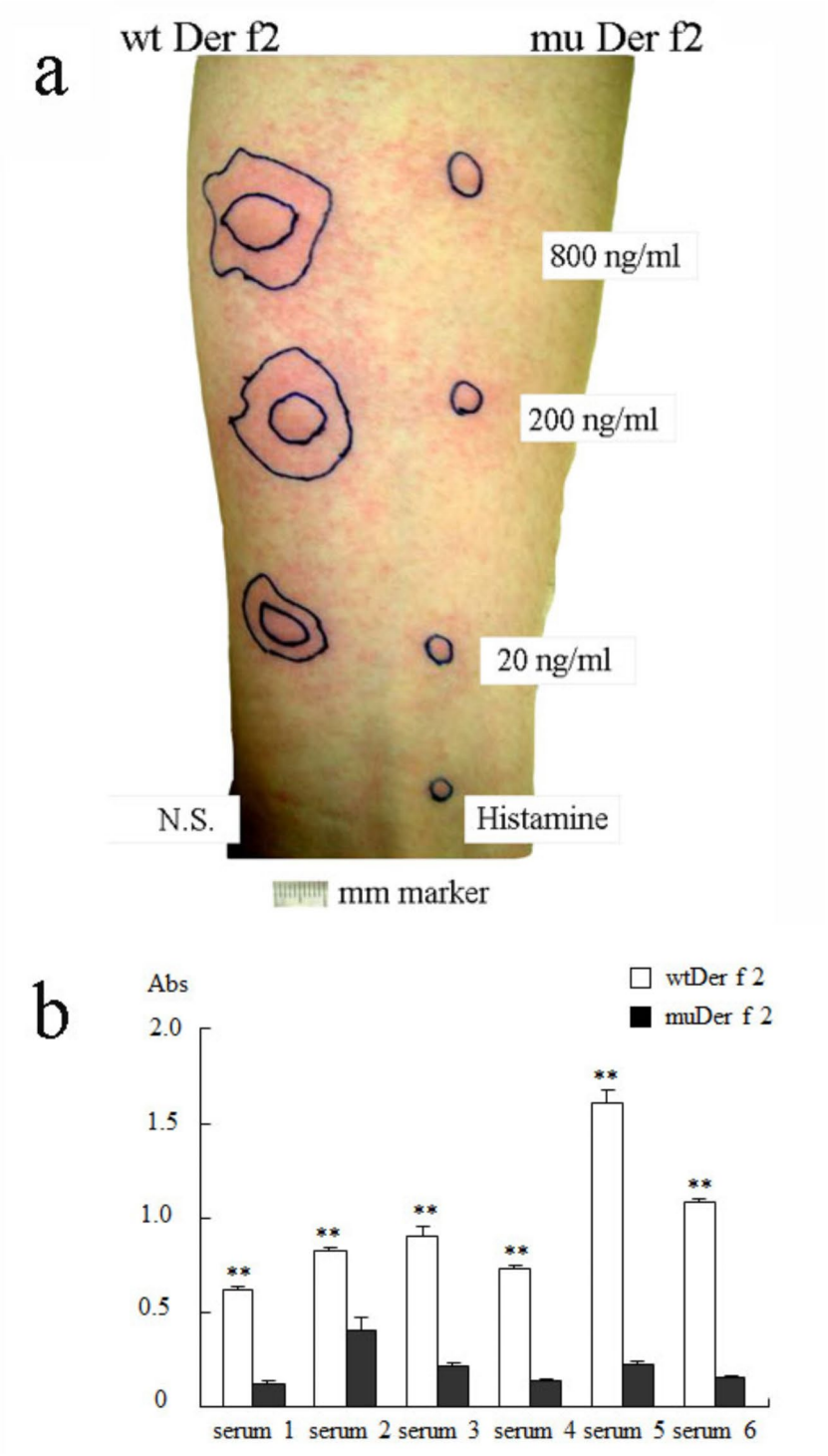

Fig. 2 Recombinant wt Der f 2 protein evokes cutaneous reactions and shows IgE-binding capacity. (**a**) Skin prick test (SPT) results: The SPT was performed on the forearm of one human volunteer. Recombinant wild-type (wt) and mutant (mu) Der f 2 were applied at concentrations of 20 ng/mL, 200 ng/mL, and 800 ng/mL, along with histamine (positive control) and normal saline (N.S., negative control). Wheal-and-flare reactions were traced with a ballpoint pen. wt Der f 2 elicited significant cutaneous reactions, whereas mu Der f 2 showed a markedly weaker response in the SPT. (**b**) IgE binding reactivity: Binding reactivity of purified wt and mu Der f 2 to IgE in sera from patients allergic to Dermatophagoides farinae was assessed. The binding capacity of wt Der f 2 to IgE was significantly higher than that of mu Der f 2 ($p < 0.01$). The antigen concentration used in the assay was 60 ng/mL.

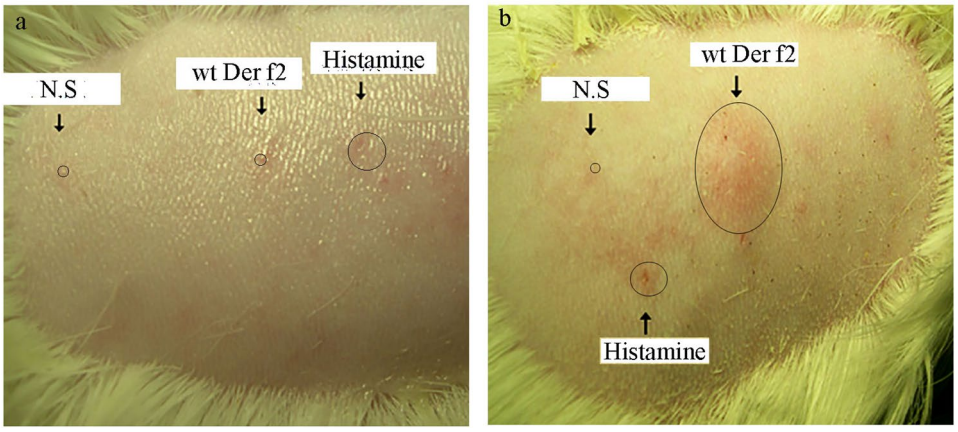

Fig. 3 Identification of sensitized guinea pigs using the cutaneous provocation test (CPT). Guinea pigs were identified as sensitized or nonsensitized using the cutaneous provocation test (CPT). Nonsensitized animals were excluded from the experimental group. The dorsal skin area of the guinea pigs was shaved the day before the CPT. On day 15, guinea pigs received an intradermal injection of 0.1 mL of 200 μg/mL Der f 2 solution into the dorsal skin. Of the 20 guinea pigs in the experimental group, 2 guinea pigs that did not exhibit significant erythema (redness) and edema (swelling), mirroring the findings in the control group (Fig. 3a), were excluded from the experimental group. Eighteen guinea pigs were CPT-positive, exhibiting significant erythema and edema at the injection site (Fig. 3b)and were thus suitable for subsequent nasal symptom assessment. (**a**) Control Group. (**b**) Experimental Group.

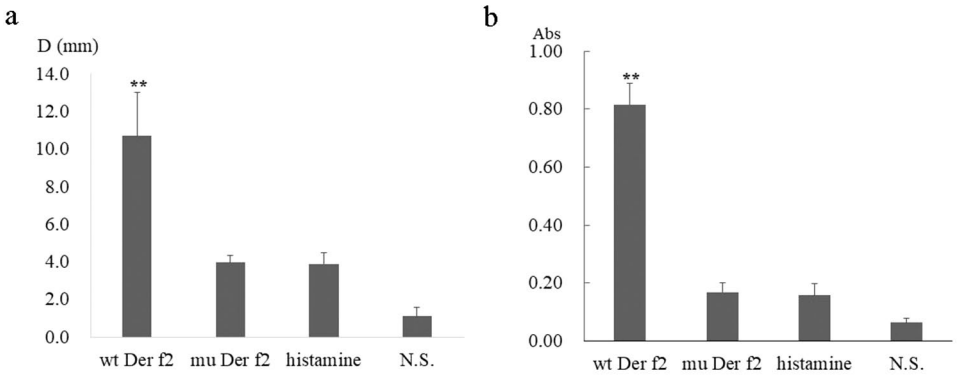

Fig. 4 Validation of the allergic airway model using an active cutaneous anaphylaxis test and Evans blue dye quantification. (**a**) Measurement of response area: Each guinea pig's back was shaved, followed by an intravenous injection of 0.5 mL of 10 mg/mL Evans blue dye (a tracer for vascular permeability). Thirty minutes later, each animal was challenged with an intradermal injection of 0.1 mL of 200 μg/mL wt Der f 2 or mu Der f 2, histamine (positive control), or normal saline (negative control) into the dorsal skin. Thirty minutes after each intradermal injection, the response area was quantified by measuring the largest diameter and the corresponding perpendicular diameter with a digital Vernier caliper, and the average of these two measurements was calculated. (**b**) Quantification of extravasated Evans blue dye: Two hours after the challenge, the animals were euthanized by exsanguination, and the blue-dyed skin patches were excised. The amount of dye was quantified using a colorimetric assay after overnight extraction in a mixture of sodium sulfate (5 mg/mL) and acetone (3:7, v/v). Absorbance was measured at 620 nm using a spectrophotometer. Quantification of extravasated Evans blue dye revealed that the absorbance of wt Der f 2 spots (0.816 ± 0.072) was significantly higher than that of mu Der f 2 spots (0.178 ± 0.033). ± *sd*, $n = 15$, ** $p < 0.01$

characteristics typical of allergic rhinitis. These included marked disruption of the columnar ciliated epithelium, ciliary loss, local goblet cell hyperplasia, nasal epithelial hypertrophy, submucosal edema, and infiltration of numerous leukocytes, particularly eosinophils (Fig. 5b). In contrast, these changes were absent in the normal saline-treated control animals (Fig. 5a).

Lung tissues

In the lungs, amorphous eosinophilic exudate and significant infiltration of eosinophils, lymphocytes, and macrophages were observed in the thickened interalveolar walls of allergen-treated animals (Fig. 5d). These findings were absent in the control group (Fig. 5c).

Elevated MUC5AC levels in allergic airway tissues

Given the histologic evidence of goblet cell hyperplasia in allergen-treated animals and the known role of goblet cells in synthesizing and secreting mucins as a protective response to various stimuli, we next sought to investigate the correlation between allergic inflammation and MUC5AC synthesis and secretion. To do this, we analyzed MUC5AC protein levels in the nasal mucosa and lung tissues using immunohistochemical staining.

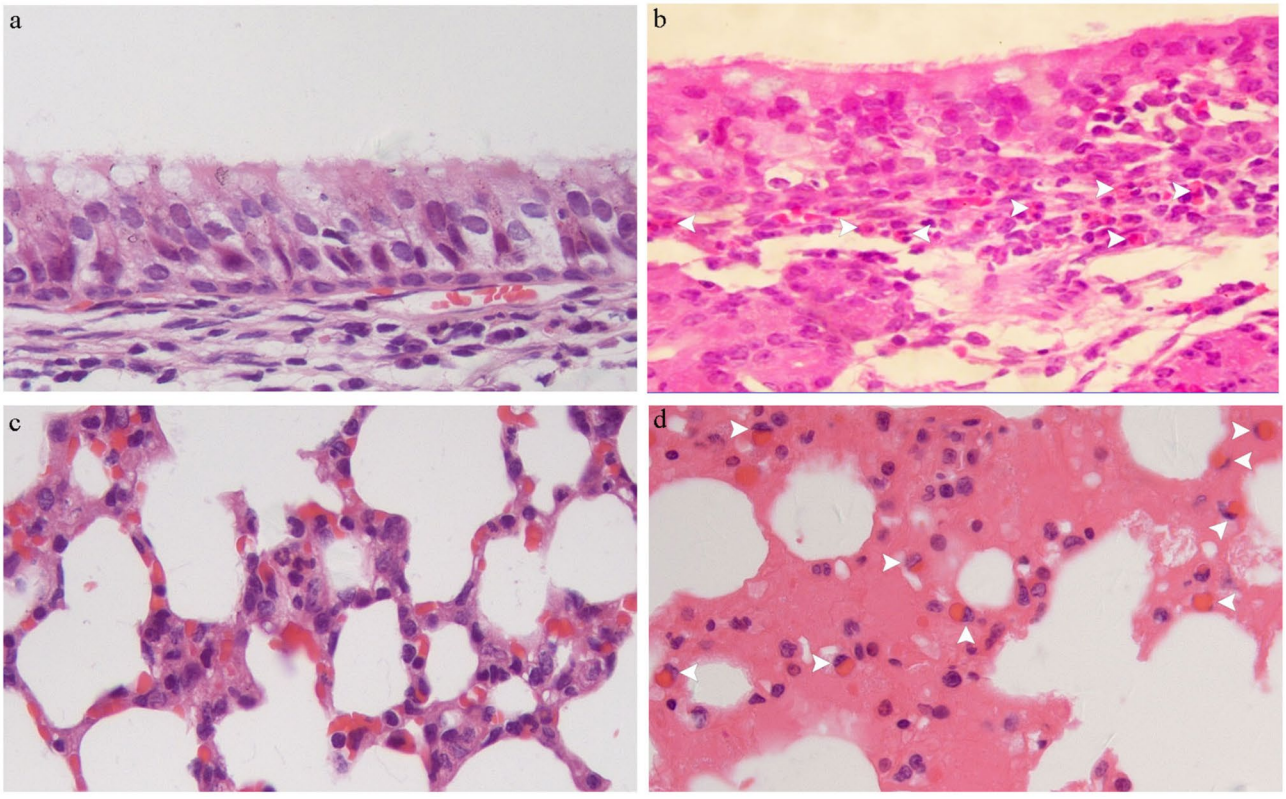

Fig. 5 Light photomicrographs of hematoxylin–eosin-stained sections from the nasal mucosa (**a**, **b**) and lung tissues (**c**, **d**) of control (**a**, **c**) and experimental (**b**, **d**) guinea pigs. (**a, b**) Nasal mucosa: Guinea pigs were euthanized 2 h after the final intranasal challenge. Nasal tissues were resected, fixed in buffered formalin, and embedded in paraffin. Tissue sections (5 μm thick) were stained with hematoxylin and eosin. Histopathological analysis was performed on randomly selected sections in a blinded manner at 400× magnification to evaluate eosinophil infiltration. The nasal mucosa of the experimental group exhibited submucosal edema and infiltration of numerous eosinophils (arrowhead), neutrophils, and lymphocytes. (**c**, **d**) Lung tissues: The lung tissues were similarly processed and analyzed. The lung tissues of the experimental group exhibited substantial infiltration of eosinophils (arrowhead), lymphocytes, and macrophages in thickened interalveolar septa.

Cells that stained positively for MUC5AC appeared brown. Immunoreactivity to MUC5AC was significantly higher in the goblet cells of the nasal mucosa in allergen-treated guinea pigs compared to control animals (Fig. 6a-c). Additionally, MUC5AC staining was significantly stronger in the intrabronchiolar and alveolar walls of allergen-treated guinea pigs compared to control animals (Fig. 6d-f).

Discussion

The global prevalence of allergic rhinitis and asthma has been rising steadily, highlighting the urgent need for the development of new therapeutic approaches. Although current treatments, such as antihistamines and corticosteroids, are effective, they have limitations, including side effects and the risk of drug resistance. Thus, there is an urgent need to screen and develop new therapeutic agents [22]. Animal models of respiratory allergic diseases are essential for evaluating the therapeutic potential of Der f 2-related therapies, such as recombinant allergens, in future clinical trials [23, 24]. These models enable the integration of high-throughput technologies, such as single-cell sequencing [25], to elucidate the pathophysiological mechanisms underlying allergic rhinitis and asthma. Additionally, these models help identify key signaling pathways involved in allergic responses and facilitate drug screening to discover potential therapeutic agents [26, 27].

To address these challenges, we successfully developed a guinea pig model of allergic airway inflammation using recombinant Der f 2. The animals were first sensitized with intraperitoneal (IP) injections of wild-type Der f 2 (wt Der f 2) and aerosol inhalation (IH), followed by intranasal challenges with wt Der f 2. This model provides a valuable platform for investigating the efficacy of novel therapeutic strategies and understanding the underlying mechanisms of allergic airway diseases.

Validation of the allergic Guinea pigs

Given the individualized responses of guinea pigs to the allergen, it was crucial to evaluate their phenotypes and exclude nonsensitized or non-allergic animals using standardized methods to ensure the model accurately mimics

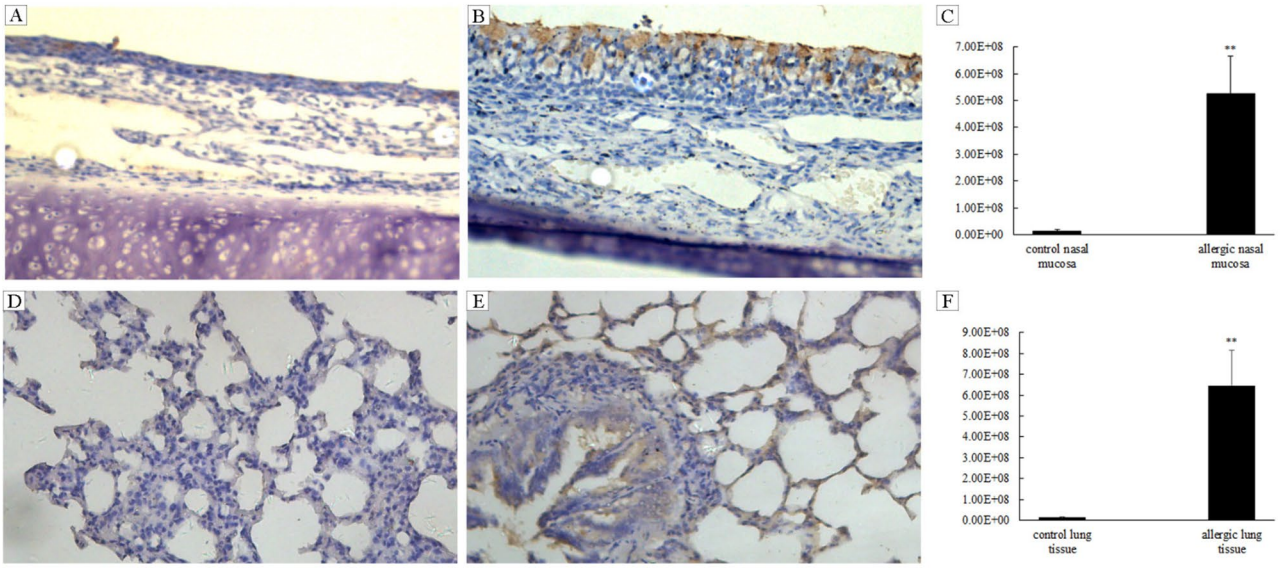

Fig. 6 Increased MUC5AC expression in airway tissues (nasal and lung) of guinea pigs sensitized and challenged with Der f 2. a - c. Nasal mucosa: Paraffin-embedded nasal mucosa from control (**a**) and experimental (**b**) guinea pigs, sacrificed 2 h after the final intranasal instillation of normal saline or Der f 2, were sectioned, incubated with a mouse anti-MUC5AC antibody, and visualized using an ABC kit. Five microscopic fields per section were captured at 200× magnification. Images were analyzed using Image-Pro Plus 12.0 (Media Cybernetics, Rockville, MD, USA), and the integrated optical density (IOD) of the positive regions was calculated to determine the mean optical density (MOD) (**c**). (**$p < 0.01$). d-f. Lung tissues: Paraffin-embedded lung tissues from control (**d**) and experimental (**e**) guinea pigs, sacrificed 2 h after the final intranasal instillation of normal saline or Der f 2, were stained with an anti-MUC5AC antibody and visualized using an ABC kit. MUC5AC expression is presented as the MOD ± SD (**f**). (**$p < 0.01$)

allergic airway responses. To achieve this, we conducted three validation tests:

1. A cutaneous provocation test following two rounds of intraperitoneal (IP) sensitization.
2. A nasal symptoms assessment after inhalation.
3. An active cutaneous anaphylaxis (ACA) test after three rounds of intranasal administration.

These tests were used to exclude nonsensitized or unchallenged guinea pigs. In this study, two guinea pigs with a negative cutaneous provocation test and three guinea pigs with low nasal symptom scores were excluded from the experimental group. The ACA test, which uses Evans blue dye extravasation, provided both qualitative and quantitative data. All 15 remaining guinea pigs in the experimental group exhibited significant Evans blue dye extravasation.

These validation procedures ensured that only qualified model animals were used for subsequent experiments, and the guinea pig model accurately reflected the pathological and pathophysiological features of allergic airway reactions.

Animal species selection

Several animal species, such as rats, dogs, swine, sheep, guinea pigs, and primates, have been used to study the pathophysiology of allergic rhinitis and asthma [8, 28]. Among these species, the guinea pig is particularly well-suited for modeling allergic airway diseases because it exhibits airway pathophysiology similar to that observed in humans [29].

In our preliminary unpublished experiments, we found that it was easier to induce AR in guinea pigs, which exhibited more pronounced nasal symptoms compared to mice. Consequently, we selected guinea pigs as the species for developing our animal model.

Der f 2 allergen delivery routes

In animal models, allergens can be introduced via various routes, including intraperitoneal (IP) injection, subcutaneous (SC) injection, intranasal instillation, or aerosol inhalation [8, 28]. To develop an animal model of allergic airway inflammation, we tested various combinations of sensitization and challenge routes. Following systemic IP sensitization, allergens were administered as a nebulized aerosol.

Aerosol challenge requires direct airway exposure to a higher dose of allergens compared to other methods, but it is less invasive and does not require animal sedation. One key advantage of aerosol inhalation is its ability to induce more intense allergic airway inflammation, as it mimics the natural route of allergen exposure that triggers allergic responses.

Our preliminary unpublished experiments showed that aerosol inhalation of Der f 2 resulted in only mild allergic

rhinitis (AR) symptoms, necessitating the use of bilateral intranasal instillation to extend allergen exposure to the airway mucosa. Combining intermittent aerosol exposure with intranasal instillation led to significant AR and allergic lung inflammation.

Histopathological assessment

In animal models of allergic airway inflammation, histopathological and immunological assessments are commonly used to evaluate the entire airway. The nasal mucosa of the experimental group exhibited typical features of allergic rhinitis, including disruption of the columnar ciliated epithelium, loss of cilia, nasal epithelial hypertrophy, submucosal edema, and infiltration of eosinophils, neutrophils, and lymphocytes. The lungs of the allergen-treated group showed significant infiltration of eosinophils, lymphocytes, and macrophages in the thickened interalveolar walls. Additionally, the alveolar walls were thickened, and small-vessel dilation was observed in the lungs of the allergen-treated group.

In a preliminary experiment, a low dose of recombinant Der f 2 and a weaker challenge led to low nasal symptom scores and mild histopathological changes (unpublished data). In another preliminary experiment, a high dose of Der f 2 for both sensitization and challenge resulted in severe pulmonary edema, leading to the death of half of the guinea pigs during the intranasal instillation phase. These findings indicate that adjusting the dose, delivery route, and administration frequency of Der f 2 was crucial for developing an appropriate allergic airway model for pathophysiological studies.

Thus, optimizing the allergen dose, delivery route, and exposure frequency is essential for developing mild, moderate, or severe allergic airway models tailored to specific pathophysiological research needs.

MUC5AC overexpression

Our experiments demonstrated that MUC5AC protein levels were significantly elevated in the allergic nasal mucosa and lung tissues compared to control tissues. MUC5AC is the primary gel-forming mucin produced by goblet cells and surface epithelial cells [30]. Excessive MUC5AC secretion in the airway and epithelium can lead to various pathological changes, including goblet cell hyperplasia, ciliated cell exfoliation, and submucosal gland hypertrophy. These changes ultimately result in airway mucus hypersecretion, with colloidal mucus plugs frequently obstructing the airways [30].

Standardizing sensitization and challenge protocols is crucial for accurately evaluating the phenotypes of guinea pigs and ensuring that only sensitized and challenged animals are included in the experimental group when developing appropriate allergic airway models. By using standardized methodologies, researchers can perform well-designed studies to investigate the molecular mechanisms underlying allergic airway diseases.

Comparison with Yasue M's study

In Yasue M's study [31], natural Der f 1 (nDer f 1) and recombinant Der f 2 (rDer f 2), along with crude dust mite extract, were used as allergens, with aluminum hydroxide (alum) serving as the adjuvant. Sensitization was performed via subcutaneous injection of a small amount of allergen, and Bordetella pertussis was used as an adjuvant for intradermal injection to enhance IgE production. Unimmunized guinea pigs were used as negative controls. The sensitized animals were challenged both intradermally and intranasally with the antigen. For the control group, naive guinea pigs were divided into three groups and each group was challenged with either mite extract, nDer f 1, or rDer f 2. After establishing the model, the author analyzed the eosinophil viability enhancing activity (EVEA) of spleen cell culture supernatant (SCCS) and measured plasma levels of anti-mite IgE, IgG1, and IgG2. Data from sensitized animals that did not exhibit significant increases in specific airway resistance (sRaw) were omitted.

Our study methodology

In our study, we employed both systemic and local sensitization methods. Systemic sensitization was achieved via intraperitoneal (IP) injection, while local sensitization was performed through aerosol inhalation of wild-type Der f 2. Control guinea pigs were exposed to normal saline (N.S.) under the same conditions. In a separate control experiment, three additional nonsensitized guinea pigs were challenged with bilateral intranasal instillation of 20 μL of wt Der f 2 adsorbed onto $Al(OH)_3$ gel. No significant symptoms, such as nose-scratching or sneezing, were observed in these control animals.

To confirm successful sensitization, we used the cutaneous provocation test (CPT). Of the 20 guinea pigs, two were CPT-negative and were excluded from the experimental group. We also assessed nasal symptoms to identify guinea pigs with allergic rhinitis; those without significant allergic symptoms were excluded from the experimental group. This rigorous validation process enhances the homogeneity of the experimental group.

Additionally, we conducted histopathological analysis and quantified the expression levels of relevant genes in the nasal mucosa and lung tissues. These analyses will aid us in conducting further research on the gene transcription regulation networks related to guinea pig allergic airway inflammation.

Conclusions

In this study, we successfully established a guinea pig model for investigating concurrent allergic rhinitis and asthma using recombinant wild-type Dermatophagoides farinae group 2 allergen (wt Der f 2).

Effective sensitization, challenge, and validation of allergic guinea pigs: Standardized methods were crucial for evaluating phenotypes and excluding nonsensitized or non-allergic animals to ensure the model accurately mimicked allergic airway responses. We conducted three validation tests: (1) a cutaneous provocation test following two rounds of intraperitoneal (IP) sensitization; (2) a nasal symptoms assessment after inhalation; and (3) an active cutaneous anaphylaxis (ACA) test after three rounds of intranasal administration. These validation procedures ensured that only qualified model animals were used for subsequent experiments, accurately reflecting the pathological and pathophysiological features of allergic airway reactions.

Pathological features: Histopathological analyses revealed typical features of allergic rhinitis and asthma in both nasal mucosa and lung tissues, such as disrupted ciliated columnar epithelium, goblet cell hyperplasia, and eosinophil infiltration.

Upregulated MUC5AC expression: Immunohistochemical analysis demonstrated significantly higher levels of MUC5AC in the nasal mucosa and lung tissues of the experimental group compared to the control group.

In conclusion, our guinea pig model offers an effective tool for studying the pathophysiology of allergic rhinitis and asthma, facilitating further advancements in therapeutic development.

Abbreviations

Der f 2	Dermotophagoides farina group 2
Der p 2	Dermotophagoides pteronyssinus group 2
nDer f 2	Natural Der f 2
rDer f 2	Recombinant Der f 2
wt Der f 2	Wild type Der f 2
mu Der f 2	Mutant Der f 2
N.S.	Normal saline
ACA test	Active cutaneous anaphylaxis test
AR	Allergic rhinitis
PAR	Perennial allergic rhinitis
HDM	House dust mite
IP	Intraperitoneal
IH	Inhalation
Il	Intranasal instillation
SPT	Skin prick test
ELISA	Enzyme linked immunosorbent assay
PBS	Phosphate buffered saline
IOD	Integrated optical density
MOD	Mean optical density
SD	Standard deviation
CPT	Cutaneous provocation test
SC	Subcutaneous
EVEA	Eosinophil viability enhancing activity
SCCS	Spleen cell culture supernatant
sRaw	Specific airway resistance

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Author contributions
Feng Xu conducted the experiments and wrote the manuscript; Lineng Zhang conducted the experiments and wrote the manuscript; Li Hu guided the part of experiments and Jianzhong Wang guided part of experiments.

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Data availability
All data used and analyzed during the current study are provided within the manuscript.

Declarations

Ethics approval and consent to participate
The present study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of Fudan University Zhongshan Hospital approved the use of sIgE serum samples from clinical patients in the indirect ELISA experiments of this study (approval number: B2023-236R). All experimental protocols and procedures received approval from the Animal Ethics Committee of Fudan University Zhongshan Hospital.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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