Mast Cell-Deficient Kit^{W-sh} Mice Develop House Dust Mite-Induced Lung Inflammation despite Impaired Eosinophil Recruitment

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Key Words

Asthma · House dust mite · Mast cells · Eosinophils · Allergic lung inflammation

Abstract

Background: Mast cells are implicated in allergic and innate immune responses in asthma, although their role in models using an allergen relevant for human disease is incompletely understood. House dust mite (HDM) allergy is common in asthma patients. Our aim was to investigate the role of mast cells in HDM-induced allergic lung inflammation. **Methods:** Wild-type (Wt) and mast cell-deficient Kit^{W-sh} mice on a C57BL/6 background were repetitively exposed to HDM via the airways. **Results:** HDM challenge resulted in a rise in tryptase activity in bronchoalveolar lavage fluid (BALF) of Wt mice, indicative of mast cell activation. Kit^{W-sh} mice showed a strongly attenuated HDM-induced recruitment of eosinophils in BALF and lung tissue, accompanied by reduced pulmonary levels of the eosinophil chemoattractant eotaxin. Remarkably, Kit^{W-sh} mice demonstrated an unaltered capacity to develop lung pathology and increased mucus production in response to

HDM. The increased plasma IgE in response to HDM in Wt mice was absent in Kit^{W-sh} mice. **Conclusion:** These data contrast with previous reports on the role of mast cells in models using ovalbumin as allergen in that C57BL/6 Kit^{W-sh} mice display a selective impairment of eosinophil recruitment without differences in other features of allergic inflammation.

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Introduction

Asthma is a disease characterized by episodes of reversible airway obstruction, dyspnea and wheezing. In Western countries asthma prevalence has reached 10% on average and remains to increase towards epidemic proportions [1]. The pathophysiology of asthma frequently involves a varying degree of allergen-induced lung inflammation that can be difficult to manage clinically and can lead to airway remodeling [2]. House dust mite (HDM) allergy – and subsequent HDM-induced allergic lung inflammation – is common in asthma patients: in most populations the majority of asthma pa-

tients are allergic for HDM [3–5]. Better understanding of the role of different cellular subsets contributing to HDM-induced allergic lung inflammation could lead to new anti-inflammatory treatment approaches.

Mast cells are resident tissue cells that are described to have important immunoregulatory functions in allergic lung inflammation and asthma [6, 7], but their precise involvement in HDM-induced allergic lung inflammation is not fully clarified [8]. Upon activation mast cells are able to release proinflammatory mediators such as histamine, tryptase, serotonin, heparin sulfates, lipid mediators, such as PGE₂ and LTB₄, and a vast range of interleukins [9, 10]. Mast cells can be activated by both IgE-dependent and IgE-independent pathways [8, 11]. A simplistic view of mast cells as ‘merely’ secretory proinflammatory and secretory cells has changed due to new insights in the involvement of mast cells in wound healing, UV irradiation protection, tumor biology [reviewed in 10] and pulmonary fibrin and fibrinolysis homeostasis in asthma [reviewed in 12]. Mast cell-deficient mice are a well-known tool for studying the role of mast cells in mouse asthma, but have not been investigated in a C57BL/6 strain-based model of HDM-induced allergic lung inflammation.

The hematopoietic system develops progenitor mast cells that further mature into mast cells at the target resident peripheral tissue. Mast cells especially reside around blood vessels, nerves and in epithelial organs such as the skin, gastrointestinal tract and lung [8, 10]. The expression of c-Kit tyrosine kinase receptor (CD117) on mast cells is essential for the appropriate development and proliferation of progenitor mast cells from the hematopoietic system [13]. Two genetic c-Kit mutant mouse strains have been investigated most frequently in asthma models: c-Kit^{W/W-v} and c-Kit^{W-sh/W-sh} (Kit^{W-sh}) mice [14]. In contrast to Kit^{W-sh} mice, which have an inversion mutation on chromosome 5 at the transcriptional site of c-Kit [15, 16], c-Kit^{W/W-v} mice have significant comorbidity (e.g. anemia, infertility, dermatitis, skin ulceration), which makes the latter mouse strain less suitable for asthma studies examining the role of mast cells.

Here, we investigated the impact of mast cell deficiency using mast cell-deficient Kit^{W-sh} mice with a C57BL/6 background in a recently developed HDM-evoked mouse asthma model [17]. We show that mast cell deficiency attenuated the recruitment of eosinophils and was associated with lower pulmonary levels of eotaxin. Remarkably, in this HDM-induced model C57BL/6 mice that lack mast cells were able to develop increased mucus production and allergic lung pathology equivalent to Wt mice.

Materials and Methods

Mice

C57BL/6 wild-type (Wt) mice were purchased from Charles River Inc. (Maastricht, The Netherlands). Mast cell-deficient Kit^{W-sh} on a C57BL/6 background were obtained from Jackson Laboratories (Bar Harbor, Me., USA), housed in standardized specific pathogen-free conditions, and sex and age matched. Experiments started when animals were 8–9 weeks old. Each group consisted of 8 mice (except for one of the Wt saline groups, n = 5; see figure legends). The Animal Care and Use Committee of the University of Amsterdam approved all experiments.

HDM Asthma Model

HDM allergen whole body extract (Greer Laboratories, Lenoir, N.C., USA), derived from the common European HDM species *Der-matophagoides pteronyssinus*, Der p, was used to induce allergic lung inflammation as previously described [17]. Briefly, mice were inoculated intranasally on day 0, 1 and 2 with 25 µg HDM (sensitization phase) and on day 14, 15, 18 and 19 with 6.25 µg HDM (challenge phase). Controls received isotonic sterile saline intranasally on each occasion. Inoculum volume was 20 µl for every HDM and saline exposure and inoculation procedures were performed during isoflurane inhalation anesthesia. The experiment was ended at day 21 by euthanizing the mice and the subsequent collection and processing of samples: in one experiment bronchoalveolar lavage fluid (BALF) and citrated blood was collected, in a separate experiment one lung was obtained for pathology and one lung for homogenization.

Bronchoalveolar Lavage and Tissue Handling

BALF was harvested after exposing the trachea through a mid-line incision and instilling and retrieving 1 ml of sterile saline 0.9% (in 500-µl aliquots) [17]. Cell counts were determined for each BALF sample in a hemocytometer (Beckman Coulter, Fullerton, Calif., USA) and differential cell counts by Cytospin preparations stained with Giemsa stain (Diff-Quick; Dade Behring AG, Düringen, Switzerland). In independent experiments nonlabeled lungs were homogenized in 5 volumes of sterile 0.9% saline using a tissue homogenizer (Biospec Products, Bartlesville, Okla., USA) or fixed in 10% formalin.

Histology

Lungs were embedded in paraffin after fixation in 10% formalin; 4-µm-thick sections were stained with hematoxylin and eosin (HE). Parameters of allergic lung inflammation were scored by an experienced histopathologist who was blinded for treatment and strain of mice. The following parameters were scored: interstitial inflammation, peribronchial inflammation, edema, endothelialitis and pleuritis, each graded on a scale of 0–4 (0: absent, 1: mild, 2: moderate, 3: severe, 4: very severe). The total pathology score was expressed as the sum of the score for all parameters. Periodic acid Schiff (PAS)-D staining for carbohydrates in mucus was performed to quantify the amount of mucus. The amount of mucus per lung section was scored by a histopathologist in a semiquantitative fashion on a scale of 0–8 (0–4 for plug formation, 0–4 for mucus extent).

Immunohistochemistry and Digital Image Analysis

Eosinophil staining was performed using a monoclonal antibody against granule major basic protein (GMBP; kindly provided by Dr. Nancy Lee and Prof. James Lee, Mayo Clinic Arizona,

Scottsdale, Ariz., USA) [17]. Entire sections were digitized with a slide scanner using the 10× objective (Olympus dotSlide, Tokyo, Japan). Images were exported in the TIFF format for quantification. Influx of eosinophils was determined by measuring the GMBP immunopositive area by digital image analysis (ImageJ 1.46, National Institute of Health, Bethesda, Md., USA), and subsequently expressed as a percentage of the total lung area. The average of ten pictures was used for analysis of eosinophilic pulmonary influx.

Assays

Plasma total IgE was measured as described using rat anti-mouse IgE as capture antibody, purified mouse IgE as standard and biotinylated rat-anti-mouse IgE as detection (all reagents from BD Biosciences Pharmingen, Breda, the Netherlands) [17]. Concentrations of lung eotaxin, IL-4, IL-5 and IL-13 were measured using Elisa (R&D Systems, Abingdon, UK). Tryptase enzyme activity was determined as described [18] using an amidolytic assay with chromogenic substrate s-2288. Shortly, 10 μ l 6-mM chromogenic tryptase substrate S-2288 (Chromogenix Instrumentation Laboratory, Milan, Italy) was added to 70 μ l 57-mM Tris-HCl with pH 8.3 in a 96-well microtiter plate. After initiating the reaction by adding 40 μ l of BALF sample the Δ A405 nm was developed in 1 h with subtraction of the baseline measurement and monitored in a plate reader at 37°C (Biotek Instruments, Winooski, Vt., USA). Differences were calculated relative to optical density at the zero time point.

Statistical Analysis

Values are expressed as mean $\pm$ SEM. Differences between groups were tested by Mann-Whitney U test. GraphPad Prism version 5.0 (GraphPad Software, San Diego, Calif., USA) was used for all analyses. Values of $p < 0.05$ were considered statistically significant.

Results

HDM Airway Exposure Results in Enhanced Tryptase Activity in BALF of Wt Mice

First, we established whether repeated HDM exposure resulted in enhanced mast cell degranulation. For this we measured tryptase activity in BALF at day 21, 2 days after the last challenge (fig. 1) [18, 19]. Tryptase activity was very low in saline-treated mice. HDM treatment increased tryptase activity in Wt mice ($p < 0.01$ vs. saline controls), but not in Kit^{w-sh} mice ($p < 0.05$ vs. HDM-treated Wt mice). These data indicate that our HDM asthma model is associated with mast cell activation in the bronchoalveolar compartment.

Kit^{w-sh} Mice Have Reduced Influx of Cells in BALF after HDM Exposure due to Decreased Recruitment of Eosinophils

Upon HDM exposure of the airways, both Wt and Kit^{w-sh} mice show increased total cell influx in BALF (fig. 2a; $p < 0.001$ and $p < 0.01$ vs. their respective saline controls). Total cell influx in BALF was significantly reduced in Kit^{w-sh} mice after HDM instillation compared

Fig. 1. Tryptase activity levels (Δ OD vs. OD at baseline): HDM airway exposure induces tryptase activity in BALF of Wt but not of Kit^{w-sh} mice. Data are means $\pm$ SEM of 8 mice per group. ** $p < 0.01$ versus saline; † $p < 0.05$ versus HDM-exposed Wt mice. OD = Optical density.

Fig. 2. Kit^{w-sh} mice have decreased total cell counts in BALF after HDM challenge due to lower eosinophil influx: total cell counts (a) and differential cell counts (alveolar macrophages, eosinophils, neutrophils and lymphocytes; b). Data are means $\pm$ SEM (10⁶ cells/ml) of 8 mice per group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus saline-challenged mice of the same genotype; † $p < 0.05$ versus Wt mice challenged with HDM.

Fig. 3. Kit^{w-sh} mice demonstrate a reduced influx of eosinophils in lung tissue after HDM challenge. **a** Percentage of lung surface stained positive for eosinophils quantified by digital imaging of GMBP staining (see Materials and Methods). Data are means $\pm$ SEM of 8 mice per group except for Wt saline ($n = 5$). * $p < 0.05$ and ** $p < 0.01$ versus saline-challenged mice of the same genotype; † $p < 0.05$ versus Wt mice challenged with HDM. **b** Representative GMBP staining of lung tissue slides of Wt mice exposed to saline, Kit^{w-sh} mice exposed to saline, Wt mice exposed to HDM and Kit^{w-sh} mice exposed to HDM. Original magnification $\times 40$.

to Wt mice (fig. 2a; $p < 0.05$). The reduction in total cell influx was explained by a decrease in HDM-evoked eosinophil recruitment in Kit^{w-sh} mice compared to Wt mice (fig. 2b; $p < 0.05$). Relative to saline controls, Wt and Kit^{w-sh} mice showed similar increases in HDM-induced influx of neutrophils (both $p < 0.01$) and lymphocytes ($p < 0.01$ and $p < 0.05$, respectively). Together, these data indicate that Kit^{w-sh} mice have decreased cell numbers in

Fig. 4. Kit^{w-sh} and Wt mice demonstrate similar lung pathology after HDM challenge. **a** Semiquantitative pathology scores (described in Materials and Methods). Data are means $\pm$ SEM of 8 mice per group except for Wt saline ($n = 5$). ** $p < 0.01$ and *** $p < 0.001$ versus saline-challenged mice of the same genotype. **b** Representative HE-stained lung tissue slides of Wt mice exposed to saline, Kit^{w-sh} mice exposed to saline, Wt mice exposed to HDM and Kit^{w-sh} mice exposed to HDM. Original magnification $\times 40$.

BALF in the HDM asthma model caused by a decreased migration of eosinophils to the bronchoalveolar compartment.

Reduced Eosinophil Accumulation in Lung Tissue in Kit^{w-sh} Mice upon HDM Administration

Lung tissue eosinophils were detected by GMBP staining, analyzed by digital imaging and expressed as the per-

Fig. 5. Kit^{w-sh} mice show unaltered mucus production in their airways upon HDM challenge. **a** Semiquantitative mucus scores (described in Materials and Methods). Data are means $\pm$ SEM of 8 mice per group except for Wt saline ($n = 5$). ** $p < 0.01$ and *** $p < 0.001$ versus saline-challenged mice of the same genotype. **b** Representative PAS-D-stained lung tissue slides of Wt mice exposed to saline, Kit^{w-sh} mice exposed to saline, Wt mice exposed to HDM and Kit^{w-sh} mice exposed to HDM. Original magnification $\times 40$.

centage of lung surface occupied by eosinophils (fig. 3). HDM instillation caused an increase in pulmonary eosinophils in both Wt and Kit^{w-sh} mice compared to saline (fig. 3a; $p < 0.01$ and $p < 0.05$, respectively). The number of eosinophils in lung tissue of Kit^{w-sh} mice was decreased by 74% compared to Wt mice ($p < 0.05$), corroborating the findings in BALF shown in figure 2 and indicating decreased HDM-induced pulmonary recruitment of eosinophils in Kit^{w-sh} mice.

Kit^{w-sh} Mice Develop HDM-Evoked Lung Pathology to a Similar Extent as Wt Mice

HE-stained slides of lung tissue were scored for parameters of allergic lung inflammation in a semiquantitative fashion as described in Materials and Methods (fig. 4). Repeated HDM exposure resulted in lung pathology in both Wt and Kit^{w-sh} mice ($p < 0.01$ and $p < 0.001$ vs. their respective saline controls). Surprisingly, there was no difference in the extent of lung pathology between Wt HDM- and Kit^{w-sh} HDM-challenged groups. Moreover, the scores of distinct pathology features (i.e. perivascular inflammation, interstitial inflammation, endothelialitis, peribronchitis and edema) were not different between HDM-exposed groups (data not shown).

HDM-Induced Pulmonary Mucus Production Is Similar in Wt and Kit^{w-sh} Mice

Lung tissue slides were PAS-D stained and subsequently scored for mucus production by procedures described in Materials and Methods (fig. 5). HDM challenge led to increased mucus scores in both Wt and Kit^{w-sh} mice compared to saline controls ($p < 0.01$ and $p < 0.001$, respectively). However, HDM-induced mucus production did not differ between Wt and Kit^{w-sh} mice.

Lung Levels of Eotaxin Are Reduced in Kit^{w-sh} Mice after HDM Airway Challenge

Since Kit^{w-sh} mice showed a specific defect in eosinophil recruitment to the lung, but not lung pathology or mucus production, upon HDM exposure, we determined whether mast cell deficiency in these mice was associated with reduced pulmonary production of cytokines implicated in eosinophil recruitment: IL-4, IL-5, IL-13 [20] and eotaxin [21]. Lung IL-4, IL-5 and IL-13 concentrations were low in all groups and not significantly different between saline- and HDM-challenged mice (data not shown). In contrast, HDM induced a significant increase in lung eotaxin levels in Wt mice, but not in Kit^{w-sh} mice (fig. 6; $p < 0.05$).

Kit^{w-sh} Mice Fail to Produce IgE upon HDM Exposure

Plasma IgE was below detection limit in saline control mice. HDM airway exposure resulted in a strong increase in plasma IgE in Wt, but not in Kit^{w-sh} mice (fig. 7; $p < 0.05$).

Discussion

Mast cells have been implicated as important players in the pathophysiology of allergic lung inflammation and asthma. Notably, however, the role of mast cells in aller-

Fig. 6. Lung concentrations of eotaxin: pulmonary eotaxin levels are decreased in Kit^{w-sh} mice after HDM exposure. Data are means $\pm$ SEM of 8 mice per group except for Wt saline (n = 5). * $p < 0.05$ versus saline-challenged Wt mice; † $p < 0.05$ versus HDM-challenged Wt mice.

Fig. 7. Plasma concentrations of IgE: Kit^{w-sh} mice do not show a plasma IgE response after HDM challenge. Data are means $\pm$ SEM of 8 mice per group. * $p < 0.05$ versus saline-challenged mice.

gic responses in the airways has been investigated predominantly in Th2-dependent ovalbumin (OVA)-based mouse models [21–23]. Important differences between OVA and HDM exist. In asthma patients allergy for HDM is highly prevalent [4], while OVA is not a relevant human allergen. Furthermore, whereas HDM can influence mast cell activity, OVA does not [24]. Moreover, relative to OVA-induced lung inflammation, the HDM-based model is characterized by epithelial involvement and mucosal defense, which is likely to be of influence on locally residing mast cells [25, 26]. Taken together, mouse models using HDM as allergen relate better to human asthma, yet are sparsely investigated in mast cell-deficient settings. Here we investigated mast cell-deficient Kit^{w-sh} mice in a recently developed HDM asthma model [17]. We showed that HDM administration via the airways resulted in a local increase in tryptase activity, indicative of mast cell activation. Kit^{w-sh} mice demonstrated decreased eosinophil numbers in BALF and lung tissue after HDM exposure, which was associated with lower eotaxin levels in the bronchoalveolar compartment. Remarkably, lung pathology and mucus production after instillation of HDM were similar in Kit^{w-sh} and Wt mice. Together, these data point to a crucial role of mast cells in HDM-induced recruitment of eosinophils to the lungs, potentially in part via an eotaxin-dependent mechanism. Our results show that mast cells do not contribute to HDM-induced lung pathology or mucus production, suggesting that these responses occur via pathways that do not rely on recruited eosinophils.

The phenotype of the Kit^{w-sh} mice in the current model of HDM-induced lung inflammation was mainly defined by a decreased pulmonary recruitment of eosinophils. This finding is in accordance with previous studies that investigated Kit^{w-sh} mice in allergic lung inflammation elicited by OVA [19, 20, 27]. While the levels of IL-4, IL-5 and IL-13 remained low in all mice after HDM challenge, pulmonary concentrations of eotaxin, a key chemoattractant for eosinophils [21], were significantly reduced in Kit^{w-sh} mice. The role of mast cells and eotaxin in eosinophil attraction was previously studied in allergen-challenged skin, identifying a role for histamine released from mast cells in inducing eotaxin expression by endothelial cells and subsequently the recruitment of eosinophils [17]. It would be interesting for future research to investigate interactions of mast cells and endothelium in HDM-induced lung inflammation.

Mast cell deficiency had no effect on HDM-evoked lung pathology in our study, which contrasts with data from earlier studies using OVA as allergen showing attenuated pulmonary inflammation in Kit^{w-sh} mice [19, 20, 27]. This illustrates differences between OVA and HDM effects in the airways: while OVA-induced responses are almost completely dependent on mast cells, the heterogeneity of the HDM extract probably induces a broader symphony of allergenic effects that also involve activation of innate immunity [reviewed in 16], initiating both mast cell-dependent and independent effects [reviewed in 3]. It is also important to recognize differences in mouse strains in this respect. While BALB/c mice are skewed for

Th-2 dependent inflammatory reactions, C57Bl/6 mice (the genetic background of the Kit^{w-sh} mice used here), are more prone to Th1 inflammation [reviewed in 28]. A potential 'Th2 bias' may occur when using BALB/c mice and/or OVA which may result in underestimation of the effect of Th1-dependent inflammatory reactions. The mouse strain used has been shown to be of importance for asthma models in a series of experiments [29], but only in an OVA Th2-dependent model. Importantly, the effects of HDM are not exclusively Th2 dependent: effects of HDM extract includes activating toll-like receptor 2- and 4-dependent pathways [30] and proteolytic activity targeted at airway epithelial tight junctions [26, 31, 32]. Additionally, HDM preparations contain protease-activated receptor agonists, which have diverse proinflammatory effects [33]. This extensive activation of multiple inflammatory pathways involves distinct cellular, epithelial and humoral components besides mast cells and they are hypothetically underestimated in OVA-based protocols. Comparable with the unaffected lung pathology between Wt and Kit^{w-sh} mice, HDM-evoked mucus production was not dependent on mast cells in our C57Bl/6-based HDM model. Lower tryptase activity observed in Kit^{w-sh} mice did not significantly impact lung pathology, indicating that tryptase-independent mechanisms were more important for the outcome of HDM-induced allergic lung inflammation. Of note, it is known that Kit^{w-sh} mice have been described to develop lung pathology resembling emphysema beyond the age of 14 weeks [34]. We used mice at a younger age and lung pathology of saline-challenged Kit^{w-sh} mice was unremarkable and not different from Wt mice.

The levels of total IgE were absent in Kit^{w-sh} mice after HDM challenge compared to Wt mice, indicating as expected that the mast-cell-dependent recruitment of eosinophils is partly IgE dependent. However, since mast cells are essential for the initiation of immunoglobulin production, it could well be that the differences in IgE are due to lack of mast cell-induced sensitization capacities of Kit^{w-sh} mice compared to Wt mice. Strongly attenuated IgE production in response to HDM was previously also shown in mast cell-deficient BALB/c mice; other parameters of allergic lung inflammation were not investigated in this study [35].

In conclusion, we have shown that mast cells play a key role in the recruitment of eosinophils to the lungs after airway exposure to HDM. Unexpectedly, mast cells did not impact on HDM-induced lung pathology or mucus production, contrasting with earlier findings in experimental allergic pulmonary inflammation elicited by OVA and adding important new information on the function of mast cells in the airway response to a clinically relevant human allergen.

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