ORIGINAL ARTICLE - EXPERIMENTAL MODELS OF ALLERGIC DISEASE

A new house dust mite–driven and mast cell–activated model of asthma in the guinea pig

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Abstract
Background: Animal models are extensively used to study underlying mechanisms in asthma. Guinea pigs share anatomical, pharmacological and physiological features with human airways and may enable the development of a pre-clinical in vivo model that closely resembles asthma.

Objectives: To develop an asthma model in guinea pigs using the allergen house dust mite (HDM).

Methods: Guinea pigs were intranasally sensitized to HDM which was followed by HDM challenges once weekly for five weeks. Antigen-induced bronchoconstriction (AIB) was evaluated as alterations in $R_n$ (Newtonian resistance), $G$ (tissue damping) and $H$ (tissue elastance) at the first challenge with forced oscillation technique (FOT), and changes in respiratory pattern upon each HDM challenge were assessed as enhanced pause (Penh) using whole-body plethysmography. Airway responsiveness to methacholine was measured one day after the last challenge by FOT. Inflammatory cells and cytokines were quantified in bronchoalveolar lavage fluid, and HDM-specific immunoglobulins were measured in serum by ELISA. Airway pathology was evaluated by conventional histology.

Results: The first HDM challenge after the sensitization generated a marked increase in $R_n$ and $G$, which was abolished by pharmacological inhibition of histamine, leukotrienes and prostanoids. Repeated weekly challenges of HDM caused increase of Penh and a marked increase in airway hyperresponsiveness for all three lung parameters ($R_n$, $G$ and $H$) and eosinophilia. Levels of IgE, IgG1, IgG2 and IL-13 were elevated in HDM-treated guinea pigs. HDM exposure induced infiltration of inflammatory cells into the airways with a pronounced increase of mast cells. Subepithelial collagen deposition, airway wall thickness and goblet cell hyperplasia were induced by repeated HDM challenge.

Conclusion and Clinical Relevance: Repeated intranasal HDM administration induces mast cell activation and hyperplasia together with an asthma-like pathophysiology...
1 | INTRODUCTION

Asthma is a heterogeneous disorder of the airways characterized by variable airflow obstruction and airway hyperresponsiveness (AHR). In allergic asthma, the exposure to innocuous airborne antigens leads to respiratory symptoms, including wheeze, cough, chest tightness and breathlessness as a consequence of excessive airway narrowing. This allergen-induced bronchoconstriction is largely mediated by mast cells, which release a broad range of preformed and de novo synthetized mediators that lead to airflow obstruction. The continuous exposure to allergens results in chronic inflammation and airway remodelling that further compromise airway function, though the mechanisms for the development of these features are still largely undefined. Access to a physiologically relevant in vivo model for mechanistic research in this complex disease involving a systemic reaction and alterations of lung function is essential for improved disease understanding and the development of novel therapies. Today, these investigations are dominated by mouse models of asthma. However, for studying functional lung responses, mouse airway smooth muscle (ASM) has limitations in that it does not respond to several important agonists such as histamine and cysteinyl-leukotrienes (Cys-LTs) and also do not show antigen-induced bronchoconstriction (AIB) since very few mast cells are located in the peripheral parts of the mouse lung. Moreover, murine airways also lack the bronchial circulation, mucus glands and the inhibitory innervation of their ASM that counteract the acute asthmatic attacks. Therefore, to investigate the mechanisms that drive airway bronchoconstriction, there is a need for new improved pre-clinical models that asthmatic features in humans.

The guinea pig has been widely used for asthma modelling because they share several resemblances with humans in lung anatomy, pharmacology and physiology. As in allergic asthma, guinea pig airways display a robust initial response to histamine and leukotrienes, which are largely released by mast cells to induce bronchoconstriction. Mast cell activation also leads to release of cytokines, chemokines and growth factors, which influence inflammatory and immunological reactions, as well as remodelling. As mast cells are distributed throughout the lung, mast cells interact with structural and immune cells along the whole airway tree. This emphasizes the value of measuring the functional responses in both the proximal and distal parts of the lung, especially as it is emerging that alterations of the peripheral part of the lung are important in asthma. Previous research in guinea pig asthma models has not studied the functional differences between different lung compartments, and there is a need to include the use of forced oscillation technique (FOT) for more accurate investigations of lung function measurement.

Although the OVA-driven model of asthma has been extensively applied in guinea pigs, OVA is not a trigger of human asthma. Instead, current asthma models in animals are moving towards using more clinically relevant allergens, particularly those from the HDM, as it has proven to induce a multifaceted immune response involving both the innate and adaptive arms of the immune system that do not appear in OVA models in mice. We have therefore developed a protocol using intranasal administration of HDM that may be used to investigate the AIB and AHR by FOT as well as to follow the early allergic reactions with whole-body plethysmograph during the whole time period. Here, we describe an asthma-like model in the guinea pig that reproduces the hallmark features of allergic asthma such as allergen-induced bronchoconstriction (AIB), AHR and type 2 inflammation with increased numbers of eosinophils and mast cells, and airway remodelling.

2 | METHODS

2.1 | Animals

Male Dunkin-Hartley guinea pigs were obtained from Envigo (Horst, The Netherlands) and housed on 12/12h light/dark cycles with food and water ad libitum. Following an acclimation period, animals with body weight range of 500-600g were used for experiments. All experiments were approved by the Stockholm ethics committee for animal research (permit number N143-14).

2.2 | Protocols of sensitization and challenge to HDM

Lyophilized Dermatophagoides pteronyssinus HDM extract was obtained from Greer Laboratories (Lenoir, NC, USA) and re-suspended in sterile PBS at a concentration of 2.1 mg/mL, and aliquots were stored at −80°C. All experiments were performed using the same HDM batch. Guinea pigs were sensitized to HDM through intranasal route on day 1 (0.5 mg/mL) followed by a booster dose given on day 4 (1.0 mg/mL). Beginning on day 15, animals were intranasally challenged with HDM (0.25 mg/mL) once per week for five consecutive weeks. All instillations were conducted in a volume of 100 µL. The timing and concentrations were based on unpublished experiments and experience with OVA-driven guinea pig models. Animals were lightly anaesthetized with 5% isoflurane (Baxter, Deerfield, Illinois, USA) mixed in oxygen followed by a maintenance dose of 2% isoflurane prior intranasal instillations. The immediate airway response induced by HDM was investigated after the first challenge at day 15 (Figure 1A), whereas airway responsiveness, inflammation and remodelling were measured after all five challenges at day 44. Age-matched PBS-treated guinea pigs were used as controls.
2.3 | Assessment of HDM-induced bronchoconstriction by invasive forced oscillatory technique

On day 15, guinea pigs were anaesthetized intraperitoneally with 40 mg/kg ketamine (Ketalar, Pfizer, Sandwich, UK) and 5 mg/kg xylazine (Bayer, Leverkusen, Germany), and the trachea was exposed and cannulated. Heart rate and oxygenation were monitored throughout the experiment, and the body temperature was maintained at 37°C with a heating pad. Animals were connected to a computer-controlled ventilator, equipped with a module 4 (flexiVent FX, SCIREQ Inc, Montreal, Qc, Canada), and ventilated at respiratory rate of 60 breaths per minute and tidal volume of 10 mL/kg with a positive end-expiratory pressure (PEEP) of 3 cmH₂O. Following 10 minutes of regular ventilation for stabilization, two baseline recordings of respiratory mechanics were obtained. Guinea pigs were initially exposed to PBS over 90 seconds followed by 3-minute measurements, and after that exposed to either HDM (0.25 mg/mL) or PBS over 90 seconds. Intratracheal challenge was by nebulization (Aerogen Ltd, Galway, Ireland), and respiratory mechanics was assessed for 30 minutes. To investigate the non-allergic effect of HDM on the bronchoconstriction, an additional group of non-sensitized (PBS) guinea pigs was exposed to HDM. All measurements were acquired using the flexiWare Software version 7.6 (Scireq Inc). Respiratory mechanics was analysed using the constant phase model to calculate the (Newtonian resistance \( R_n \)); tissue damping \( G \)) and tissue elastance \( H \) parameters. An additional group of HDM-sensitized guinea pigs was treated with a combination of the selective histamine H₁ receptor antagonist pyrilamine (10 mg/kg of body weight), the non-selective COX inhibitor indomethacin (2 mg/kg of body weight) (both obtained from TOCRIS, Abingdon, UK) and the 5-LOX inhibitor MK-886 (3 mg/kg body weight) (Cayman Chemicals, Ann Arbor, MI, USA). Pharmacologic intervention was given intraperitoneally 30 minutes before the HDM challenge.

2.4 | Assessment of HDM-induced changes in respiratory pattern by non-invasive barometric plethysmography

Non-invasive measurements of respiratory responses caused by intranasal challenges with HDM were performed using barometric

| FIGURE 1 | HDM-induced bronchoconstriction in sensitized guinea pigs is mediated by mast cell activation. (A) Protocol for HDM sensitization and first challenge. (B) Temporal course of average responses at the first challenge with HDM or PBS, as determined by invasive measurement of respiratory mechanics using flexiVent FX. Changes in \( R_n \), \( G \) and \( H \) are shown as percentage of increase in baseline values. Recordings were given for 30 minutes. (C) Serum samples were analysed to determine the levels of HDM-specific IgE, IgG₁ and IgG₂ by antigen-capture ELISA. Serum samples were reacted with or without capturing HDM to achieve the ΔABS at 490 nm. Data are representative of two independent experiments. (D) Differential cell count in BALF obtained after PBS or HDM challenge and 30 min of respiratory measurements. Data are mean ± SEM. *P < .05, **P < .01 and ***P < .001. PBS/PBS group (n = 7), sensitized and challenged with PBS; PBS/HDM group (n = 5), sensitized with PBS and challenged with HDM; HDM/HDM group (n = 10–13), sensitized and challenged with HDM; HDM/HDM + pyr + indo + MK-886 group (n = 4), sensitized with HDM and treated with antagonists to histamine, prostaglandins and leukotriene receptors prior to HDM challenge. \( R_n \), Newtonian resistance; \( G \), tissue damping; \( H \), tissue elastance; BALF, bronchoalveolar lavage fluid; HDM, house dust mite; pyr, pyrilamine; indo, indomethacin.
plethysmography for unrestrained animals. Guinea pigs were acclimated to a single-chamber whole-body plethysmograph (EMMS, Hampshire, UK) for two 15-minute sessions prior to starting the experiments. On the challenge days, that is 15, 22, 29, 36 and 43, guinea pigs were placed inside the plethysmography chamber for 15 minutes of habituation and basal readings were then recorded for 10 minutes. Afterwards, guinea pigs were intranasally challenged with either HDM or PBS, and following a few seconds of anaesthesia recovery, animals were reintroduced into the plethysmography chamber and the respiratory pattern was monitored for 90 minutes. Pressure signals inside the chamber were processed with eDaq Software version 1.8 (EMMS), and respiratory parameters, including the enhanced pause (Penh), were acquired. Penh was obtained according to principles previously described \(^{14-16}\) and expressed as percentage increase over baseline values.

### 2.5 | Assessment of airway responsiveness to methacholine

Respiratory mechanics were assessed 24 hours after the fifth challenge using forced oscillation technique. Guinea pigs were anaesthetized with ketamine and xylazine, and additional doses were administered as needed. Animals were placed on a warm pad, and the trachea was exposed and connected via a cannula to flexiVent FX as described above. Following standard lung volume history, two baseline measurements were acquired. Animals were then exposed to PBS aerosols for 10 seconds followed by respiratory measurements. Increased doses of methacholine (Sigma-Aldrich, St. Louis, MO, USA) (0.018-0.32 mg/mL) were given at 7-minute intervals. Because guinea pig airways are extremely reactive to contractile agonists, low doses of methacholine were chosen based on previous studies.\(^ {17,18}\) \(R_n\) was considered to calculate the provocative dose 200\% (PD\(_{200}\)), that is the interpolated methacholine dose that caused a threefold increase of basal value.

### 2.6 | Cell counts in bronchoalveolar lavage fluid

Following lung function measurements, guinea pigs were killed with an overdose of ketamine and xylazine, and blood was collected by cardiac puncture. Lungs were flushed two times with 5 mL of sterile saline (0.9% NaCl), and the recovered bronchoalveolar lavage fluid (BALF) was pooled. BALF was centrifuged and separated into cell-free BALF for measurement of inflammatory mediators and BALF cells for cell counting and cytospins. Total cells were counted using a Bürker haemocytometer, and ≤50 000 cells were used for cytospins. Cells were stained with May-Grünwald-Giemsa (Histolab Products AB, Gothenburg, Sweden) according to the manufacturer’s protocol. Leucocytes were identified based on morphologic criteria and quantified by counting 300 cells per slide.

### 2.7 | Cytokine analysis

Cytokines were quantified in BALF by sandwich ELISAs (Nordic BioSite; Täby Sweden) according to the manufacturer’s protocol.

### 2.8 | Quantification of serum HDM-specific immunoglobulin levels

HDM-specific immunoglobulins were measured using the antigen-capture ELISA method according to standard protocols. Anti–guinea pig IgE (Nordic BioSite AB, Täby, Sweden), and anti–guinea pig IgG\(_1\) (246-GAGp/IgG1/Bio) and IgG\(_2\) (246-GAGp/IgG2/Bio) (both from Nordic Immunological Laboratories, Susteren, The Netherlands) were used for the detection of immunoglobulin subclasses. Diluted serum samples were added to wells with or without capturing antigen, and the delta absorbance at 490 nm (\(\Delta\)ABS 490 nm) was calculated as the difference between wells with or without antigen capture.

### 2.9 | Lung tissue histology

Lungs were perfused with 5 mL of saline solution through the trachea and harvested immediately. Left caudal lobe was excised and fixed with 10% neutral buffered formalin. To identify mast cells in airway tissue, right caudal lobe was fixed with Carnoy’s fixative (60% ethanol, 30% chloroform and 10% acetic acid). Tissues were dehydrated and embedded in paraffin for sectioning. Caudal left lobe sections were stained with haematoxylin and eosin (H&E) (Histolab, Göteborg, Sweden), Picro-Sirius Red staining (Abcam, Cambridge, UK) and Periodic Acid-Schiff (PAS) (Sigma-Aldrich). Caudal right lobe sections were stained with Astra blue (Sigma-Aldrich), which is a cationic dye that binds specifically to heparin contained in mast cell granules.\(^ {19}\) Stained airways were randomly selected under low power light microscopy (x40 magnification) and captured by using an Olympus UC50 camera (Olympus Australia, Melbourne, VIC, Australia) attached to an Olympus BX51 microscope. Areas (µm\(^2\)) of infiltrated inflammatory cells into the airways as well as the subepithelial region (SER) thickening and the ASM layer were estimated in H&E-stained lungs. Subepithelial connective tissue was identified as red-stained areas in Sirius red sections. All measurements were adjusted by length of the corresponding basement membrane (BM) perimeter (µm). PAS-positive mucus-containing cells within airway epithelial cells layer and Astra blue–stained mast cells were counted under microscope and expressed as the number of goblet cells or mast cells per mm of BM. All measurements were conducted by three independent persons blinded to the experimental groups using ImageJ Software (version 1.51j8, Wayne Rasband, National Institutes of Health, Bethesda, USA).
2.10 | Statistical analysis

All data were analysed using GraphPad Prism 8 Software (GraphPad Software Inc, La Jolla, CA, USA) and are presented as the mean ± SEM. Statistical analysis was performed using one-way or two-way ANOVA followed by Dunnett or Tukey’s multiple comparison test. In addition, unpaired Student’s t test was included. Significant differences were defined as *P < .05, **P < .01 and ***P < .001.

3 | RESULTS

3.1 | Mast cell mediators drive the HDM-induced bronchoconstriction in sensitized guinea pigs

To validate the effect of sensitization, we first investigated the respiratory mechanics in response to challenge with HDM using FOT (Figure 1A). The animals were challenged with either PBS or HDM delivered intratracheally as aerosols. HDM challenge caused an immediate and transient increment in the resistance of conducting airways (Rn) in HDM-sensitized guinea pigs compared with both groups of PBS-sensitized guinea pigs challenged with either PBS or HDM (Figure 1B). In contrast, the tissue damping (G) was persistent in HDM-sensitized guinea pigs compared with both PBS-sensitized groups (Figure 1B). Aerosol challenge with either PBS or HDM did not affect overall tissue elastance (H) in neither PBS- nor HDM-sensitized animals (Figure 1B).

To explore the contribution of mast cell mediators to the response, HDM-sensitized guinea pigs were treated with a combination of a selective histamine H1 receptor antagonist (pyrilamine), an inhibitor of prostaglandin production (indomethacin) and a leukotriene synthesis inhibitor (MK-886) prior to challenge with HDM. This pharmacological intervention completely abolished the HDM-induced bronchoconstriction in HDM-sensitized guinea pigs (Figure 1B).

To approach the immunological response in the allergic reactions induced by HDM, allergen-specific immunoglobulins were quantified. HDM-treated guinea pigs showed a significant increase of HDM-specific IgG1 and IgG2, but not IgE, as compared with the PBS-sensitized groups (Figure 1C). Moreover, differential cell count of immune cells in BALF following the first challenge revealed that HDM induced a robust and significant increase in the number of eosinophils and lymphocytes in both HDM- and PBS-sensitized guinea pigs as compared with PBS-challenged animals (Figure 1D). In contrast, HDM challenge induced a marked increase in the number of neutrophils only in PBS-sensitized guinea pigs (Figure 1D). The number of macrophages was similar among the groups (data no shown).

3.2 | Repeated intranasal HDM challenges induce changes in the respiratory pattern of guinea pigs

To investigate the effect of prolonged exposure to HDM on the airway responses, we adopted a long-term protocol of repeated intranasal challenges applied for up to 5 weeks (Figure 2A). The respiratory pattern of unrestrained guinea pigs was assessed at each challenge by non-invasive whole-body plethysmography, monitoring breathing pattern with no interference by anaesthetic agents or animal restriction. HDM challenge elicited a noticeable increase in Penh over the baseline value (Figure 2B), and responses over 200% of basal Penh were detected after 25 minutes with maximum Penh responses (Rmax) observed between 40 and 70 minutes post-challenge. Recovery was complete by 90 minutes (Figure 2B). Rmax induced by HDM reached values over 200% of baseline and was significantly higher than Rmax from PBS-treated controls at each challenge (Figure 2C). PBS instillation did not induce bronchoconstriction in control animals (Figure 2), and baseline Penh values remain unchanged throughout the study and were similar among the groups (data no shown).

3.3 | HDM administration induces AHR in guinea pigs

To investigate whether the exposure to HDM affects the AHR, we assessed the airway responses to methacholine after five weeks of challenges using FOT. HDM-treated guinea pigs showed marked increase in Rn compared with PBS-treated controls (Figure 3A). Moreover, HDM-challenged animals showed both higher G (Figure 3B) and H (Figure 3C) values than PBS-treated controls upon methacholine aerosols, indicating that HDM affects both conducting airways and tissue mechanics. In addition, HDM-treated animals displayed a marked decrease of methacholine PD50 compared with PBS animals (Figure 3D).

3.4 | Exposure to HDM promotes a marked increase of immunoglobulins and mast cell numbers in sensitized guinea pigs

We next examined whether repeated exposure to HDM through the respiratory mucosa alters the production of allergen-specific immunoglobulins and induces mast cell accumulation. HDM-treated guinea pigs exhibited increased levels of specific IgE, IgG1 and IgG2 as compared with PBS-treated controls (Figure 4A). In addition, lung sections stained with Astra blue revealed a robust increase of mast cells around both proximal and distal airways in HDM-treated guinea pigs (Figure 4B).

3.5 | Repeated intranasal HDM challenges result in airway inflammation

Analysis of cells in BALF revealed a significant induction of eosinophils in response to HDM challenge, but not of macrophages, neutrophils and lymphocytes (Figure 5A). Histological examination of H&E-stained lung sections showed an extensive infiltration of inflammatory cells in HDM-challenged guinea pigs (Figure 5B).
To investigate potential mediators that might play a role in the induction of allergic airway inflammation in this model, pro-inflammatory cytokines were quantified in BALF obtained 24 hours after the last challenge. HDM-treated guinea pigs exhibited significantly increased levels of the type 2 cytokine IL-13 but not IL-4 (Figure 6A-B). Levels of the eosinophil chemotactic mediators, IL-5 and CCL11 (eotaxin-1), and the pro-inflammatory cytokine IL-6 in BALF were not significantly affected by repeated exposure to HDM (Figure 6C-E).

3.6 HDM challenge induces airway structural changes

Analysis of H&E-stained lung tissues revealed a significant increase of the airway subepithelial region of HDM-treated animals compared to PBS-treated controls (Figure 7A). In addition, the ASM layer was slightly increased in HDM-treated animals but did not reach significance between the groups (Figure 7A).

To further confirm that repeated HDM challenges promote structural changes in the airways, we assessed collagen deposition by using Picro-Sirius Red staining. Challenge with HDM exhibited a marked accumulation of collagen in the wall of distal airways (representative lung sections in Figure 7B, collagen stained red). The morphometric analysis demonstrated that HDM-treated animals had a significant increase of connective tissue compared with PBS-treated controls (Figure 7B). Furthermore, the examination of PAS-stained lung sections revealed a significant increase in the number of mucus-producing cells in HDM-treated animals as compared with PBS-treated controls (Figure 7C).

4 DISCUSSION

Here, we present a new pre-clinical asthma model, where exposure of the respiratory mucosa of the guinea pig to HDM extract elicits an asthma-like pathology. The observed AIB was caused by release of mast cell mediators, indicating that HDM exposure triggers mast cell activation, allergic inflammation and airway remodelling, as well as increasing the number of mast cells in the lung. Moreover, for the first time, it was possible to demonstrate HDM-induced AHR in the guinea pig. This asthma model meets a need where mast cell involvement can be studied and evaluate how these cells and their mediators influence the bronchial constriction in asthma.

By using FOT measurements for the first time in guinea pigs, the functional lung response to HDM exposure was investigated at the first challenge after the sensitization. HDM caused a transient increase of Rn, indicating contraction of the conducting airways, and a sustained increase of G, reflecting the resistance of the peripheral airways. In asthmatic individuals, the distal airways are the main site of airflow obstruction, where smooth muscle contraction results in the reduction of diameter and length of airways.10,11 Another important similarity between human and guinea pig airways is that the acute response to allergen provocation is largely mediated by histamine and leukotrienes.20,21 In agreement with this, we were able to abrogate the effects of HDM on lung mechanics through pharmacological
FIGURE 3  HDM induces airway hyperresponsiveness in the guinea pig. Airway responsiveness was assessed 24 h after the last challenge with HDM or PBS by invasive measurements using flexiVent FX. (A) Resistance in conducting airways ($R_n$), (B) tissue damping ($G$) and (C) tissue elastance ($H$) were measured in response to increasing aerosols of methacholine (0.018 - 0.32 mg/mL). (D) Airway responsiveness to methacholine was evaluated by means of the methacholine dose that causes a threefold increase (200%) of basal airway resistance, that is the provocative dose 200% ($PD_{200}$). A decrease in $MCh \; PD_{200}$ indicates an increase in airway reactivity. Data are mean ± SEM of $n=8$ animals each group. * $P < .05$ and ** $P < .01$ compared with PBS control. $R_n$, Newtonian resistance; $PD_{200}$, provocative dose 200; MCh, methacholine; HDM, house dust mite extract.

FIGURE 4  HDM-sensitized guinea pigs exhibit a marked increase of mast cells in the airways. (A) HDM-specific IgE, IgG$_1$ and IgG$_2$ levels in serum were measured by antigen-capture ELISA method. Serum samples were reacted with or without capturing HDM to achieve the $\Delta$ABS at 490 nm. Data are representative of two independent experiments. (B) Representative Astra blue–stained lung sections for mast cells in HDM- or PBS-treated guinea pigs (left). The number of mast cells in distal airways was related to BM perimeter (mm) (right). Data are mean ± SEM of $n=8$ animals each group. * $P < .05$ and ** $P < .001$. HDM, house dust mite; Delta absorbance ($\Delta$ABS) at 490 nm; basement membrane (BM).
intervention with histamine H<sub>1</sub> receptor antagonist together with leukotriene and prostanoid synthesis inhibitors, demonstrating that HDM-induced bronchoconstriction was caused by mast cell activation. Thus, the anatomic co-localization of mast cells and smooth muscle in the airway is an important factor to consider for accurate modelling of the allergen-induced bronchoconstriction that occurs in asthma.

To verify that HDM challenge induces allergic activation of mast cells, we quantified the levels of HDM-specific immunoglobulins following the first challenge and found that HDM-treated guinea pigs were indeed sensitized to the allergen as shown by increased levels of HDM-specific IgG<sub>1</sub> and IgG<sub>2</sub> compared with PBS-treated guinea pigs. The induction of both IgG subclasses in response to allergens, including dust mites, has previously been described in guinea pigs. Furthermore, it has been shown that both immunoglobulins can induce the ASM contraction, also in line with our study. In contrast, no differences in the HDM-specific IgE levels following the sensitization
phase were found. Furthermore, the HDM administration led to increased numbers of inflammatory cells in the BALF. Eosinophils and lymphocytes were found to be elevated irrespectively of the sensitization status, whereas neutrophils were only increased in non-sensitized animals. The increase in the number of neutrophils infiltrated into the airways most likely is due to the activation of innate immune responses at the first contact with the irritative components of HDM extract rather than an allergic response. As non-sensitized guinea pigs did not respond to HDM challenge, this supports the notion that the immediate bronchoconstriction was caused by sensitization to HDM and not by increase of cells in BALF.

The allergic responses to the repeated once-weekly intranasal HDM administrations were further followed by Penh measurements with unrestrained whole-body plethysmography during the five-week challenge period. Penh showed a progressive rise that reached its maximum response within 40-70 minutes and then resolved by 90 minutes after challenge. Penh may not reflect the changes in the airway resistance, but it may be used to monitor changes in airflow. Thus, as the long-term effect of HDM on bronchoconstriction measured by FOT was associated with increased G values, it can be speculated that constriction of the peripheral airways is the cause also of the increase in Penh.

In the current study, we showed that the repeated intranasal challenge with HDM extract leads to AHR in guinea pigs. Although HDM was introduced for sensitization of guinea pigs already 1973 and found to elicit AIB after repeated intradermal injection or five days of aerosol challenge, no AHR to histamine or methacholine was found. These observations suggest that HDM extract instilled directly into the airways likely induces a more intense mucosal-driven allergic response that causes AHR. Furthermore, as the responses were measured using FOT, the AHR was found in both central and peripheral airways. As this technique has not been used for airway responsiveness measurements before in guinea pigs, it opens for investigating new dimensions in the coming studies.

One potential driver of AHR in our model is the extensive airway remodelling characterized by significant thickening of the airway wall by accumulation of connective tissue in the subepithelial region and increase of club cells observed in response to continued
exposure to HDM. Mucosal fibrosis and goblet cell metaplasia and hyperplasia in the airway epithelium are often observed in patients with asthma, and they have been recognized as markers of lung remodelling.32,33 In guinea pig OVA models, this has been shown after 15 days or after 13 weeks of OVA exposure.34 In addition, we observed an increase in the density of ASM upon repeated HDM exposures, although this did not reach statistical significance. The thickness of ASM layer is the major abnormality that affects the airway physiology in asthmatic subjects.35 In many animal models, high doses of allergen are used to achieve structural changes in the airways. However, prolonged exposure to high doses of allergen, particularly in OVA-driven models, may induce the development of tolerance,36 whereas low doses of allergen may result in too mild allergic responses.37-39 In our study, the allergen tolerance was avoided by using increasing doses of HDM during the sensitization phase followed by several challenges with low HDM doses sufficient to cause airway remodelling.

Evaluation of the airway inflammation, which also is implicated in the development of AHR, showed that HDM challenge induced eosinophilic inflammation in BALF, without altering the number of total inflammatory cells. Histologic examination of lung tissue showed that the continuous exposure to HDM leads to sustained inflammation in the proximal and distal airways. This is in agreement with other asthma models, including those in guinea pigs, where HDM challenge has been reported to induce eosinophilic airway inflammation,40 recapitulating the increased eosinophil levels observed in BALF obtained from asthma patients.35,39 To further characterize the allergic inflammation in our HDM-driven model, inflammatory mediators were quantified in the BALF of guinea pigs. IL-13 was found to be elevated in HDM-challenged guinea pigs. Moore et al showed that IL-13 is required for the expression of AHR and maintenance of airway eosinophilia in the guinea pig OVA model.41 In addition, human recombinant IL-13, but not IL-4, can induce the differentiation of guinea pig tracheal epithelial cells into mucus-producing goblet cells.42 Consistent with this, the repeated HDM challenge led to up-regulation of IL-13, but not other type 2 mediators such as IL-4 and IL-5. Notably, airway eosinophilia was not associated with the IL-5 and CCL11 production. However, this likely was not the optimal time point to detect elevated levels of cytokines as eosinophilic inflammation was already established in the airways.

Although the underlying mechanisms of AHR remain poorly understood, the induction of IgG1 sensitization has been related to a greater contractile response of ASM in the guinea pig.13,43 In our study, intranasal HDM administration induced high levels of allergen-specific IgG1 and IgG2 in both the short- and long-term protocols and increased allergen-specific IgE level following the repeated challenges. In agreement with our findings, Nabe and colleagues found that repeated inhalations of OVA induced an IgG1 response already at an early phase of the protocol, whereas IgE appeared only after repeated challenges.44 Thus, in our HDM-driven model, multiple doses of the allergen over time might be required to induce IgE-mediated responses, whereas IgG1- and IgG2-mediated responses are activated by low levels of HDM. As mast cells from guinea pig sensitized with IgG1 release higher levels of histamine than those sensitized with IgE, IgG1 has frequently been associated with allergic responses in the guinea pig.22,45,46 These data suggest that mast cells may drive the airway dysfunction in our HDM-driven asthma model in guinea pig. Given that mast cells play a key role in the pathobiology of asthma, one of the major advantages of using guinea pigs for asthma studies is that distribution and function of mast cells is similar to that in human.7,47 In our model, mast cell–dependent reactions, AHR and airway inflammation, were achieved upon the repeated intranasal challenge.

Taken together, the purpose of this paper was to establish a new asthma model by the use of a species that physiologically, anatomically and pharmacologically has great similarities to humans4 using a method for sensitization with a relevant allergen through respiratory mucosa that activates both the adaptive and the innate immunity in the airways. The study provides evidence that the used protocol generates several key features of asthma such as AIB, AHR, remodelling and recruitment of inflammatory cells (including mast cells) together with an increase of IL-13. The model is therefore clearly relevant to human asthma and provides new opportunities to investigate the immunological processes and the mechanisms behind AHR, remodelling and recruitment of inflammatory cells. With this first step in this direction, we wish to demonstrate a model that can be used for future opportunities to study specific cell types and/or mediators, including the involvement of mast cells in asthma.

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