

Allergic airway inflammation induces migration of mast cell populations into the mouse airway

David Schmit¹ · Duc Dung Le¹ · Sebastian Heck¹ · Markus Bischoff² · Thomas Tschernig³ · Christian Herr⁴ · Christoph Beisswenger⁴ · Peter Kobelt⁵ · Philipp Moritz Lepper⁴ · Kian Fan Chung⁶ · Robert Bals⁴ · Quoc Thai Dinh^{1,4}

Received: 6 June 2016 / Accepted: 23 February 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract Mast cells (MCs) and airway nerves play an important role in allergic asthma. However, little is known about the MCs and their interaction with airway nerves during allergic airway inflammation. This study aims to investigate the distribution and proliferation of MC populations in different lung compartments, along with the association of mast cells with nerve endings, using a house dust mite (HDM) model for allergic airway inflammation. BALB/c mice were exposed to HDM extract intranasally (25 µg/50 µl) for 5 consecutive days a week over 7 weeks. Immunofluorescence and Edu stains were used to examine the colocalisation of MCs and nerves and the proliferation of MCs, respectively. HDM treatment caused an increased migration of MCs into bronchi, alveolar parenchyma and airway vessels. The proportions of tryptase-chymase expressing MC (MC_{TC}) increased significantly in the bronchi and the alveolar parenchyma but not in

the vascular tissues, by allergic airway inflammation. The association of MCs with nerves was found only in the bronchi and there were no changes in comparison of controls to HDM-treated animals. The present study shows a strong migration of tryptase expressing MC (MC_T) and MC_{TC} into the bronchi and the alveolar parenchyma, as well as of MC_T in the vascular compartment under HDM treatment. This supports the hypothesis that these mast cell populations may contribute to allergic airway inflammation.

Keywords Mast cells · Allergic airway inflammation · Tryptase · Chymase · Neuroimmune Interaction David Schmit and Duc Dung Le contributed equally to this work.

David Schmit and Duc Dung Le contributed equally to this work.

✉ Quoc Thai Dinh
Thai.Dinh@uniklinikum-saarland.de

¹ Department of Experimental Pneumology and Allergology, Saarland University Hospital and Saarland University Faculty of Medicine, Kirrberger Strasse, Geb. 61.4, 66421 Homburg/Saar, Germany

² Institute of Medical Microbiology and Hygiene, Saarland University Hospital, Homburg, Germany

³ Institute of Anatomy and Cell Biology, Saarland University Hospital, Homburg, Germany

⁴ Department of Internal Medicine V, Saarland University Hospital, Homburg, Germany

⁵ Center for Internal Medicine and Dermatology, Division of General Internal and Psychosomatic Medicine, Charité – Universitätsmedizin, Berlin, Germany

⁶ Thoracic Medicine, National Heart & Lung Institute, Imperial College, London, UK

Introduction

Throughout recent decades, the prevalence of bronchial asthma has increased dramatically and especially in industrialized countries (Braman 2006). About 300 million people worldwide are suffering from bronchial asthma. Asthma is characterized by symptoms such as increased airway hyper-responsiveness, bronchial obstruction, secretion of mucus, chronic airway inflammation and airway remodeling. It is now considered as a chronic complex disease consisting in different phenotypes (Wenzel 2006, 2012). As the immunology of the mouse has been well characterized and genetically modified or altered mice are available, the mouse is frequently used as model for allergic airway inflammation and may help to reveal this heterogeneity of the disease.

Mast cells play a key role in allergic diseases by releasing amounts of biologically active molecules from their secretory granules. These include histamine, prostaglandins (PGs), leukotrienes (LTs), nerve growth factor (NGF) and various MC-specific proteases, such as tryptase and chymase (Galli

et al. 2005; Leon et al. 1994; Brown et al. 2008; Wernersson and Pejler 2014; Xiang and Nilsson 2000). In humans, only one chymase has been identified and the mast cells are classified into two subpopulations: MC_T containing only tryptase and MC_{TC} that contains chymase in addition to tryptase (Pawankar and Ra 1996; Bradding and Arthur 2016). In contrast, rodents have been shown to express a number of chymases such as mast cell protease (mMCP)-1, mMCP-2, mMCP-4 and mMCP-5 with distinct proteolytic properties. With respect to their biological effects, mMCP-4 expressed by mouse MCs have been suggested to be likely the functional homologue of human chymase (Tchougounova et al. 2003). In the airway, MC populations are not homogenous and each lung compartment contains its own specific sub-group (Andersson et al. 2009). Thus, these cells differ remarkably in their morphology, mediator content and histochemical characteristics as well as their response to external stimuli (Kitamura 1989; Oskeritzian et al. 2005). Although the important role of MCs in allergic asthma is undoubted, their precise function in asthma remains controversial. The reports about MCs in the airway in recent years have renewed the interest in the role of MCs in allergic asthma (Robinson 2004; Cyphert et al. 2011). MCs have been found to be increased within the airway smooth muscle in asthmatic patients (Brightling et al. 2002).

Activation of MCs leads to the release of different mediators, which can affect various cell types, such as immune cells, epithelium, endothelium and airway smooth muscle in different compartments of the airway (Hatanaka et al. 1986; Carter and Bradding 2011; Robinson 2004). Mast cell mediators can also influence neuronal activity as well as the release of neuropeptides (Weigand et al. 2009; Forsythe and Bienenstock 2012). Reciprocally, MCs can be activated by various stimuli, including neuropeptides (Persson et al. 1998; Busse and Lemanske 2001). MCs were found to be associated with nerve fibers in animal and human tissue under physiological and pathophysiological conditions (Jarvikallio et al. 2003; Alving et al. 1991; Stead et al. 1987). Human airways are innervated by numerous cholinergic, adrenergic and non-adrenergic non-cholinergic neurons that may contribute to inflammatory processes (Barnes 1986, 1992; Dinh et al. 2004a, b). The potential role of MCs is well documented and includes their interaction with neuropeptides, neurotrophins, and tachykinins (van Houwelingen et al. 2002; Groneberg et al. 2007; Dinh et al. 2005). Additionally, MCs have been suggested to play a particular role in interaction with the nervous system in chemical- and irritant-induced asthma (Hox et al. 2013; Devos et al. 2016).

This study aims to investigate the distribution, proliferation and proportion of MC subpopulations in different lung compartments (bronchi, alveolar parenchyma and vessels) by using a HDM mouse model of allergic airway inflammation.

Furthermore, the mast cell–nerve contacts in the airways are studied in different lung compartments.

Methods

Animals

Female wild-type BALB/c-mice (6–8 weeks old; Janvier Labs, France) were held in regular 12-h dark/light cycles at a temperature of 22 °C and received laboratory food and tap water ad libitum. The animals were adapted to the new environment for at least 2 weeks prior to the study. All animal experiments were performed in strict concordance with the German animal protection law and approved by the authorities of Saarland (No. 14/2013).

The HDM-mouse model for allergic airway inflammation

Chronic allergic airway inflammation was induced by exposing 8 mice on 5 consecutive days a week over a total period of 7 weeks to HDM extract intranasally (Greer). A dose of 25 µg protein in 50 µl saline was used. A second group of animals ($n = 8$) served as control and was treated via the same route with 50 µl saline. Analyses were performed 24 h after the last allergen challenge (Fig. 1).

In vivo proliferation study with EdU (5-ethynyl-2'-deoxyuridine)

EdU is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis. The animals received an i.p. injection of 1 mg EdU (Invitrogen) in a volume of 200 µl 24 h before sacrifice.

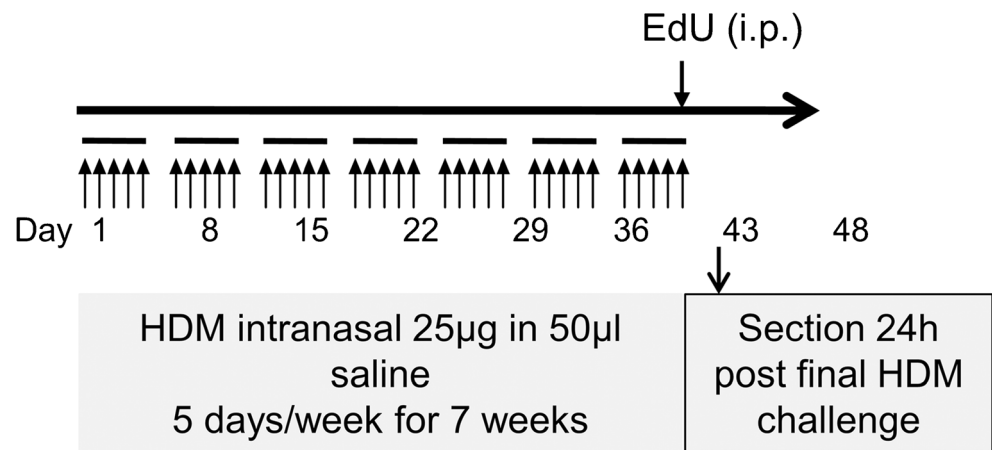
Bronchoalveolar lavage fluid (BALF)

To perform bronchoalveolar lavage, 0.8 ml ice-cold PBS was installed twice. The total cell count in BALF was determined. The BALF was centrifuged (320g, 10 min, 4 °C) and the supernatants were removed. Cytospots were prepared and stained according to Diff Quick (Medion Diagnostics) and differential cell counts were evaluated.

Histological stains

Zamboni fixed lung cryosections (8 µm) were made using a cryostat (CM1950; Leica Cryostat, Germany). Airway inflammation was assessed by staining cryosections with hematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) according to standard protocols. The measurement of airway epithelium thickness (area measurement) and the length of basement membrane was performed by using software Axio Vision

Fig. 1 HDM treatment protocol. The animals were systemically sensitized by intranasal application of house dust mite 5 days a week for 7 weeks. EdU was injected (i.p.) 1 day before analysis



(Carl Zeiss). The thickness of airway epithelium was expressed as mean of area (μm^2) per 1 μm basement membrane. The assessment of airway epithelial goblet cells was expressed as mean of number of goblet cells per 1 mm basement membrane.

Immunofluorescence staining

Immunofluorescence staining has been described previously (Le et al. 2014). Briefly, cryosections of the lungs were dried at room temperature for 15 min and then rehydrated in PBS for 5 min. To reduce nonspecific antibody binding, the sections were incubated for 15 min at room temperature in 5% normal serum of the host species of the secondary antibody diluted in PBS. The sections were incubated with primary antibodies (chicken anti-mouse PGP 9.5, 1:400; Acris Antibodies; rabbit anti-mouse tryptase, 1:500; Abcam; goat anti-mouse mMCP-4, 1:400; Acris Antibodies) or the appropriate isotope control antibodies for 1 h at room temperature and then overnight at 4 °C. After rinsing twice with 0.1 M PBS, the sections were incubated with secondary fluorescein-conjugated antibodies (Jackson ImmunoResearch) (donkey anti-rabbit Cy3, 1:400; donkey anti-chicken Cy2, 1:400; donkey anti-goat, 1:400) for 2 h at room temperature. Finally, the sections were washed twice with 0.1 M PBS, once with double-distilled water, mounted with fluorescent mounting medium Fluoroshield (Sigma Aldrich) and covered with cover slips.

Quantitative analysis of mast cells was visualized with epifluorescence microscopes (Olympus BX5 and Axio Imager M2; Carl Zeiss) and cell counting was performed manually. Sixteen lung slices per animals were analyzed and all quantifications were carried out on blinded sections and controlled by a second investigator. The evaluations of solely tryptase ir-cells (MC_T) and of tryptase ir- and mMCP-4 ir-cells (MC_TC) were analyzed in bronchi, alveolar parenchyma and vessels and expressed as densities (cells/mm^2) and as percentages of the total MC numbers ($\text{MC}_\text{TOT} = \text{MC}_\text{T} + \text{MC}_\text{TC}$). The

numbers of MC_T and MC_TC with direct contact to PGP 9.5 ir-neurons was expressed as percentages of the specific MC subtype (total MC_T or total MC_TC).

EdU staining for proliferating analysis

As previously described, EdU staining was conducted using Click-iT™ Cell Reaction Buffer Kit and Alexa Fluor 594 azide according to the manufacturer's protocol (Invitrogen) (Le et al. 2014). The lung sections were rehydrated for 5 min in PBS and then blocked with 5% normal serum. The sections were incubated with 200 μl prepared Click-iT reaction cocktail for 30 min and then incubated with antibody rabbit anti-mouse tryptase (1:500; Abcam) and secondary antibody donkey anti-rabbit Cy2 (1:400; Jackson ImmunoResearch) as described above. DAPI was used for counterstaining.

Measurement of serum total IgE and cytokines in BALF

The concentration of total IgE in serum and IL4 and IL13 in the BALF supernatants was assessed by using Mouse IgE Ready-SET-Go, Mouse IL-4 ELISA Ready-SET-Go and Mouse IL-13 ELISA Ready-Set-Go (eBioscience, San Diego, CA, USA), as per the manufacturer's instructions. The limits of detection were 4 ng/ml for IgE and 4 pg/ml for IL-4 and IL-13.

Statistical analysis

Data are given as mean $\pm$ SEM. The Kolmogorov–Smirnov test analyzed the Gaussian distribution. Statistical significance between groups was analyzed with an unpaired *t* test using GraphPad Prism 4.03. *P* values < 0.05 were considered significant.

Results

HDM treatment induces allergic inflammation in the airways

HDM treatment-induced allergic airway inflammation is characterized by the recruitment of eosinophils, neutrophils, lymphocytes and macrophages into the airways (Fig. 2a, b). HDM-treated mice showed increased levels of total serum IgE (HDM 82.64 ± 13 ng/ml $n = 8$ vs. saline 674.3 ± 62.75 ng/ml $n = 8$, $P < 0.0001$) and of Th2 cytokines IL4 (HDM 22.19 ± 2.32 pg/ml $n = 8$ vs. saline 1.84 ± 1.11 pg/ml $n = 8$, $P < 0.0001$) and IL13 in the BALF (HDM 59.93 pg/ml ± 12.25 vs. saline undetectable, $P < 0.0001$) (Fig. 2c–e)

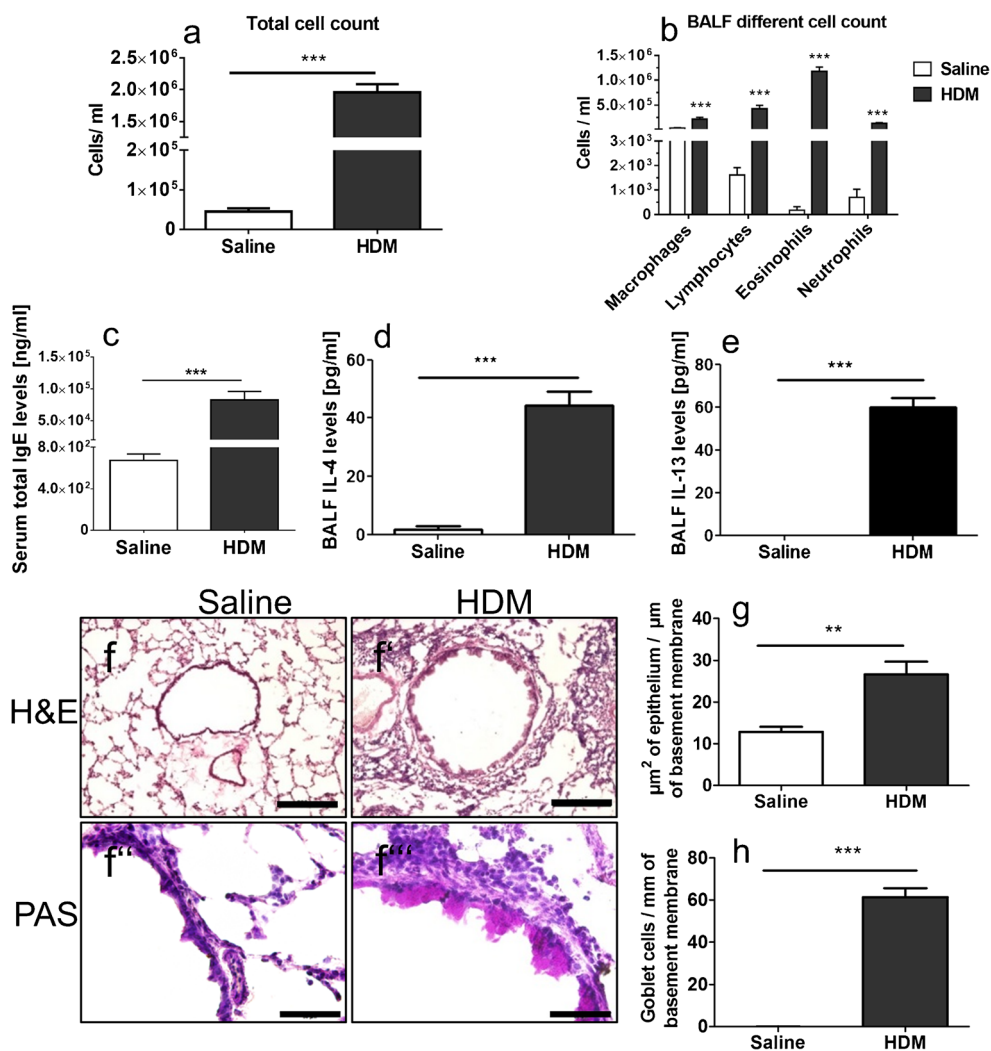
The histological analysis using H&E and PAS staining of different lung tissues showed massive infiltration of mononuclear cells and a distinct mucus secretion (Fig. 2f, f'') in HDM-treated mice. In contrast, the lung sections of control mice did not contain inflammatory cell aggregates and no

mucus secretion was detected (Fig. 2f, f''). Semi-quantitative analysis also showed a significant increase of airway epithelium thickness (HDM 12.85 ± 1.23 $\mu\text{m}^2/\text{mm}$ $n = 5$ vs. saline 26.66 ± 3.06 $\mu\text{m}^2/\text{mm}$ $n = 5$, $P < 0.0031$) and the number of airway epithelial goblet cells (HDM 0.05 ± 0.03 cells/mm $n = 5$ vs. saline 61.37 ± 4.23 cells/mm $n = 5$, $P < 0.0001$) in HDM-treated mice in comparison to controls (Fig. 2g, h).

Mast cells increased significantly during allergic inflammation in most lung compartments

Mast cells were detected by staining antibody against mouse tryptase. The contribution of MC in lung compartments was quantified and expressed as cells per mm^2 . Under inflammatory conditions, the highest density of total MCs was found in conducting airways with the lowest concentration in alveolar tissue. Allergic airway inflammation induced a significant increase of MCs in the bronchi (HDM 24.48 ± 2.49 $n = 8$ vs. saline 4.78 ± 0.98 $n = 8$, $P < 0.0001$), alveolar parenchyma (HDM 5.21 ± 0.70 $n = 8$ vs. saline 0.40 ± 0.09 $n = 8$,

Fig. 2 HDM induces allergic airway inflammation. Bronchoalveolar lavage fluid (BALF) total cell count (a) and cell differentiation (b). Eosinophils, neutrophils, lymphocytes and macrophages in BALF demonstrated inflammation in the lungs of HDM-exposed mice, in comparison with control mice. Total serum IgE level (c) and the cytokine levels of interleukin IL-4 (d) and IL-13 (e) in the bronchoalveolar lavage (BAL) fluid. f–f'' Representative photomicrographs of H&E- and PAS-stained lung sections. The lung sections from HDM-sensitised and -challenged mice showed an infiltration of mononuclear cells (H&E) and a highly mucus secretion (PAS) in the airways, whereas the lungs of the control mice do not contain aggregated inflammatory cells and mucus secretion. Quantification of airway epithelial thickness (g) and airway epithelial goblet cells (h) as mean per 1 mm of basement membrane. Results are expressed as mean $\pm$ SEM. ** $P < 0.01$, *** $P < 0.001$ (unpaired two-tailed t test). Scale bars (H&E) 200 μm , (PAS) 50 μm



$P < 0.0001$) and airway vessels (HDM 18.98 ± 2.95 $n = 8$ vs. saline 1.47 ± 0.40 $n = 8$, $P < 0.0001$) (Fig. 3)

HDM treatment induced the migration of MC into the airways

To examine whether MCs proliferated in the lung or migrated from outside into the airways during allergic airway inflammation, the animals were injected with EdU, which was incorporated with the DNA of dividing cells. Systemic administration of EdU 24 h before analysis assured the detection of proliferating cells. The proliferating MCs in two groups were examined (double-positive with EdU and tryptase). The results showed that proliferating MCs were not detected in the lung of controls and HDM treated animals (Fig. 4a, b–b’). Furthermore, experiments were carried out to examine the survival of MCs during allergic inflammation. Lung tissues were stained with survival marker phosphor Akt1 (Ser473). All mast cells in controls and HDM group were found negative with Akt1 (Fig. 4c, d–d’, e).

Proportional changes of MC populations in allergic airway inflammation

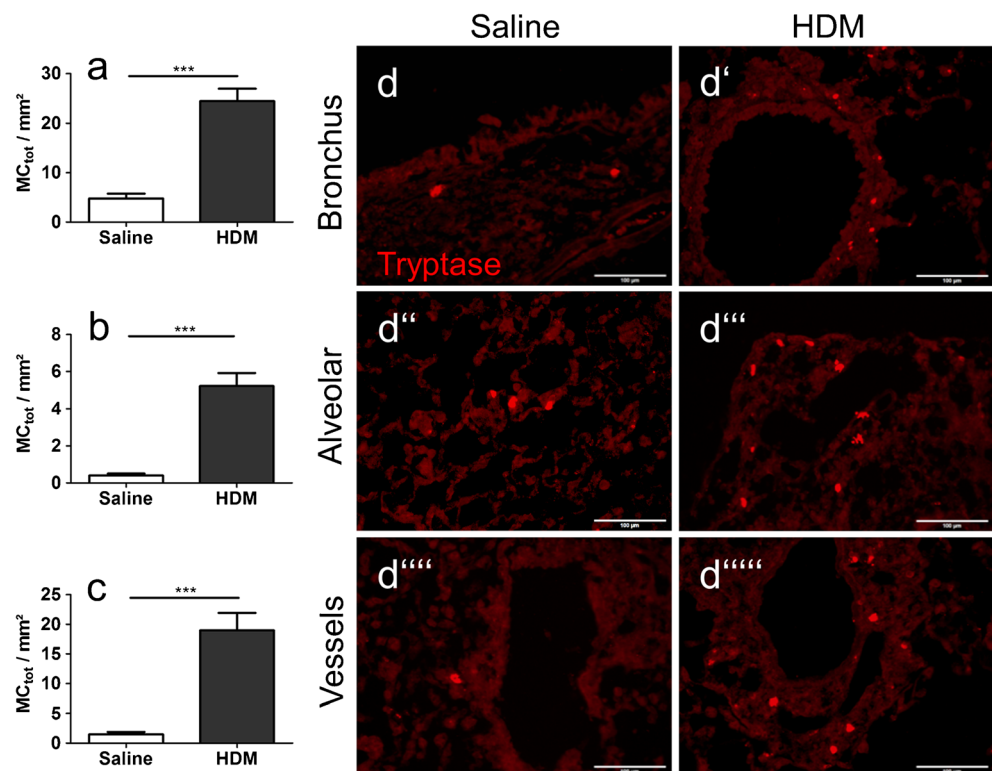
MCs were differentiated using staining against tryptase and mMCP-4 (mouse chymase) followed by immunofluorescence imaging. MC_T were positive only for tryptase,

whereas MC_{TC} expressed both enzymes. Under baseline conditions, MC_T were the predominant subtype in all lung compartments with the highest proportion in vascular tissue, whereas MC_{TC} were found only in the bronchus and alveolar lung parenchyma but not in the vessels. The highest fraction of MC_{TC} appeared in the bronchi. The proportion of MC_{TC} in lung compartments was quantified and expressed as a percentage (MC_{TC}/MC_{TOT}). Allergic airway inflammation induced significant increases in the proportions of MC_{TC} in changes in the bronchi (HDM $7.37 \pm 0.75\%$, $n = 8$ vs. saline $2.0 \pm 1.30\%$ $n = 8$, $P = 0.0032$) and alveolar parenchyma (HDM $4.00 \pm 0.94\%$ $n = 8$ vs. saline $1.00 \pm 0.86\%$ $n = 8$, $P = 0.0346$). In vascular tissue, these changes were not detectable (Fig. 5).

MC-nerve interaction in the lung

The pan-neuronal marker PGP 9.5 was used to detect nerves in the conducting airways. Under physiological conditions, a minority of MC_T could be found in the proximity of nerve fibers. MC_{TC} did not form any anatomic link. During lung inflammation, proportional changes of MC_T and MC_{TC} with contact to nerves were not detectable (% MC_T having contact with nerve: HDM $13.75 \pm 1.34\%$, $n = 8$ vs. saline $23.13 \pm 5.09\%$ $n = 8$, $P = 0.0969$) (% MC_{TC} having contact with nerve: HDM $5.87 \pm 4.24\%$, $n = 8$, saline were not detectable) (Fig. 6)

Fig. 3 Contribution of mast cells in the lung. Quantification of mast cells in bronchus (a), alveolar (b) and vessels. Results are expressed as cells per ml. Microphotograph of mouse lungs showing present of MCs (red, stained with antibody against tryptase) in bronchus, alveolar and vessels. Results are expressed as mean $\pm$ SEM, *** $P < 0.001$ (unpaired two-tailed t test). Scale bars 100 μ l



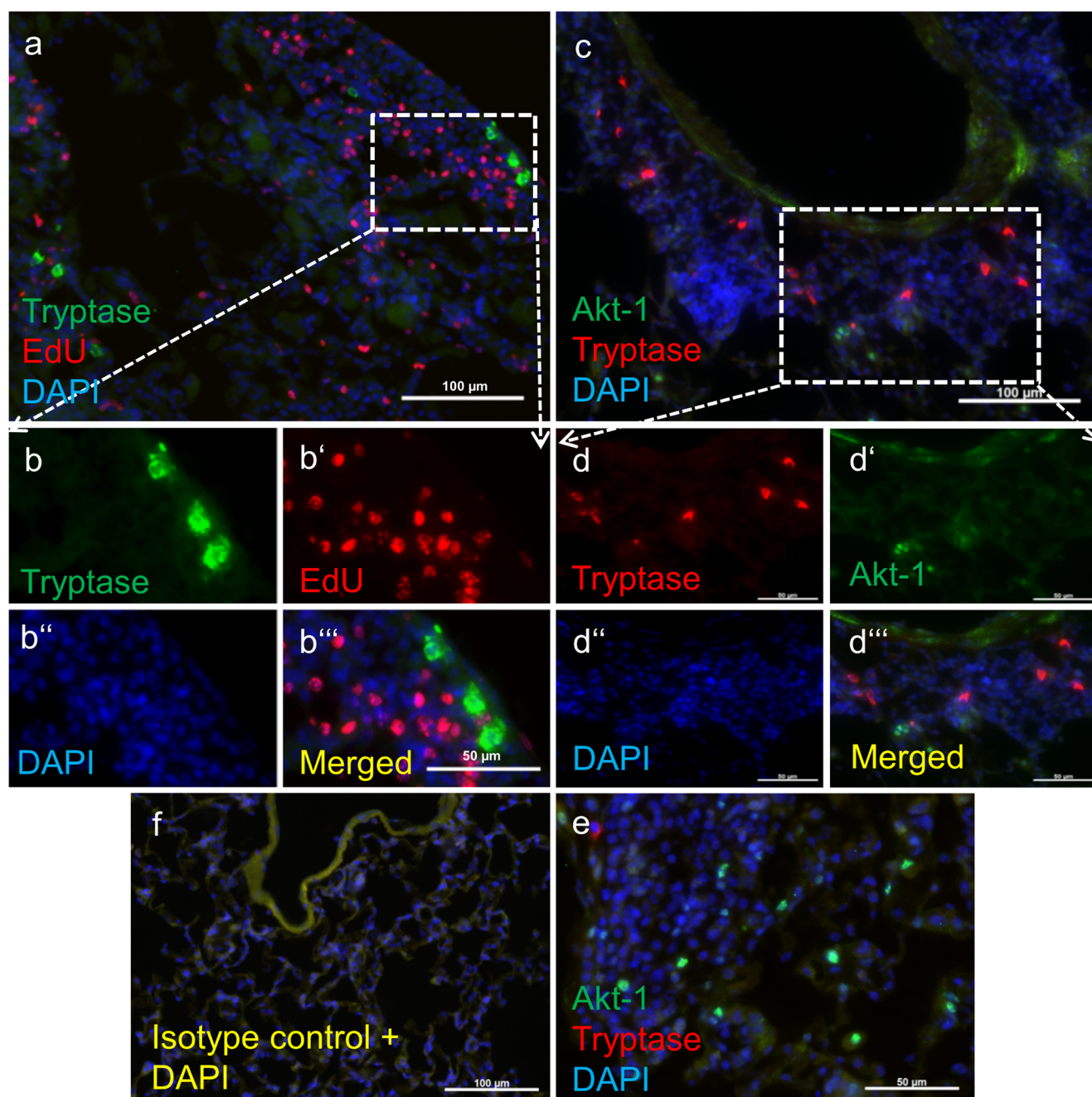


Fig. 4 Proliferating analysis of MCs in the lungs. **a** Fluorescence staining for tryptase (green), Edu (red) and DAPI (blue). No proliferating mast cells (green) were found in the lungs of controls and HDM-treated mice. **b–b''** Large magnifications of mast cells in (a). **c** Immunofluorescence staining of survival marker Akt1 (green), tryptase (red) and DAPI (blue)

in the mouse lung tissues. All mast cells in controls and HDM-treated mice were negative for Akt1. **d–d''** Large magnifications of (c). **e** The Akt1-positive cells were found in the lung tissues. **f** Negative control for immunofluorescence staining. Scale bars (a, c, f) 100 μm, (b–b'', d–d'', e) 50 μm

Discussion

Our study revealed a significant increase of MCs in the bronchi, alveolar and vascular tissues during allergic airway inflammation. The number of MC_{TC} subpopulations rose significantly in bronchi and alveolar parenchyma but not in vascular tissues. The anatomic associations between MCs and

nerves were found only in bronchi and there were no changes after HDM treatment. Mast cells form a very heterogeneous cell population displaying multiple functions (Erjefalt 2014). Therefore, investigating their contribution and the proportion in anatomical compartments is essential for the study of the role of mast cells in allergic airway inflammation. Their particular distribution and phenotypical appearance (MC_T and

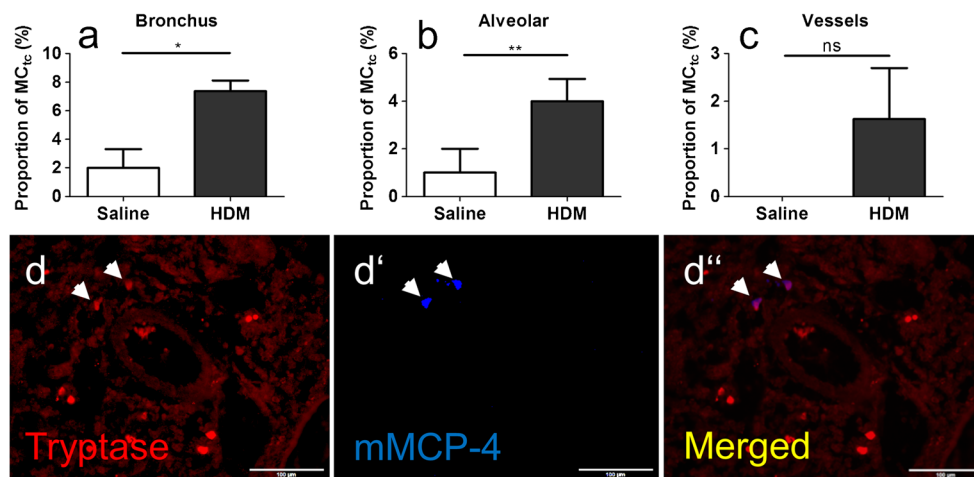


Fig. 5 Proportion of MC_{TC} subpopulations **a–c** The proportion of MC_{TC} bronchus, alveolar and vessels in the lung of controls and HDM-treated mice. The results are expressed as the percentage of MC_{TC} to the number of MC_{tot} ($MC_T + MC_{TC}$). **d–d''** Fluorescence staining showing tryptase (red), mMCP4 (mouse chymase) (magenta, stained with antibody against

mMCP4) and tryptase-mMCP-4 double-positive mast cells (arrows), indicating two subpopulations of MCs in the lung: tryptase-positive (MC_T) and tryptase-mMCP-4 (chymase)-positive (MC_{TC}). Results are expressed as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ (unpaired two-tailed t test). Scale bars 100 μ m

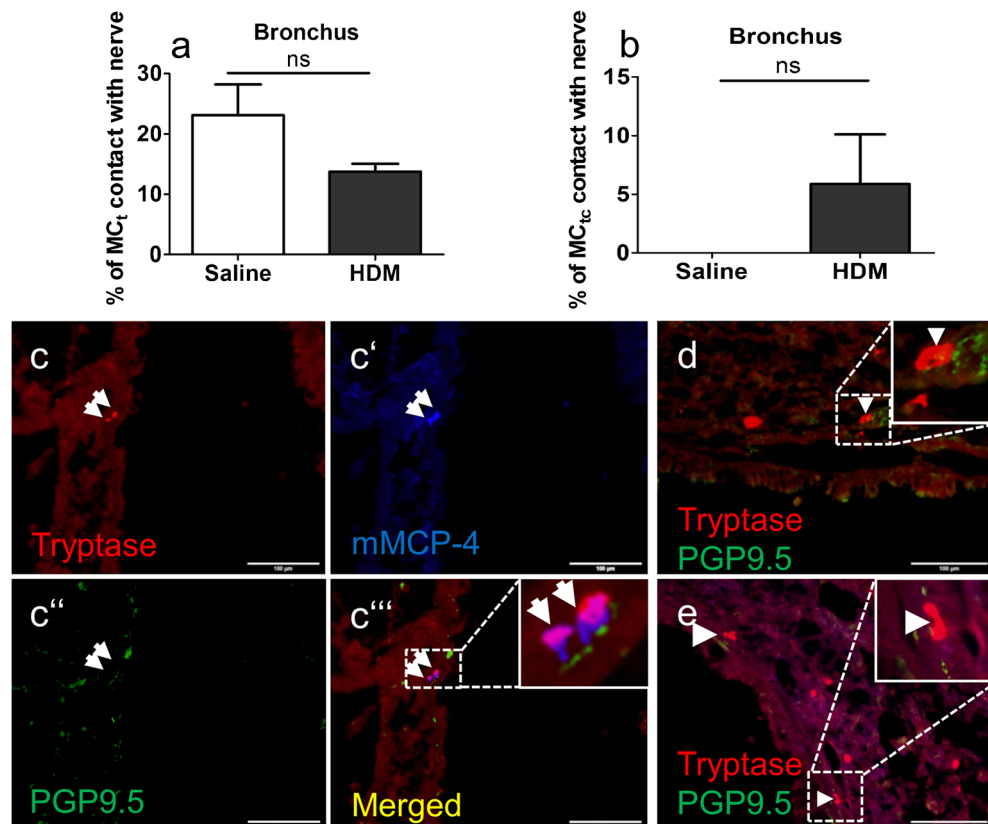
MC_{TC}), as well as their interaction with the nervous system, are still of interest and remain unexplored in mouse models for allergic airway inflammation.

Analysis of mast cell numbers in human asthma show divergent results. While some authors report decreasing numbers, others reveal higher numbers of MCs and their subtypes

in different parts of asthmatic airways (Andersson et al. 2009). These differences may be due to variations in asthma severity, the use of different methods and analysis criteria or effects of glucocorticoid treatments. The results of this study show some important features described in human asthma. Under physiological conditions, human MCs can be found in all lung

Fig. 6 Mast cell–nerve contacts in the lung. The anatomic associations between MCs and nerves were only found in bronchus. The quantitative analysis of MC_T (**a**) and MC_{TC} (**b**) contact with nerves. The results are expressed as the percentage of MC_T and MC_{TC} associating with nerves to the numbers of MC_T and MC_{TC} , respectively. **c–c''**

Microphotograph showing the association between MC_{TC} (arrows) and nerve fibers (green, stained with antibody against PGP 9.5) in bronchus. **d, e** The association between MC_T (arrows) and nerve fibers (green)



compartments, with MC_T being the predominant subtype in bronchi and alveolar parenchyma (Andersson et al. 2009). Our HDM mouse model shows a similar distribution, whereas direct comparison elicits fewer MC numbers and even smaller proportions of MC_{TC} in the murine airways under baseline conditions. These findings could point to different roles in promoting early allergic inflammation.

The proliferation study of MCs showed that MCs did not proliferate and survive after HDM treatment. Therefore, we believe that these MCs migrate from the systemic circulation into the airways and lung during allergic inflammation.

Under inflammatory conditions, MCs increased in human lung tissue especially towards the periphery and the alveolar parenchyma (Andersson et al. 2011). This increase seems to be accompanied by an elevated proportion of MC_{TC} in small airways and alveolar compartments (Balzar et al. 2005). This HDM model also shows a rise of MC_{TC} in bronchi and even alveolar parenchyma and therefore reproduces a key feature of human asthma. Furthermore, we demonstrate a proportional increase of MC_{TC} in conducting airways as well as in the alveolar compartment. This seems to represent the human situation in severe uncontrolled asthma, as has been described before in asthmatics (Andersson et al. 2011). As it is known that under some conditions the number of MC_{TC} in small airways and alveolar parenchyma correlates especially with better lung function (Balzar et al. 2005), their regulative role has to be analyzed in the HDM mouse model. Nevertheless, it has to be taken into consideration that the total MC numbers have been shown to be much higher in asthmatic lung and airways, especially in distal airways, than in HDM-treated mice.

The roles of MCs in allergic asthma are still not fully understood. Previous studies have shown that MCs may have an essential role in airway remodeling, especially on increased smooth muscle mass, subepithelial fibrosis, epithelial alterations and neovascularization, by releasing proteases and growth factors (Okayama et al. 2007). MCs have been found to infiltrate the airway smooth muscle in asthma and to have a relationship with airway smooth muscle hypertrophy (Brightling et al. 2002; Amin et al. 2005). Tryptase has been shown to have the capacity to induce the proliferation of airway epithelial cells (Cairns and Walls 1996). Asthmatics as well as HDM-treated mice show increased vascularity that correlates with disease severity (Rydell-Tormanen et al. 2008; Salvato 2001). Especially, human MC_{TC} secrete vascular endothelial growth factor (VEGF), which contributes significantly to the vasculature angiogenesis (Chetta et al. 2003; Abdel-Rahman et al. 2006). Our results show that there is an increase of MC_T but not MC_{TC} in the vascular compartment under inflammatory conditions and that MC_{TC} are even absent under baseline conditions. Comparisons to the human situation are difficult due to a lack of data. Nevertheless, the role of MC_{TC} in the vascular remodeling process has to be questioned in this model.

Moreover, mast cells proteases have been reported to increase the migration of immune cells, including neutrophils and eosinophils (Terakawa et al. 2006; He and Walls 1998). Mast cell chymase was revealed to be a potent secretagogue for airway serous gland cells (Sommerhoff et al. 1989).

With respect to MC–nerve interaction, histological studies show that these cells are closely related to the central and peripheral nervous systems (Bienenstock et al. 1988). This connection is not limited solely to anatomical but also shares numerous functional aspects (Bienenstock et al. 1991; Weigand et al. 2009). In the context of an “axon reflex”, MCs activate nerve fibers, which in turn modulate the inflammatory processes via the release of tachykinins and neuropeptides (e.g., SP, NKA, CGRP) (Barnes 1986). Our analysis reveals an anatomical connection between MC_T and bronchial nerve fibers in the HDM mouse model. Under morphological aspects, this connection was not changed following HDM treatment. Whether this colocalization therefore comes out by chance is very unlikely in view of the previous investigations (Alving et al. 1991) but it implicates the need for functional analysis with the focus on the expression of specific tachykinin (SP, NKA) and CGRP and neuropeptide receptors on MCs and in electrophysiological experiments.

In conclusion, the present study reveals for the first time that there is a migration of MC_T and MC_{TC} into the bronchi and the alveolar parenchyma as well as an increase of MC_T in the vascular compartment following HDM treatment. This supports the idea that MCs contribute to allergic airway inflammation in the HDM mouse model and may also implicate the key features of severe human asthma. Furthermore, it shows that at least MC_T interacts with airway nerves but that there is the need for further functional investigations concerning this interaction in bronchial asthma.

Acknowledgements This project was supported and funded by the foundation ‘Stiftung Bergmannshilfswerk Luisenthal’ to Prof. Dr. Quoc Thai Dinh. We would like to thank Ms Janine Schlicker for her technical assistance.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

- Abdel-Rahman AM, el-Sahrigy SA, Bakr SI (2006) A comparative study of two angiogenic factors: vascular endothelial growth factor and angiogenin in induced sputum from asthmatic children in acute attack. *Chest* 129:266–271
- Alving K, Sundstrom C, Matran R, Panula P, Hokfelt T, Lundberg JM (1991) Association between histamine-containing mast cells and sensory nerves in the skin and airways of control and capsaicin-treated pigs. *Cell Tissue Res* 264:529–538

- Amin K, Janson C, Boman G, Venge P (2005) The extracellular deposition of mast cell products is increased in hypertrophic airways smooth muscles in allergic asthma but not in nonallergic asthma. *Allergy* 60:1241–1247
- Andersson CK, Mori M, Bjermer L, Lofdahl CG, Erjefalt JS (2009) Novel site-specific mast cell subpopulations in the human lung. *Thorax* 64:297–305
- Andersson CK, Bergqvist A, Mori M, Mauad T, Bjermer L, Erjefalt JS (2011) Mast cell-associated alveolar inflammation in patients with atopic uncontrolled asthma. *J Allergy Clin Immunol* 127:905–912
- Balzar S, Chu HW, Strand M, Wenzel S (2005) Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 171:431–439
- Barnes PJ (1986) Asthma as an axon reflex. *Lancet* 1:242–245
- Barnes PJ (1992) Modulation of neurotransmission in airways. *Physiol Rev* 72:699–729
- Bienenstock J, Perdue M, Blennerhassett M, Stead R, Kakuta N, Sestini P, Vancheri C, Marshall J (1988) Inflammatory cells and the epithelium. Mast cell/nerve interactions in the lung in vitro and in vivo. *Am Rev Respir Dis* 138:S31–S34
- Bienenstock J, MacQueen G, Sestini P, Marshall JS, Stead RH, Perdue MH (1991) Mast cell/nerve interactions in vitro and in vivo. *Am Rev Respir Dis* 143:S55–S58
- Bradding P, Arthur G (2016) Mast cells in asthma—state of the art. *Clin Exp Allergy* 46:194–263
- Braman SS (2006) The global burden of asthma. *Chest* 130:4S–12S
- Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID (2002) Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 346:1699–1705
- Brown JM, Wilson TM, Metcalfe DD (2008) The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy* 38:4–18
- Busse WW, Lemanske RF Jr (2001) Asthma. *N Engl J Med* 344:350–362
- Cairns JA, Walls AF (1996) Mast cell tryptase is a mitogen for epithelial cells. Stimulation of IL-8 production and intercellular adhesion molecule-1 expression. *J Immunol* 156:275–283
- Carter RJ, Bradding P (2011) The role of mast cells in the structural alterations of the airways as a potential mechanism in the pathogenesis of severe asthma. *Curr Pharm Des* 17:685–698
- Chetta A, Zanini A, Foresi A, Del DM, Castagnaro A, D'Ippolito R, Baraldo S, Testi R, Saetta M, Olivieri D (2003) Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 167:751–757
- Cyphert JM, Kovarova M, Koller BH (2011) Unique populations of lung mast cells are required for antigen-mediated bronchoconstriction. *Clin Exp Allergy* 41:260–269
- Devos FC, Boonen B, Alpizar YA, Maes T, Hox V, Seys S, Pollaris L, Liston A, Nemery B, Talavera K, Hoet PH, Vanoirbeek JA (2016) Neuro-immune interactions in chemical-induced airway hyperreactivity. *Eur Respir J* 48:380–392
- Dinh QT, Groneberg DA, Peiser C, Springer J, Joachim RA, Arck PC, Klapp BF, Fischer A (2004a) Nerve growth factor-induced substance P in capsaicin-insensitive vagal neurons innervating the lower mouse airway. *Clin Exp Allergy* 34:1474–1479
- Dinh QT, Groneberg DA, Witt C, Peiser C, Cifuentes LB, Frossard N, Klapp BF, Fischer A (2004b) Expression of tyrosine hydroxylase and neuropeptide tyrosine in mouse sympathetic airway-specific neurons under normal situation and allergic airway inflammation. *Clin Exp Allergy* 34:1934–1941
- Dinh QT, Mingomataj E, Quarcio D, Groneberg DA, Witt C, Klapp BF, Braun A, Fischer A (2005) Allergic airway inflammation induces tachykinin peptides expression in vagal sensory neurons innervating mouse airways. *Clin Exp Allergy* 35:820–825
- Erjefalt JS (2014) Mast cells in human airways: the culprit? *Eur Respir Rev* 23:299–307
- Forsythe P, Bienenstock J (2012) The mast cell-nerve functional unit: a key component of physiologic and pathophysiologic responses. *Chem Immunol Allergy* 98:196–221
- Galli SJ, Nakae S, Tsai M (2005) Mast cells in the development of adaptive immune responses. *Nat Immunol* 6:135–142
- Groneberg DA, Fischer TC, Peckenschneider N, Noga O, Dinh QT, Welte T, Welker P (2007) Cell type-specific regulation of brain-derived neurotrophic factor in states of allergic inflammation. *Clin Exp Allergy* 37:1386–1391
- Hatanaka K, Imakita M, Go S, Yamamoto A (1986) Effects of degranulation of mast cells on proliferation of mesenchymal cells in the mesentery of mice. *Cell Tissue Res* 246:53–56
- He S, Walls AF (1998) Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells in vivo. *Br J Pharmacol* 125:1491–1500
- Hox V, Vanoirbeek JA, Alpizar YA, Voedisch S, Callebaut I, Bobic S, Sharify A, De Vooght V, Van Gerven L, Devos F, Liston A, Voets T, Vennekens R, Bullens DM, De VA, Hoet P, Braun A, Ceuppens JL, Talavera K, Nemery B, Hellings PW (2013) Crucial role of transient receptor potential ankyrin 1 and mast cells in induction of nonallergic airway hyperreactivity in mice. *Am J Respir Crit Care Med* 187:486–493
- Jarvikallio A, Harvima IT, Naukkarinen A (2003) Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. *Arch Dermatol Res* 295:2–7
- Kitamura Y (1989) Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu Rev Immunol* 7:59–76
- Le DD, Rochlitz S, Fischer A, Heck S, Tschernig T, Sester M, Bals R, Welte T, Braun A, Dinh QT (2014) Allergic airway inflammation induces the migration of dendritic cells into airway sensory ganglia. *Respir Res* 15:73
- Leon A, Buriani A, Dal TR, Fabris M, Romanello S, Aloe L, Levi-Montalcini R (1994) Mast cells synthesize, store, and release nerve growth factor. *Proc Natl Acad Sci U S A* 91:3739–3743
- Okayama Y, Ra C, Saito H (2007) Role of mast cells in airway remodeling. *Curr Opin Immunol* 19:687–693
- Oskeritzian CA, Zhao W, Min HK, Xia HZ, Pozez A, Kiev J, Schwartz LB (2005) Surface CD88 functionally distinguishes the MCTC from the MCT type of human lung mast cell. *J Allergy Clin Immunol* 115:1162–1168
- Pawankar R, Ra C (1996) Heterogeneity of mast cells and T cells in the nasal mucosa. *J Allergy Clin Immunol* 98:S248–S262
- Persson CG, Erjefalt JS, Greiff L, Andersson M, Erjefalt I, Godfrey RW, Korsgren M, Linden M, Sundler F, Svensson C (1998) Plasma-derived proteins in airway defence, disease and repair of epithelial injury. *Eur Respir J* 11:958–970
- Robinson DS (2004) The role of the mast cell in asthma: induction of airway hyperresponsiveness by interaction with smooth muscle? *J Allergy Clin Immunol* 114:58–65
- Rydell-Tormanen K, Johnson JR, Fattouh R, Jordana M, Erjefalt JS (2008) Induction of vascular remodeling in the lung by chronic house dust mite exposure. *Am J Respir Cell Mol Biol* 39:61–67
- Salvato G (2001) Quantitative and morphological analysis of the vascular bed in bronchial biopsy specimens from asthmatic and non-asthmatic subjects. *Thorax* 56:902–906
- Sommerhoff CP, Caughey GH, Finkbeiner WE, Lazarus SC, Basbaum CB, Nadel JA (1989) Mast cell chymase. A potent secretagogue for airway gland serous cells. *J Immunol* 142:2450–2456
- Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J (1987) Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci U S A* 84:2975–2979
- Tchougounova E, Pejler G, Abrink M (2003) The chymase, mouse mast cell protease 4, constitutes the major chymotrypsin-like activity in peritoneum and ear tissue. A role for mouse mast cell protease 4 in thrombin regulation and fibronectin turnover. *J Exp Med* 198:423–431

- Terakawa M, Tomimori Y, Goto M, Fukuda Y (2006) Mast cell chymase induces expression of chemokines for neutrophils in eosinophilic EoL-1 cells and mouse peritonitis eosinophils. *Eur J Pharmacol* 538:175–181
- van Houwelingen AH, Kool M, de Jager SC, Redegeld FA, van Heuven-Nolsen D, Kraneveld AD, Nijkamp FP (2002) Mast cell-derived TNF- α primes sensory nerve endings in a pulmonary hypersensitivity reaction. *J Immunol* 168:5297–5302
- Weigand LA, Myers AC, Meeker S, Undem BJ (2009) Mast cell-cholinergic nerve interaction in mouse airways. *J Physiol* 587:3355–3362
- Wenzel SE (2006) Asthma: defining of the persistent adult phenotypes. *Lancet* 368:804–813
- Wenzel SE (2012) Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 18:716–725
- Wernersson S, Pejler G (2014) Mast cell secretory granules: armed for battle. *Nat Rev Immunol* 14:478–494
- Xiang Z, Nilsson G (2000) IgE receptor-mediated release of nerve growth factor by mast cells. *Clin Exp Allergy* 30:1379–1386