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Mast Cell-Derived Tumor Necrosis Factor is Essential for Allergic Airway Disease

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Abstract

Mast cells are thought to contribute to allergic airway disease. However, the role of mast cell produced mediators like TNF for the development of allergic airway disease is unclear.

To define the role of mast cells in acute allergic airway disease we studied 2 strains of mast cell deficient mice ($Kit^{W/Wv}$ and $Kit^{W\text{-}sh/W\text{-}sh}$).

Compared to their wild-type littermates, $Kit^{W/Wv}$ and $Kit^{W\text{-}sh/W\text{-}sh}$ mice developed significantly lower airway responsiveness to methacholine and less airway inflammation and goblet cell metaplasia, following sensitization in the absence of adjuvant and airway challenge. Transfer of bone marrow-derived mast cells (BMMCs) from wild-type mice to $Kit^{W\text{-}sh/W\text{-}sh}$ mice reconstituted both airway responsiveness and inflammation to levels similar in sensitized and challenged wild-type mice. In contrast, sensitized $Kit^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with BMMCs from TNF deficient mice were still severely impaired in their ability to develop airway hyperresponsiveness, inflammation, or goblet cell metaplasia following allergen challenge.

These results demonstrate the significance of mast cells in the development of airway disease and highlight the importance of mast cell-derived TNF in these responses.

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Introduction

Asthma is a complex syndrome characterized by airway hyperresponsiveness (AHR), airway inflammation and airway obstruction [1]. An additional feature of allergic asthma is the increased production of IgE in response to common environmental allergens, and a relationship between atopy and allergic asthma has been demonstrated in several studies [2,3]. Many inflammatory cells have been implicated in the allergic airway response, but especially mast cells, predominantly located at the mucosal interface between host and environment, are regarded as important effector cells in the allergic immune response. This is mainly due to the allergen-specific activation of these cells through the IgE-loaded high-affinity IgE receptor (FcεRI), following contact with allergen. Indeed, in human asthmatics increased numbers of mast cells have been found in close proximity to airway smooth muscle suggesting a potential role for the development and maintenance of allergic airway disease [4].

Several studies in murine models support potential roles for mast cells in allergic airway disease [5-7]. Mast cells, following activation, are able to degranulate and produce a plethora of different mediators [8]. Some of those mediators have been implicated in chemotaxis of T-cells which are important for the development of allergic airway disease [9-11]. One of the many proinflammatory cytokines is tumor necrosis factor (TNF) which can be preformed and stored in mast cells and released upon demand within minutes [12-14]. Interestingly, mast cell-derived TNF has been found to promote T-cell activation and proliferation [15,16] as well as the migration of dendritic cells [17]. However, the role of mast cell-derived TNF for the development of allergic airway disease is not well described.

The aim of the current study was to investigate the role of mast cells and mast cell produced TNF in the induction of allergic airway disease in a model of allergen sensitization without adjuvant. We show that mast cell deficient mice do not develop allergic airway disease following sensitization in the absence of adjuvant and airway challenge. Additionally,

we demonstrate that TNF produced by mast cells is essential for the development of AHR, airway inflammation and goblet cell metaplasia.

Methods

Mice

WB/ReJ-W/$^{+}$ and C57Bl/6J-W$^{v/+}$ mice were obtained from Jackson Laboratory and the mast cell-deficient c-kit mutant F_1-generation mice WBB6F_1-Kit$^{W/W\text{-}v}$ and the congenic WBB6F_1-Kit$^{+/+}$ were bred in the Zentrale Tierzuchtanstalt of the Johannes-Gutenberg-University Medical Center. In addition, mast cell-deficient C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice and congenic C57Bl/6-Kit$^{+/+}$ wild-typ littermates were obtained by intercrossing C57Bl/6-*Kit*$^{W\text{-}sh}$/$^{+}$ mice kindly provided by Marcus Maurer, Berlin, Germany. C57Bl/6 mice deficient for TNF were obtained from Kerstin Steinbrink, University of Mainz, Germany. Mice were used at the age of 8-12 weeks. For reconstitution experiments mice were 14 weeks old at time of sensitization. Animal procedures were conducted in accordance with current institutional guidelines and performed according to the Helsinki convention for the use and care of animals.

Experimental protocols

Experimental groups consisted of three or four mice per group, and each experiment was performed at least twice. Mice were sensitized by intraperitoneal (i.p.) injection of 100 µl of 20 µg ovalbumin (OVA, Sigma-Aldrich, St. Louis, MO) solution in phosphate buffered saline (PBS) on days 0 and 14. Mice were then challenged via the airways on days 28, 29 and 30, using nebulized OVA (1% in PBS), with an ultrasonic nebuliser (NE-U17, Omron, Hoofdorp, The Netherlands).

Mast cell reconstitution

To obtain bone marrow derived mast cells (BMMC), bone marrow from C57Bl/6 mice was cultured for 4 to 5 weeks in a modified IMDM-Medium (IMDM, 10%FCS, 50µM β-Mercaptoethanol, 2mM glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 20 U/ml

mIL-3, and 200 ng/ml kit-ligand) as described previously [18]. Non-adherent cells were transferred to fresh culture plates every 2–3 days for a total of at least 21 days to remove adherent macrophages and fibroblasts. After 4 weeks of culture, >95 % of nonadherent cells contained granules that stained positively with toluidine blue, and >95 % expressed c-Kit on their surface as determined by FACS analysis using anti-c-Kit mAb. To reconstitute the mast cell deficient mice (6 week old C57Bl/6-Kit$^{Wsh/Wsh}$), 5x10^6 BMMCs were injected in the tail vein of each mouse. 8 weeks following the injection sensitization was started and airway challenges were performed at 12 weeks following BMMCs administration.

Measurement of airway reactivity

Measurements of the airway resistance (R_L) were performed on anesthetized, intubated and mechanically ventilated mice (FlexiVent, Scireq, Montreal, QC) in response to increasing doses of inhaled methacholine (MCh) (6.25, 12.5, 25, 50 and 100 mg/ml). Measurements of R_L were performed every 15 seconds following each nebulization step until a plateau phase was reached.

Broncho-alveolar lavage

After assessment of airway function, lungs were lavaged via the tracheal tube with PBS (1 x 1 ml). Numbers of BAL cells were counted by using trypan blue dye exclusion. Differential cell counts were made from cytocentrifuged preparations fixed and stained with the Microscopy Hemacolor ®-Set (Merck, Darmstadt, Germany). Percentage and absolute numbers of each cell type were calculated. The numbers of CD3-, CD4-, and CD8-positive cells was assessed by flow cytometry analysis using fluorescein isothiocyanate–conjugated monoclonal rat anti-mouse CD3 and phycoerythrin-conjugated rat anti-mouse CD4 or CD8 (all BD Bioscience, Heidelberg, Germany). Absolute numbers of CD4-positive (CD3+/CD4+)

and CD8-positive (CD3+/CD8+) T cells were calculated by multiplying the total cell count and the percentage of either CD3/CD4 or CD3/CD8 cells.

Histology

Lungs were fixed by inflation (1 ml) and immersion in 10% formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS). To assess reconstitution efficiencies, sections were used for fluorescence staining of tissue specific for mast cells with avidin-Alexa-488 (Molecular probes) [19-21] or were stained with toluidine blue. Slides were examined in a blinded fashion with a microscope (BX40, Olympus, Hamburg, Germany). Using avidin-Alexa-488 and PAS stained slides number of mast cells and goblet cells were analyzed respectively using the imaging software (analysis, Soft Imaging Systems, Stuttgart, Germany). For the assessment for mast cell numbers in each slide mast cells were counted by a blinded investigator in 5 different fields and in each field the lung area was measured using an image analysis **system.** Numbers of mast cells are expressed per cm^2 [6,22].**.** Numbers of goblet cells are expressed as number of cells per mm basement membrane (BM).

Antigen-specific ELISA

Serum was obtained 48 hours following the last challenge. OVA specific IgG1- and IgG2b-titres were determined via ELISA. Biotin conjugated detection antibodies, Streptavidin-horseradish-peroxidase and substrat-reagent (BD-Pharmingen) were used in concentrations recommended by the manufacturer. OVA-specific IgE was assessed using a method described by Spergel et al. [23]. Briefly, plates were coated the with rat anti–mouse IgE (clone R35-72; PharMingen). Following administration of 3% BSA-PBS for 2 h, serial dilutions of sera were incubated overnight at 4° C. Then biotin-labeled OVA was added for 2 h and absorption was read after adding Streptavidin–horseradish peroxidase and *o*-

phenylenediamine. The antibody titer was defined as the reciprocal serum dilution yielding an absorbance reading of OD=0.2 after linear regression analysis.

Statistical analysis

ANOVA was used to determine the levels of difference between all groups. Comparisons for all pairs was performed by the Tukey-Kramer honest significant difference test. *P* values for significance were set at 0.05. Values for all measurements are expressed as the mean ± SEM.

Results

Mast cells are not required for allergen-specific B cell and T cell responses

To assess the role of mast cells following sensitization without adjuvant, two different strains of mast cell- deficient mice were sensitized with OVA and then challenged with inhaled OVA on 3 consecutive days. Following sensitization and challenge WBB6F_1-Kit$^{W/Wv}$ and C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice showed an increase in OVA-specific serum IgG1 and IgE titers, similarly to sensitized and challenged respective wild-type (WBB6F1-Kit$^{+/+}$ and C57Bl/6-Kit$^{+/+}$) mice (Table 1). In addition, $CD4^+$ T cells derived from the spleens of sensitized and challenged mast cell-deficient and wild type mice displayed a comparable response with respect to Th2 cytokine production after restimulation *in vitro* (online depository table 1).

Mast cells are necessary for the development of allergic airway disease

To investigate the role of mast cells in the development of AHR, lung function was assessed 48 hrs following the last allergen inhalation. Both strains of mast cell deficient WBB6F_1-Kit$^{W/Wv}$ mice and C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice showed no increase in airway responsiveness compared to respective wild-type mice which displayed increased airway responsiveness to MCh following sensitization and challenge (Figure 1 A and B). As depicted in Figure 2a, wild type mice developed an eosinophilia in the airways, whereas in mast cell-deficient mice only few eosinophils appeared in the BAL fluid. Additionally, numbers of $CD4^+$ and $CD8^+$ T cells in BAL fluid were significantly lower in sensitized and challenged C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice compared to wild-type mice (Figure 2 B). A reduction in tissue inflammation was also evident in HE-stained sections of lung tissue derived from mast cell-deficient mice compared to their congenic littermates after sensitization and challenge (Figure 3, left panel). Using PAS staining (Figure 3, right panel), significantly reduced numbers ($p<0.01$) of goblet cells in airway epithelia of sensitized and challenged C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$

mice were seen (mean ± SEM: 27 ± 8 PAS-positive cells/mm BM in C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ versus 93 ± 8 PAS-positive cells/mm BM in wild-type mice, $p<0.01$, n=12). Using the same model, we were also able to confirm our findings, decreased airway inflammation, failure to develop AHR, and reduced numbers of goblet cells- using WBB6F$_1$-Kit$^{W/Wv}$ animals, a different mouse strain which is also devoid of mast cells (data not shown).

Mast cell-derived TNF is pivotal for the development of AHR

Previous studies have shown that mast cell function can be restored in mast cell deficient mice by transfer of BMMC [5,6]. Taking advantage of this technique, we examined the role of mast cell-derived TNF in our model of acute allergic airway disease. To this end, BMMC were obtained from either C57Bl/6-TNF$^{+/+}$ or C57Bl/6-TNF$^{-/-}$ donors. Following crosslinking of FcεRI *in vitro* no differences in degranulation and production of cytokines (IL-4, IL-6 and IL-13) were found between the TNF-deficient mast cells and wild-type mast cells except for the production of TNF, which was absent in the BMMCs from C57Bl/6-TNF$^{-/-}$ donors (data not shown).

BMMCs derived from C57Bl/6-TNF$^{+/+}$ or C57Bl/6-TNF$^{-/-}$ donors were transferred to C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ recipient mice. Eight weeks following reconstitution, recipient mice were sensitized and challenged. In mice reconstituted with mast cells from C57Bl/6-TNF$^{+/+}$ animals (n=9) (mean ± SEM mast cells per cm^2) 35 ± 12 mast cells were detected compared to 20 ± 5 cells following reconstitution with mast cells from C57Bl/6-TNF$^{-/-}$ donors (n=9) ($p>0.05$ compared to all other groups) and 30 ± 12 cells in sensitized and challenged wild-type mice (n=9) (Figure 6 and Figure 1 in the online depository).

C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with wild-type BMMC showed airway responsiveness similar to sensitized and challenged wild-type mice (Figure 4). In contrast, reconstitution of mast cell-deficient mice with BMMC derived from C57Bl/6-TNF$^{-/-}$ donors

did not restore airway hyperresponsiveness (Figure 4). This clearly indicates an important role of mast cell-derived TNF for the development of AHR.

Airway inflammation and goblet cell metaplasia in mast cell-deficient mice cannot be restored upon transfer of TNF deficient mast cells

Using reconstitution experiments, we next analyzed the influence of mast cells and mast cell-derived TNF on airway inflammation and goblet cell hyperplasia. As summarized in Figures 5 and 6, numbers of eosinophils and T cells in BAL (Figure 5) as well as cellular infiltration in the lungs (Figure 6, upper panel) and number of goblet cells (Figure 6, middle panel and table 2) were restored to wild type levels upon transfer of wild-type BMMC into mast cell-deficient mice. However, reconstitution of mast cell-deficient mice with BMMC derived from C57Bl/6-TNF$^{-/-}$ donors did not or only marginally restore airway inflammation (Figures 5 and 6) and goblet cell metaplasia (Figure 6 and Table 2).

Discussion

Several lines of evidence support a pivotal role of mast cells for the development of allergic asthma, but so far very little is known about the underlying mechanisms and mediators involved in these mast cell dependent responses. In the present study we show in an acute model of allergic airway disease that following sensitization without adjuvant and challenge of the mice, mast cells are necessary for the development of AHR and airway inflammation. Furthermore we show that mast cell-derived TNF is an essential mediator for the development of these responses in this model.

Mast cells have been postulated as important effector cells in allergic airway disease for a long time. However, in murine models the role and contribution of mast cells to the development of allergic airway disease appears to be highly dependent on the sensitization and allergen exposure protocol. Studies using models of systemic sensitization with adjuvant have repeatedly shown a similar degree of AHR and airway inflammation in mast cell or IgE-deficient mice compared to respective wild type mice [24-26]. In several different models with probably less potent sensitization protocols mast cells have been implicated to be necessary for the induction of non-allergic [27] but also allergen-induced airway disease [5-7,28,29]. In the present study, we show that following systemic allergen sensitization without adjuvant and airway challenge, levels of allergen-specific IgE and IgG1 and allergen-induced proliferation of T cells remained intact in both mast cell deficient mouse strains. Also increased levels of OVA-specific IgG2 b were detected. However these were much lower compared to OVA-specific IgG1 levels, suggesting a predominant Th2 response in these animals. These findings and previous studies [6] suggest that following systemic introduction of the allergen, sensitization is not impaired in mast cell deficient mice. In contrast, development of key features of allergic airway disease including AHR, airway inflammation, migration of T cells into the lung, and goblet cell metaplasia are decreased in both mast cell-

deficient mouse strains, suggesting an important role for mast cells in mediating allergen-induced responses.

Transfer of BMMCs to mast cell deficient mice has been shown to reconstitute mast cells in many organs but also the lungs of the recipient mice [22,30]. Previous studies by Williams et al. [5] and Yu et al. [6] in a more chronic model of allergic airway disease have also demonstrated that BMMC administration to mast cell deficient mice reconstituted the features of allergic airway disease. Similarly, here using a more acute model, we showed that transfer of BMMCs derived form C57Bl/6-Kit$^{+/+}$ mice to C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ recipients resulted in detectable mast cells in lung tissue accompanied by AHR, increased airway inflammation, and goblet cell metaplasia in sensitized and challenged recipients, to levels seen in sensitized and challenged wild-type mice.

Mast cells can be activated through different stimuli [31], and in the context of allergic airway disease is primarily through IgE/allergen-mediated crosslinking of FcεRI [32,33]. Yu et al. showed that the expression of the Fc receptor γ chain in mast cells, which is necessary for the surface expression of the Fcγ receptor I, Fcγ receptor III and FcεRI receptor, is critical for the induction of most features of allergen induced lung pathology [6]. Studies in a model of allergen inhalation have suggested that activation through FcεRI, which in mice is only displayed on mast cells and basophils [32], contributed to increased airway reactivity [7]. Despite several studies demonstrating a contribution of mast cells in the development of acute and chronic allergic airway disease, little is known about which mast cell-derived mediator(s) may be necessary for these responses. Following activation mast cells are capable to produce a wide variety of mediators, including histamine, lipid mediators, chemokines and cytokines. Some of these mediators have been linked to the development of allergic airway disease, but it remains unclear if mast cells are an essential source for them as other cells types might also contribute to their production. Indeed, IL-13 has been repeatedly shown to be a central effector cytokine in allergic airway disease [34-36]. However, the production of IL-13 by

mast cells seems not to be a necessity for the induction of increased airway reactivity following inhaled allergen challenge [7]. On the other hand specialized adoptive transfer models have suggested a role of mast cell produced lipid mediators for recruitment of allergen specific CD8-positive T effector cells into the lung [11], which are necessary for development of allergen specific lung pathology [10]. In the current study a decrease in CD4-positive as well CD8-positive T-cells was detected in BAL fluid of the sensitized and challenged mast cell deficient mice suggesting impaired T-cell migration or local expansion in the lung consequent to the missing mast cell stimuli.

A mediator which has been implicated in many mast cell dependent inflammatory responses is TNF. Well in line with these reports in the present study mast cell deficient mice reconstituted with BMMC from TNF deficient donors failed to develop significant AHR following sensitization and challenge. In addition other features of allergic airway disease like airway inflammation and goblet cell metaplasia were not reconstituted in mice engrafted with TNF deficient mast cells, implicating a critical role of mast cell-derived TNF for the development of allergic airway disease. Mast cells have the capability to store and rapidly release TNF following activation [37,38]. In different disease models mast cell-derived TNF has been identified to be important for the induction and promotion of initial inflammatory events, e.g. in models of immune complex-induced inflammation [39], acute septic peritonitis [40], cutaneous inflammation [41], colitis [42], delayed-type hypersensitivity reactions [43], and pulmonary hypersensitivity reactions [44]. Several studies have also suggested a contribution of TNF during the induction of allergic airway disease. Following IgE-dependent stimulation of human lung tissue TNF is produced in amounts sufficient to induce biological effects [45]. Also, administration of TNF into the lungs of mice induces increased airway mucus gene expression [46] and a late airway response [47]. Recently, in TNF deficient mice a reduction in airway hyperresponsiveness and airway inflammation following a protocol of systemic sensitization without an additional adjuvant and airway challenge was demonstrated

[48]. Following allergen challenge WBB6F$_1$-Kit$^{W/Wv}$ mice express lower levels of TNF in BAL fluid, and reconstitution with wild-type BMMCs restored BAL fluid levels of TNF and AHR [49]. Independently confirming our results, Nakae et al. reported that mast cell-deficient mice reconstituted with BMMCs deficient in TNF failed to reconstitute the development of AHR [50]. In a model of allergic airway disease different from the one used in our experiments the authors showed that development of airway inflammation and goblet cell hyperplasia was impaired following reconstitution of C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice with TNF deficient BMMCs but not with wild-type BMMCs. The findings in the present study are in line with these results and extend them to a different model of allergic airway disease. The comparable results in two independent studies underscore the importance of mast cell produced TNF. The clinical relevance of these latter findings was strengthened by recent studies in patients with moderate [51] and also more severe asthma [52,53] where beneficial effects of treatment with TNF neutralizing antibodies were described. The fact that treatment with TNF neutralizing antibodies has shown first promising results in humans further supports that TNF is a potent therapeutic target for patients with allergic asthma.

The underlying mechanisms by which mast cell-derived TNF affects the different features of allergic airway disease remain to be elucidated. Based on current knowledge it is conceivable that migration of dendritic cells from the lung to the regional lymph nodes is impaired in the presence of TNF-deficient mast cells [54]. This assumption is substantiated by reports showing that IgE- dependent mast cell activation induces Langerhans cell migration [55] and that mast cell-derived TNF directly influences dendritic cell migration from the lung [17]. However, a direct effect on T-cells, which are thought to orchestrate the allergic response [1], is just as conceivable, as TNF from mast cells can directly influence T-cell activation and proliferation [15,16]. Indeed, Nakae et al suggest an increase in T-cell activation triggered by mast-cell produced TNF as the main modulator for the development of AHR and airway inflammation [50].

In summary the present study demonstrates a critical role of mast cells for the development of acute allergic airway disease following sensitization without an adjuvant. This finding is in line with models of more chronic protocols [5,6]. Based on the reconstitution experiments with TNF deficient mast cells the pivotal role for mast cell-derived TNF in the allergen-induced development of AHR, airway inflammation, and goblet cell metaplasia after allergen exposure of the sensitized host was identified.

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FIGURE LEGENDS

Figure 1: Airway responsiveness in mast cell deficient mice

Panel A: Airway responsiveness (resistance) in challenged only WBB6F$_1$-Kit$^{W/W\text{-}v}$ (WBB6F$_1$-Kit$^{W/W\text{-}v}$ chall, n=10), sensitized and challenged WBB6F$_1$-Kit$^{W/W\text{-}v}$ (WBB6F$_1$-Kit$^{W/W\text{-}v}$ sens/chall, n=10), challenged only (WBB6F$_1$-Kit$^{+/+}$ chall, n=10), and sensitized and challenged respective wild-type mice (WBB6F$_1$-Kit$^{+/+}$ sens/chall, n=10); * $p<0.05$ compared to all other groups. Results are expressed as mean±SEM

Panel B: Airway responsiveness in challenged only C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ chall, n=12), sensitized and challenged C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ sens/chall, n=12), challenged only (C57Bl/6-Kit$^{+/+}$ chall, n=12), and sensitized and challenged wild-type mice (C57Bl/6-Kit$^{+/+}$ sens/chall, n=12); # $p<0.05$ compared to all other groups. Results are expressed as mean±SEM

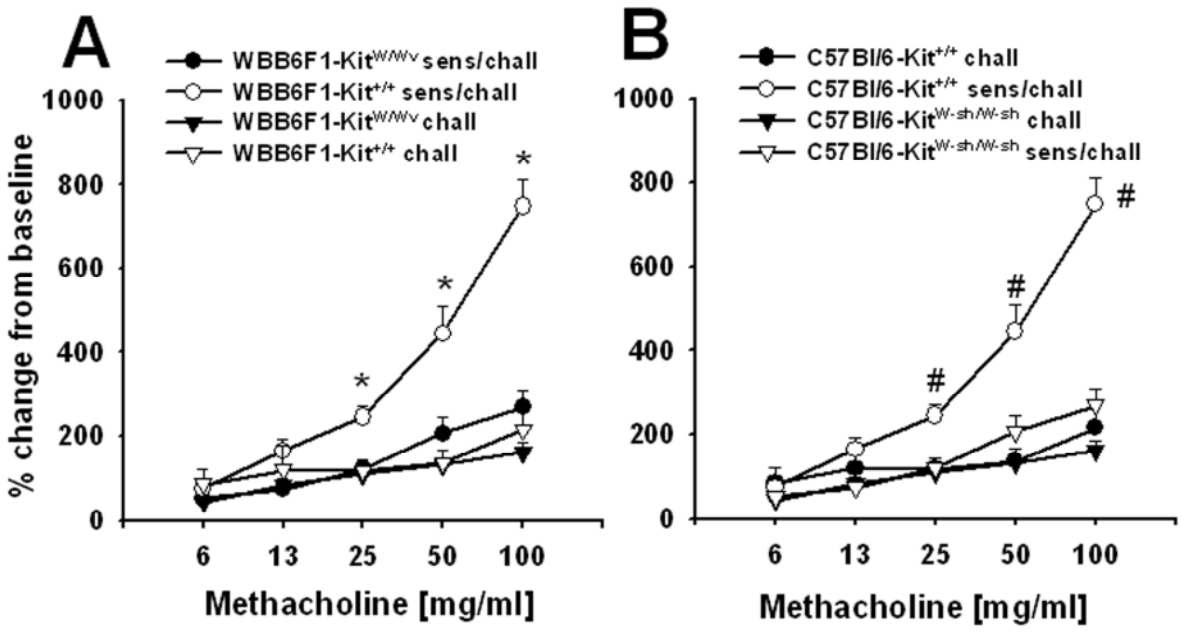
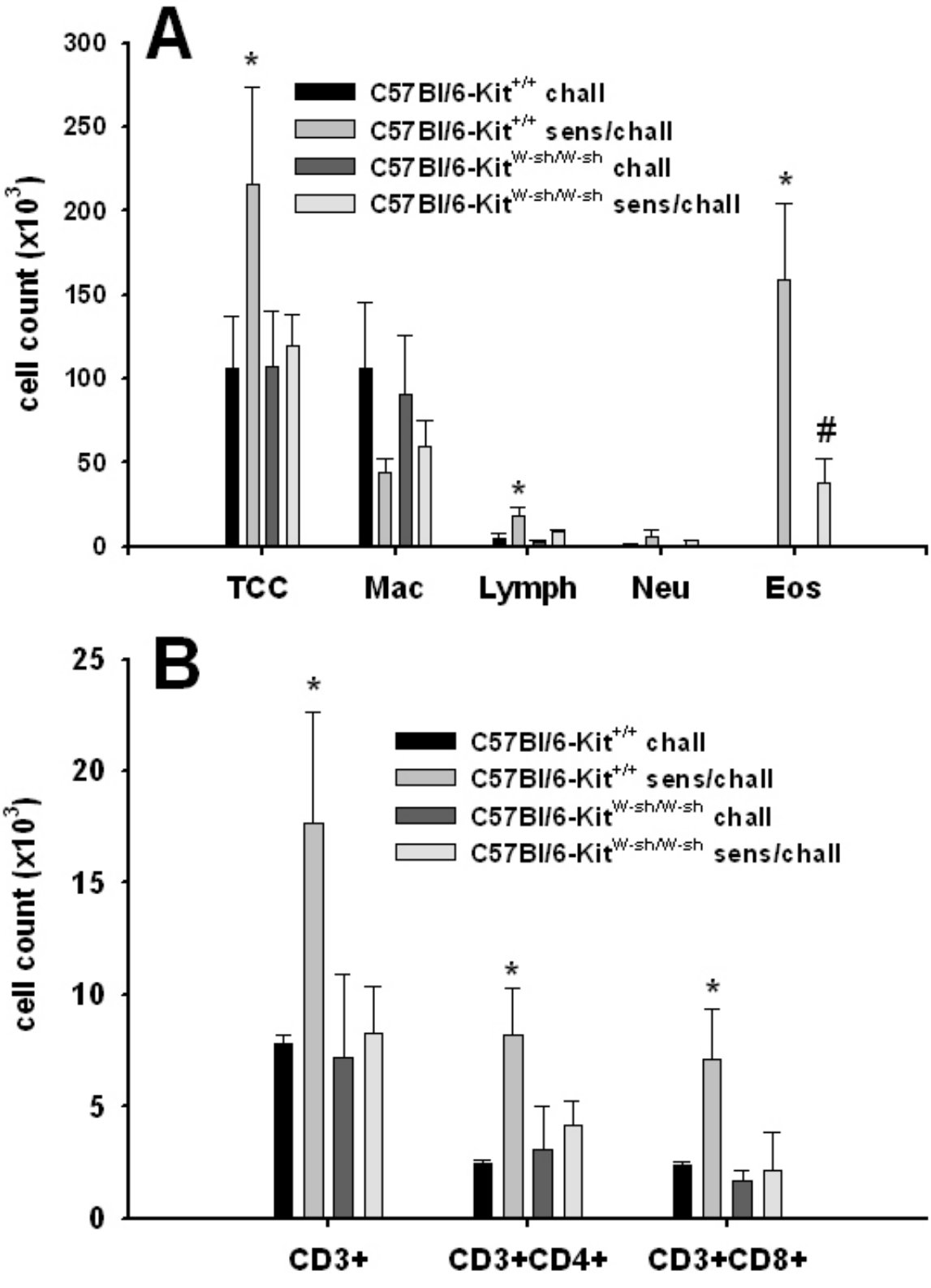


Figure 2: Airway inflammation in BAL fluid.

Differential cell count (Panel A) and number of CD4$^+$ and CD8$^+$ T-cells (Panel B) were assessed in BAL fluid of challenged only C57Bl/6-Kit$^{+/+}$ (C57Bl/6-Kit$^{+/+}$ chall, n=12) mice,

sensitized and challenged C57Bl/6-Kit$^{+/+}$ (C57Bl/6-Kit$^{+/+}$ sens/chall, n=12) mice, challenged only C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ chall, n=12) and sensitized and challenged C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ sens/chall, n=12) mice. Results are expressed as mean±SEM. *$p<0.05$ compared to all other groups; #$p<0.05$ compared to C57Bl/6-Kit$^{+/+}$ chall and C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ chall.

Figure 2


A
cell count (x10^3)
300
250
200
150
100
50
0
C57Bl/6-Kit+/+ chall
C57Bl/6-Kit+/+ sens/chall
C57Bl/6-KitW-sh/W-sh chall
C57Bl/6-KitW-sh/W-sh sens/chall
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TCC
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Lymph
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cell count (x10^3)
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C57Bl/6-Kit+/+ chall
C57Bl/6-Kit+/+ sens/chall
C57Bl/6-KitW-sh/W-sh chall
C57Bl/6-KitW-sh/W-sh sens/chall
*
*
*
CD3+
CD3+CD4+
CD3+CD8+

Figure 3: Tissue inflammation and goblet cell metaplasia in mast cell deficient mice

Tissue inflammation was evaluated 48 hrs following the last challenge using hematoxylin and eosin staining (HE) and PAS staining for goblet cells in challenged only C57Bl/6-$Kit^{+/+}$ mice ($Kit^{+/+}$ chall), sensitized and challenge C57Bl/6-$Kit^{+/+}$ ($Kit^{+/+}$ sens/chall), challenged only C57Bl/6-$Kit^{W\text{-}sh/W\text{-}sh}$ ($Kit^{W\text{-}sh/W\text{-}sh}$ chall) and sensitized and challenged C57Bl/6-$Kit^{W\text{-}sh/W\text{-}sh}$ ($Kit^{W\text{-}sh/W\text{-}sh}$ sens/chall). Final magnifications 100x and 400x for inserts.

Figure 3

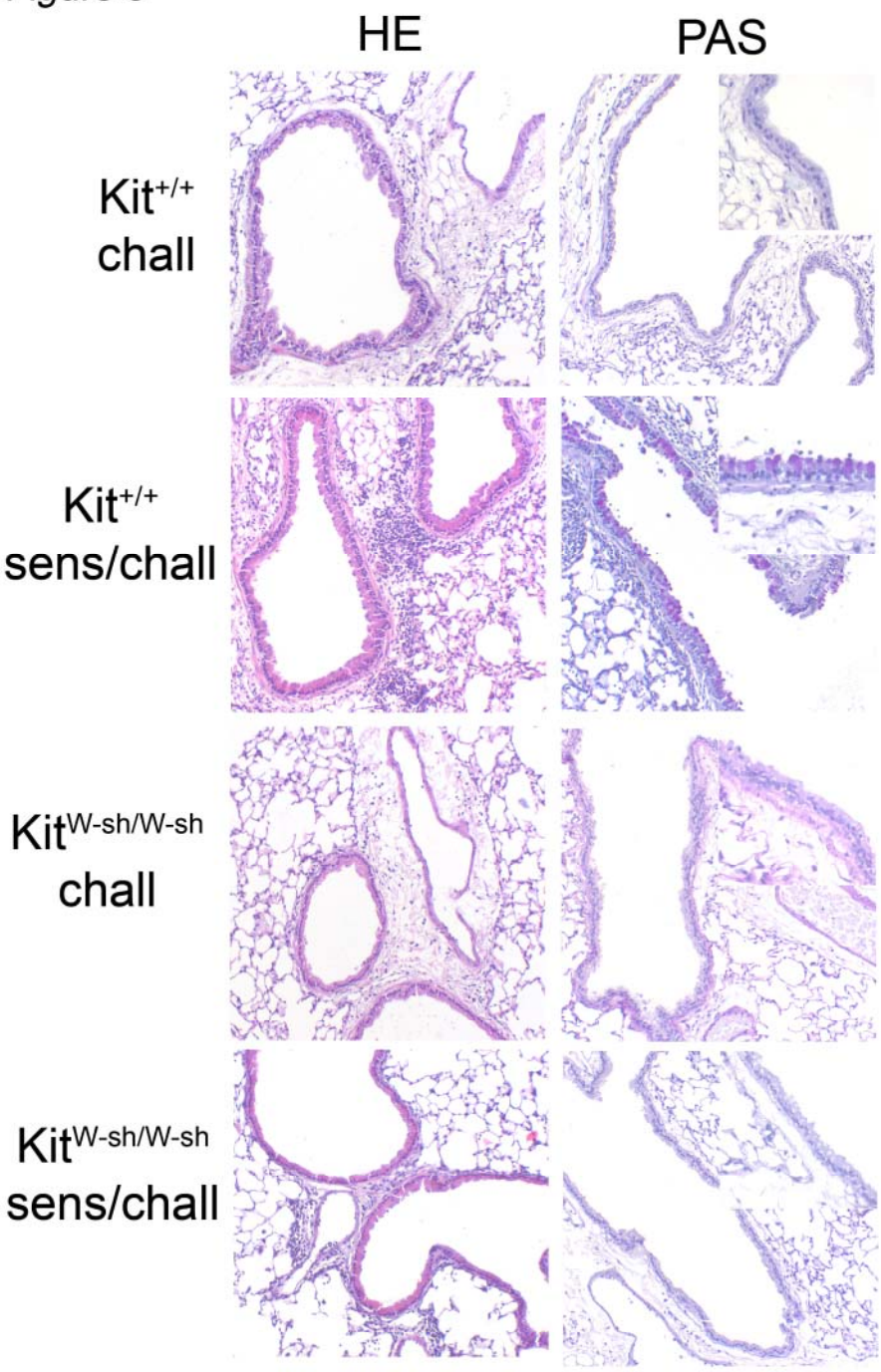

Figure 4: Airway responsiveness in mast cell deficient mice following reconstitution with wild-type and TNF-α deficient BMMCs

Airway reactivity was assessed following sensitization and challenge in C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ reconstituted with either BMMCs derived from wild-type mice (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$+ WT

BMMCs, n=9) or TNF-deficient mice (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$+ TNF BMMCs, n=9) and compared to challenged wild type mice (C57Bl/6-Kit$^{+/+}$ chall, n=9), sensitized and challenged wild type mice (C57Bl/6-Kit$^{+/+}$ sens/chall, n=9), challenged C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ chall, n=9) and sensitized and challenged C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice which were not reconstituted with mast cells (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ sens/chall, n=9). Each condition contained nine mice, and the mean±SEM from three independent experiments are given. * $p<0.05$ compared to C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$+ TNF BMMCs, C57Bl/6-Kit$^{+/+}$ chall, C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$, and C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ sens/chall.

Figure 4

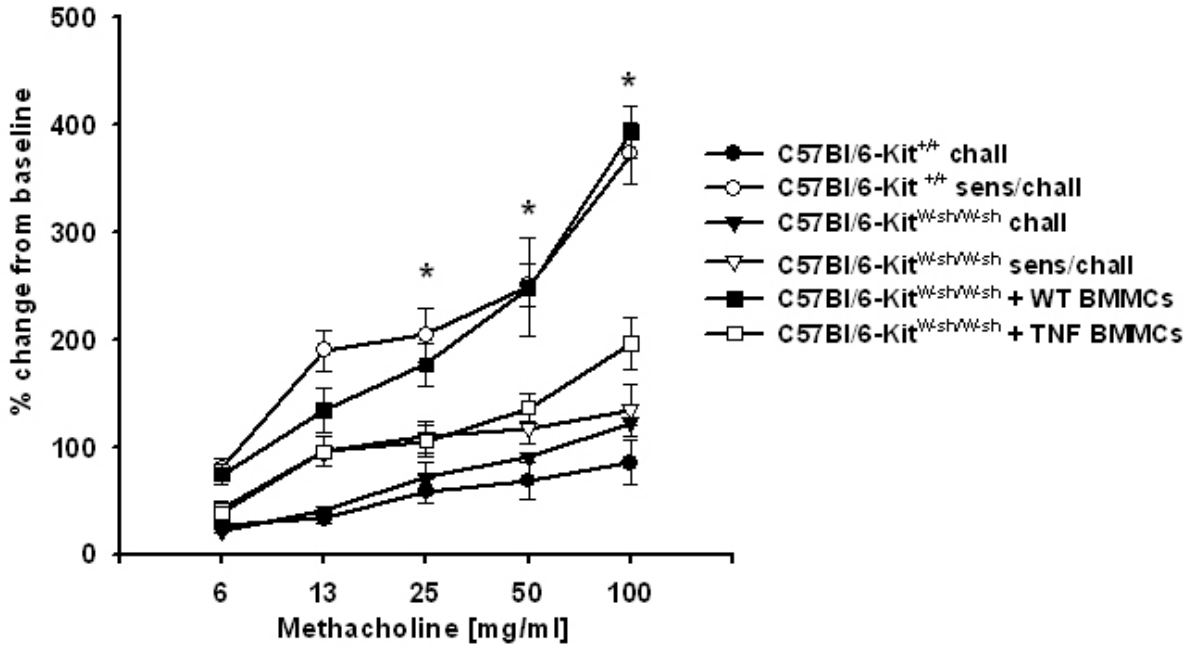

Figure 5: Eosinophil, CD4+ an CD8+ T-cell numbers in BAL fluid

Number of eosinophils (Panel A) and CD4+ and CD8+ T-cells (Panel B) were assessed in BAL of challenged only (chall) and sensitized and challenged (sens/chall) wild-type mice (C57Bl/6-Kit$^{+/+}$). Also challenged only (chall), sensitized and challenged (sens/chall), sensitized and challenge reconstituted with wild-type BMMCs (+WT BMMCs) or TNF-deficient BMMCs (TNF BMMCs) C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice (C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$). Each

condition contained nine mice, and the mean±SEM from three independent experiments are given. * $p<0.05$ compared to all other groups C57Bl/6-Kit$^{+/+}$ chall, C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ chall, C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ sens/chall and C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ +TNF-α BMMCs. # $p<0.05$ compared to C57Bl/6-Kit$^{+/+}$ chall and C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ chall.

Figure 5

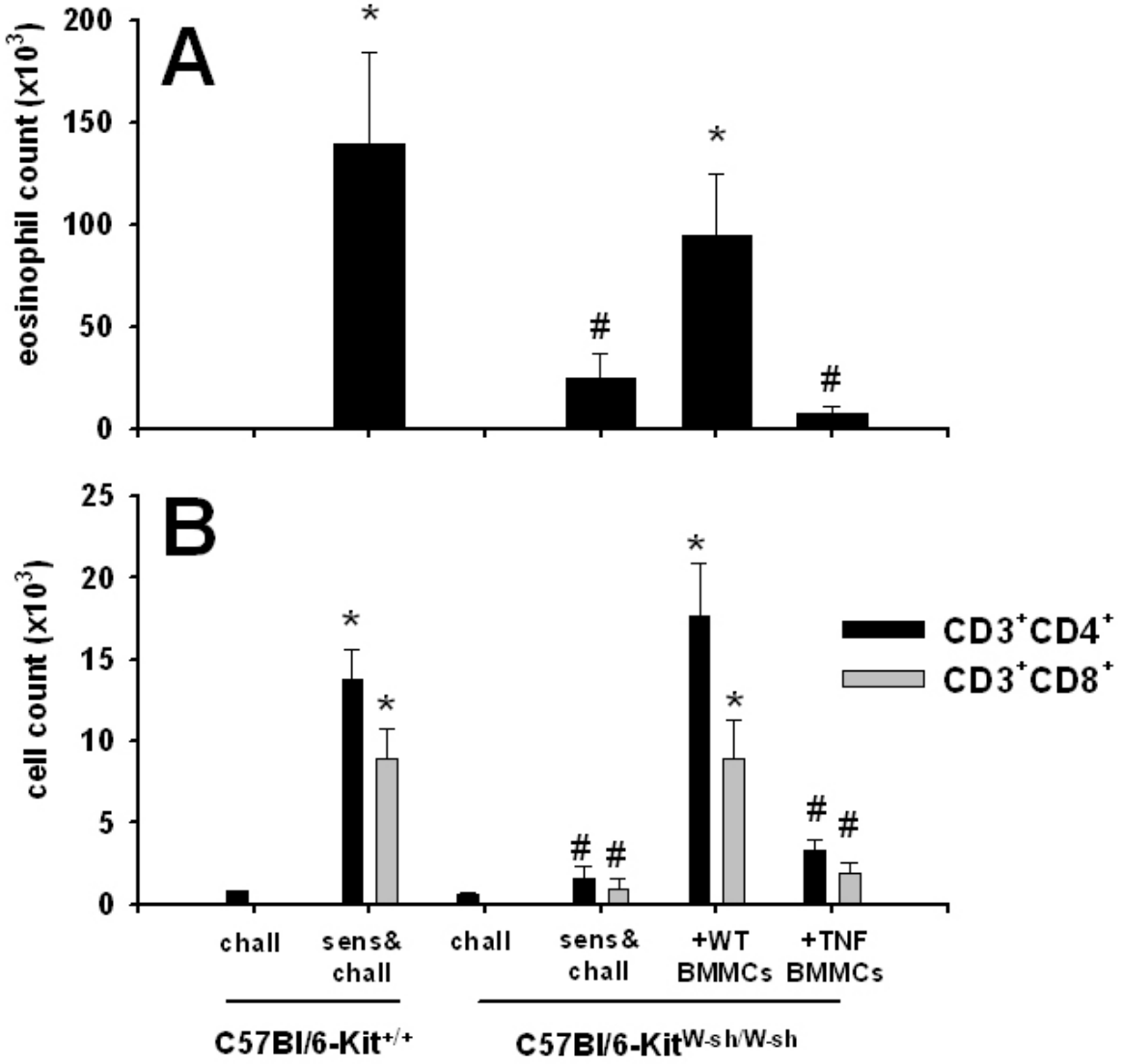

Figure 6: Tissue inflammation and goblet cell metaplasia in mast cell deficient mice

Tissue inflammation was evaluated 48 hrs following the last challenge using hematoxylin and eosin staining (HE), PAS staining for goblet cells and Toluidine blue and Avidin-Alexa-488 staining for mast cells in challenged only C57Bl/6-Kit$^{+/+}$ mice (Kit$^{+/+}$ chall), sensitized and challenge C57Bl/6-Kit$^{+/+}$ mice (Kit$^{+/+}$ sens/chall), sensitized and challenge C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice (Kit$^{W\text{-}sh/W\text{-}sh}$ sens/chall), sensitized and challenge C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with BMMCs from wild-type mice (Kit$^{W\text{-}sh/W\text{-}sh}$ WT BMMCs) and sensitized and challenge C57Bl/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with BMMCs from TNF-deficient mice (Kit$^{W\text{-}sh/W\text{-}sh}$ TNF BMMCs). Arrows mark toluidine blue stained cells. Final magnifications 100x and 400x for inserts.

Figure 6

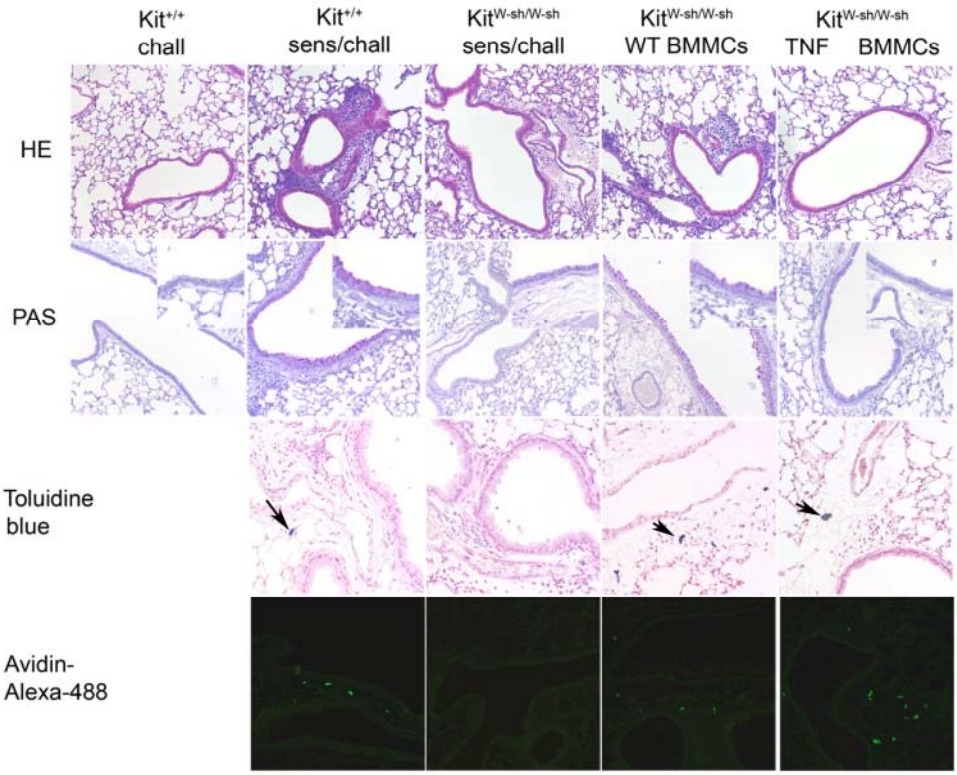

Table 1: Serum immunoglobulin titers

	WBBF1/Kit $^{+/+}$ chall	*WBBF1/Kit $^{+/+}$ sens/chall*	*WBBF1/Kit $^{W/Wv}$ chall*	*WBBF1/Kit $^{W/Wv}$ sens/chall*	*C57Bl/6-Kit $^{+/+}$ chall*	*C57Bl/6-Kit $^{+/+}$ sens/chall*	*C57Bl/6-Kit$^{Wsh/Wsh}$ chall*	*C57Bl/6-Kit$^{Wsh/Wsh}$ sens/chall*
OVA-specific IgE (Titer)	N.D.	352.7±64.4#	N.D.	315.2±57.9#	N.D.	42.3±12.7*	N.D.	39.5±12.1*
OVA-specific IgG1 (Titer) (x10^3)	N.D.	117.7±25.5#	N.D.	90.6±28.7#	N.D.	45.7±11*	N.D.	38.3±19*
OVA-specific IgG2b (Titer)	18±4	1393±287#	25±7	1412±402#	19±4	584±117*	15±3	426±119*

Mice were sensitized and challenged as described in Methods. Serum levels of immunoglobulins were assessed 48 h after the last challenge. Mean values±SEM are given; WBBF1/Kit $^{W/Wv}$: mast cell deficient mice; WBBF1/Kit $^{+/+}$: congenic wild-type control mice; C57Bl/6-Kit$^{Wsh/Wsh}$: mast cell deficient mice; C57Bl/6-Kit$^{+/+}$ congenic wild-type controls; chall: challenged only mice; sens/chall: sensitized and challenged mice. N.D.: not detectable. # $p<0.05$ compared to WBBF1/Kit $^{+/+}$ chall and WBBF1/Kit $^{W/Wv}$ chall. * $p<0.05$ compared to C57Bl/6-Kit $^{+/+}$chall and C57Bl/6-Kit$^{Wsh/Wsh}$ chall.

Table 2: Number of PAS-positive cells in airway epithelium

	C57Bl/6-Kit $^{+/+}$ chall	*C57Bl/6-Kit $^{+/+}$ sens/chall*	*C57Bl/6-Kit$^{Wsh/Wsh}$ chall*	*C57Bl/6-Kit$^{Wsh/Wsh}$ sens/chall*	*C57Bl/6-Kit$^{Wsh/Wsh}$ WT BMMC*	*C57Bl/6-Kit$^{Wsh/Wsh}$ TNF BMMC*
PAS-positive cells/ mm BM	N.D.	65 ± 11 #	N.D.	20 ± 11 *	62 ± 10 #	21 ± 9 *

Goblet cell metaplasia expressed as number of PAS-positive cells per mm of basement membrane (BM). Mean values±SEM are given, n=9 per group. C57Bl/6-Kit$^{Wsh/Wsh}$: mast cell deficient mice; C57Bl/6-Kit$^{+/+}$ congenic wild-type controls; chall: challenged only mice; sens/chall: sensitized and challenged mice. WT BMMC: reconstitution with BMMC from wild-type donors, TNF BMMC: reconstitution with BMMC from TNF deficient donors. N.D.: not detectable. # $p<0.05$ compared to C57Bl/6-Kit $^{+/+}$ chall, C57Bl/6-Kit$^{Wsh/Wsh}$ chall, C57Bl/6-Kit$^{Wsh/Wsh}$sens/chall and C57Bl/6-Kit$^{Wsh/Wsh}$ TNF-$\alpha^{-/-}$ BMMC. * $p<0.05$ compared to C57Bl/6-Kit $^{+/+}$ chall, C57Bl/6-Kit$^{Wsh/Wsh}$ chall.