Mast cell engraftment of the peripheral lung enhances airway hyperresponsiveness in a mouse asthma model

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Asthma is a chronic inflammatory disease with airway hyperresponsiveness (AHR) as a main characteristic (6). AHR is defined as an exaggerated airway narrowing in response to a variety of chemical, physiological, and pharmacological stimuli (20). Several causative factors have been proposed such as chronic inflammation, airway remodeling, smooth muscle growth, and epithelial damage, but the mechanisms are not yet clear (9). This is probably because AHR is multifactorial and different in distinct phenotypes of asthma (42). Mast cells have been implicated in the development of AHR; however, their role is not clear (42).

Mast cells play a central role in the allergic asthmatic response, which can be life threatening within minutes of encountering an allergen (7). Activation of mast cells via cross-linking of FcεRI-bound allergen-specific immunoglobulin (Ig) E induces degranulation and release of mediators, particularly histamine and lipid mediators, chemokines, and cytokines that evoke the different symptoms of the asthmatic response (10). Several studies have demonstrated elevated numbers of mast cells at different sites in the lungs of asthmatic patients, including within the intraepithelial cell layer (13, 17), the alveolar parenchyma (2), and the bronchial smooth muscle (8). This compartmental increase and altered distribution of lung mast cells have been correlated with inflammation and impairment of pulmonary function (1, 2, 4, 8, 23).

The role of mast cells in the development of AHR has been investigated by using mast cell-deficient mice in different mouse asthma models by measuring the response to methacholine. The initial studies, using short protocols with adjuvant, showed similar AHR in both mast cell-deficient and wild-type mice (40, 43), indicating that, in mice, mast cells are not involved in the development of AHR. On the other hand, mast cell-deficient mice did not have the same increased methacholine response as wild-type mice when a single dose of anti-IgE was given (11, 27). Elaborating with the sensitization procedure by excluding adjuvant in the short protocol, a mast cell-dependent AHR was obtained (43). However, the lack of mast cells generally decreased the effect of methacholine both with and without allergen challenge compared with wild-type mice. In addition, in a chronic protocol with a sensitization procedure without adjuvant, Yu et al. (46) also showed an AHR that was dependent on mast cells, which was reestablished when the mast cell-deficient mice were engrafted with mast cells. Thus it appears that the development of AHR can be either mast cell dependent or independent in mouse models of asthma and it has been suggested that the former is due to a sensitization without adjuvant and the latter with adjuvant (43). However, these earlier studies measured the AHR with whole body plethysmography or by whole lung resistance (40, 43, 46) and did not separate the AHR in proximal and distal parts of the lung, which is of particular importance since mast cells in mouse airways are not evenly distributed along the airway tree and are mainly found around the proximal airways (19, 44, 45). Moreover, reconstitution of mast cell-deficient mice with bone marrow-derived mast cells leads to a more distal distribution of airway mast cells compared with wild-type mice (18). This phenomenon creates a model that can be used to address important hypotheses regarding the functional role of mast cells in allergic airway inflammation.

The present study addresses the hypothesis that the localization and number of pulmonary mast cells determines the level of inflammation and AHR. For this purpose, inbred mast cell-deficient B6.Cg-Ki6+ (Wsh) mice, which carry a spon-
taneous mutation in the Kit/CD 117 receptor for stem cell factor (26), were employed, with and without engrafment of congenic mast cells derived from the bone-marrow of wild-type C57BL/6 mice (18). The mice were then exposed to an experimental model of chronic asthma involving both inflammation and airway remodeling (14). In addition to measuring the whole lung resistance, a forced-oscillation technique was used to assess physiological changes in both proximal and distal regions of the lung. Inflammation was evaluated by monitoring leukocyte infiltration into bronchoalveolar lavage (BAL) fluid (BALF) and the pulmonary tissue, as well as tissue levels of inflammatory mediators. Our findings demonstrate, in a chronic murine asthma model, that AHR is maintained in mast cell-deficient mice and enhanced following engrafment, especially in the peripheral lung possibly because of an increase of mast cells in the parenchyma.

**MATERIALS AND METHODS**

**Animal experiments.** At 6 wk of age, female Wsh mice were engrafted by intranasal injection of mast cells (5x10^6/100 µl; Wsh+MC) derived from the bone marrow of 6-wk-old C57BL/6 mice, as described previously (34). At 18–20 wk of age, wild-type C57BL/6 (Charles River, Sulzfeld, Germany), Wsh, and Wsh+MC mice were subjected to a 91-day protocol designed to produce chronic lung inflammation through repeated challenges with ovalbumin (OVA, Sigma-Aldrich, Stockholm, Sweden), as described elsewhere (14, 24) (Fig. 1). These intranasal challenges with OVA or PBS were performed under anesthesia with isoflurane. Diclofenac sodium [1 mg/kg body wt; nonselective cyclooxygenase (COX) inhibitor; Cayman Chemicals, Ann Arbor, MI] was given intraperitoneally. At 1 h before each OVA-challenge, 1 h before the methacholine challenge and as an intravenous injection at the start of the anesthesia. The dose of diclofenac has previously been described to be effective (38, 39). All experiments were preapproved by the Regional Committee of Animal Experimentation Ethics (Stockholm, Sweden).

**Pulmonary mechanics.** Following anesthesia with pentobarbital (70 mg/kg body wt ip), the pulmonary mechanics of the mice were assessed as described previously (37). Using the forced-oscillation technique, lung resistance was determined assuming a single compartment linear model. Newtonian airway resistance, tissue resistance, and tissue elastance were determined by assuming a constant-phase compartment linear model. A single com-

**Quantification of allergic sensitization.** Plasma levels of OVA-specific IgE were measured by ELISA (MD Bioscience, Zurich, Switzerland; lower limit of quantification = 62.5 ng/ml).

**Characterization of inflammation, numbers of mast cells, amount of collagen, smooth muscle layer thickness, and mast cell phenotype.** BAL and differential leukocyte counts were performed as described previously (38). After preparation as reported elsewhere (38), 5-µm-thick paraffin sections of the left lung taken 1 or 2 mm distal of the hilum were stained to detect inflammatory cell infiltration (hematoxylin and eosin; Histolab, Gothenburg, Sweden), mast cells (1% toluidine blue; Sigma-Aldrich), and collagen (0.1% Sirius red; Histolab). Pulmonary inflammation was assessed in a blinded manner by semiquantitative evaluation of the cellular infiltrate, using the following scale: 0 = no; 1 = slight; 2 = moderate; 3 = abundant infiltration by cells. The numbers of mast cells in central airways, smaller airways, perivascular space, and parenchyma were counted in sections taken at least 20 µm apart. Antibodies against carboxypeptidase A3 (CPA3) and mouse mast cell protease 6 (mMCP-6) (kindly provided by Gunnar Pejler, Uppsala, Sweden), and toward α-smooth muscle actin (α-SMA; Dako, Stockholm, Sweden) were examined immunohistochemically after antigen retrieval. For detection, LSAB+ System-HRP (Dako) and AEC Staining Kit (Sigma-Aldrich) were utilized. The dilutions of the primary antibodies prior to use were as follows: α-CPA, 1:300; α-mMCP-6, 1:1,100; α-SMA, 1:100; and nonspecific rabbit serum: 1:300. The amounts of collagen and α-SMA present in the vicinity of the main bronchi were calculated by employing Image Pro Plus, v.6.2 (Media Cybernetics, Bethesda, MD).

**Quantification of cytokines and chemokines in lung homogenates.** Protein was extracted from snap-frozen samples of pulmonary tissue with tissue extraction buffer (Invitrogen) supplemented with proteinase inhibitors (Sigma-Aldrich), IL-5, IL-9, IL-13, IL-33, CXCL1, and CXCL10 were quantified with the multiplex technique (Millipore, Billerica, MA) and IL-17A by ELISA (Ebioscience, San Diego, CA). The corresponding lower limits of quantification were 18, 21, 17, 945, 32, 20, 3, and 5 pg/100 mg tissue, respectively.

**Statistical analyses.** One-way or two-way ANOVA, the chi² test, and Bonferroni’s post hoc test were employed for statistical analyses (Graph Pad Prism 5, La Jolla, CA). A P value of <0.05 was considered statistically significant.

**RESULTS**

Mast cells engraft the peripheral lung and are further increased in the parenchyma during chronic inflammation. To examine how allergic inflammation influences the localization and number of mast cells, wild-type mice and mast cell-deficient mice without (Wsh) or with (Wsh+MC) engrafment with mast cells were first sensitized toward OVA and subsequently challenged with either PBS or OVA (Fig. 2, A and B). The number of pulmonary mast cells in wild-type animals.
treated in either manner was low (Fig. 2B) and, as expected, no mast cells were present in Wsh mice (Fig. 2B). In the Wsh+MC mice, the number of pulmonary mast cells was 15-fold higher than in the wild-type animals (Fig. 2B), both with and without OVA challenge.

Examination of the distribution of mast cells within different pulmonary compartments revealed that in wild-type mice, these cells were located primarily around the central airways and in the perivascular space (Fig. 2C), whereas in Wsh+MC animals mast cells were found predominantly in the alveolar parenchyma and perivascular space (Fig. 2C). With the exception of the central airways, the numbers of mast cells in all compartments examined were higher in the Wsh+MC mice (Fig. 2D–G).

Under conditions of allergic airway inflammation in Wsh+MC mice, the number of mast cells in the parenchyma was elevated (Fig. 2F), with parallel reductions in the numbers in the central airways and in the perivascular space (Fig. 2D and G). In contrast, challenging wild-type mice with OVA had no influence on mast cell distribution (Fig. 2, D–G).

With respect to phenotype, in OVA-challenged Wsh+MC mice the number of cells staining positively for the mast cell-specific proteases CPA3 and mMCP-6 were the same as the total numbers of mast cells detected by toluidine blue staining (Fig. 2H). In all groups, the mast cells in the vicinity of the central and peripheral airways were found primarily in the submucosa, with a small proportion in the adventitial layer and none in the intraepithelial region.

Fig. 2. Staining and localization of mast cells in pulmonary sections from wild-type C57BL/6 mice and B6.Cg-KitW−/Wsh mice with and without mast cell engraftment. A: representative toluidine blue-staining of lung sections of the different groups of mice. Arrows point to stained mast cells and scale bar represents 100 μm. Red arrows shows the mast cells, which are shown in a higher magnification in the insets. B: quantification of the total numbers of lung mast cells and of mast cell relocation following challenge with the allergen (OVA) (examined in 3 lung sections per mouse). C: proportion (%) of mast cells in different compartments of the lung and numbers in the central airway (D), smaller airways (E), perivascular space (F), and lung parenchyma (G). Means ± SE are shown (n = 12 per group). *P < 0.05 for comparison of the allergen-challenged groups, as analyzed by 2-way ANOVA, with the Bonferroni correction. #P < 0.05 for comparison of the control and allergen-challenged groups, as analyzed by 2-way ANOVA, with the Bonferroni correction. H: immunohistochemical staining for the mast cell proteases carboxypeptidase A3 (CPA3; top left) and mouse mast cell protease 6 (mMCP-6; top right) in pulmonary sections from Wsh+MC mice challenged with OVA. Bottom: corresponding control staining. The numbers of cells staining positively for these proteases were the same as the total numbers of mast cells detected by toluidine blue staining. No epithelial mast cells were detected. A total of 3 animals in each group were analyzed. Scale bar represents 25 μm.
Distinct AHR is related to the localization of airway mast cells. After intranasal treatment with PBS alone, wild-type and mast cell-deficient mice with or without mast cell-engraftment exhibited the same lung resistance as well as central airway resistance (Newtonian resistance), tissue resistance, and tissue elastance upon exposure to methacholine.

In all allergen-challenged groups the lung resistance was higher than in the control animals (Fig. 3A). Wild-type and Wsh mice exhibited a similar lung resistance, whereas this was further increased in Wsh+MC mice. Using a forced-oscillation technique, which enables separation of the resistance, we found that the Newtonian resistance was increased in all OVA-challenged animals, but with slightly altered pattern compared with lung resistance. In Wsh mice, the Newtonian resistance was elevated compared with wild-type animals. As for the lung resistance, the Newtonian resistance was more pronounced in Wsh+MC than in either wild-type or Wsh mice following allergen challenge. Tissue resistance was also enhanced in all OVA-challenged animals, again with the strongest and most marked elevation being observed in the Wsh+MC mice (Fig. 3B).

To elucidate how the presence of mast cells influences prostanoid-induced effects on AHR, the OVA-challenged mice demonstrated a significant enhanced tissue elastance.

A mast cell-dependent release of prostanoids partly protects from AHR in wild-type mice. As has been shown before, both in a short protocol with adjuvant included during sensitization (31, 39) and in a long protocol without adjuvant (38), a release of prostanoids partly protects from the development of AHR. To elucidate how the presence of mast cells influences prostanoid-induced effects on AHR, the OVA-challenged mice were treated with the nonselective cyclooxygenase inhibitor diclofenac. Notably, in the wild-type mice diclofenac treatment caused an increase of the Newtonian resistance that reached the same level as the Wsh mice (Fig. 4), suggesting that the difference in AHR between these groups was due to a higher release of protective prostanoids in the wild-type mice. Diclofenac also had an effect on tissue resistance, but in this case AHR was decreased in the Wsh+MC mice (Fig. 4).

Allergen-specific sensitization is not dependent on mast cells, but is nonetheless amplified in Wsh+MC mice challenged with OVA. Plasma levels of OVA-specific IgE, a measure of allergen-specific sensitization, were low and not significantly different in wild-type (368 ± 82 ng/ml), Wsh (523 ± 76 ng/ml), and Wsh+MC (835 ± 110 ng/ml) mice sensitized intraperitoneally toward OVA and subsequently challenged with PBS. Allergen challenge resulted in values of 20,300 ± 2,900, 21,900 ± 2,500 and 43,000 ± 7,400 ng/ml (Fig. 5), i.e., the most pronounced response was again exhibited in Wsh+MC animals.

Allergen-challenged wild-type, Wsh and Wsh+MC mice all exhibit airway inflammation. Allergic asthma is associated with airway inflammation and remodeling of the airway walls. To assess the extent of cellular inflammation, the leukocytes in both BALF and lung tissue were analyzed. BALF leukocytes were more abundant in all OVA-challenged mice than controls (Fig. 6A). Differential cell counts revealed increased numbers of all leukocytes examined, i.e., macrophages, neutrophils, lymphocytes, and eosinophils, in all OVA-challenged groups (Fig. 6B). However, the increase for lymphocytes in the Wsh mice and eosinophils in the Wsh+MC mice did not reach significance (P = 0.07 and P = 0.17, respectively). Furthermore, the level of both macrophages and eosinophils after allergen-challenge were higher in Wsh mice compared with wild-type mice after allergen challenge.

When the degree of cellular infiltration in lung sections was analyzed (Fig. 7A), all OVA-challenged groups exhibited significantly higher numbers of inflammatory cells than the mice...
challenged with PBS alone, with no difference between wild-type, Wsh and Wsh/H11001 MC values (Fig. 7, A and B). Same inflammatory pattern was observed in all challenged groups. Neither the smooth muscle layer nor collagen deposition around the airways was influenced by allergen challenge (Fig. 7, C and D). Localization of mast cells exerts an impact on allergen-induced alterations in the levels of cytokines and chemokines. When the levels of various cytokines at the site of the actual immune response, i.e., in the lung tissue, were assessed (Fig. 8, A–F), the levels of IL-5 were found to be low in the PBS-challenged groups but elevated following challenge of wild-type and Wsh mice with OVA (Fig. 8A). Furthermore, the levels of CXCL10 (Fig. 8B) and CXCL1 were significantly higher in all three OVA-challenged groups (Fig. 8C). The levels of IL-13 were low and did not differ significantly between the all groups, whereas IL-9 was below the detection limit for the assay (data not shown).

The pulmonary levels of IL-33, a proinflammatory cytokine released primarily from structural cells (25), were increased following chronic allergen challenge in all three groups of mice (Fig. 8D). However, levels were significantly lower in Wsh OVA than in either wild-type OVA or Wsh+MC OVA mice. In wild-type, but not Wsh or Wsh+MC, mice, chronic intranasal administration of OVA elevated the pulmonary level of IL-23, a cytokine associated with the promotion of IL-17 production (41) (Fig. 8E). Allergen challenge also induced higher levels of IL-17 in wild-type and Wsh+MC mice, with significantly lower levels in both Wsh and Wsh+MC mice than in the wild-type group (Fig. 8F).

**DISCUSSION**

In a chronic murine model of asthma, we could demonstrate that pulmonary mast cells were localized around the central airways in wild-type mice whereas engraftment of mast cells in Wsh mice caused an increase in lung mast cell number with a prominent localization in the alveolar parenchyma. The AHR was similar in wild-type and Wsh mice but was more pronounced and also present in the tissue elastance in Wsh+MC mice. In the wild-type and Wsh+MC mice, the levels of IgE, IL-17, and IL-33 were higher than in Wsh mice. Cellular influx into BALF and the lung tissue was increased after challenge but not different between the groups. Thus these data demonstrate that AHR and several inflammatory features of importance for asthma are promoted by mast cells.

The mast cells in the lungs of wild-type C57BL/6 mice are few in number and restricted to the central airways and trachea (16), in contrast to the human lung, where these cells are evenly distributed along the airways and vessels, as well as in the parenchyma (3). It has also been shown that subjects with asthma have higher number of mast cells than healthy subjects (4, 8, 13, 17). However, even though we employed a relatively long period of challenge to induce chronic inflammation, this did not alter mast cell number or distribution in the lungs of the wild-type animals. Our results differ from previous reports showing that BALB/c exhibit increased numbers of pulmonary mast cells in association with chronic inflammation (21, 29). However, this difference probably reflects the less extensive

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**Figure 4.** Peak responses of AHR to methacholine for the Newtonian resistance of the central airways (A), the tissue resistance (B), and tissue elastance (C) in PBS-challenged mice and OVA-challenged mice, treated with or without diclofenac. All values indicated are means ± SE (n = 12). Bars between groups represent *P < 0.05, as analyzed by 1-way ANOVA, with the Bonferroni correction.

**Figure 5.** Allergic sensitization in the different groups of mice, as reflected in plasma levels of OVA-specific IgE (quantified by ELISA). The individual values and means (horizontal lines) for a total of n = 9–12 animals in each group are shown. *P < 0.05 for comparison of the allergen-challenged groups, as analyzed by 2-way ANOVA, followed by t-test with Bonferroni correction.

#P < 0.05 for comparison of the control and allergen-challenged groups, as analyzed by 2-way ANOVA, with the Bonferroni correction.
induction of Th2 responses in C57BL/6 mice compared with BALB/c mice (29).

Mast cells injected intravenously into Wsh mice populated the lung with a 15-fold higher number compared with wild-type animals. These cells were predominantly localized in the parenchyma but also in the small airways and perivascular space. Elevated numbers of parenchymal mast cells have been demonstrated previously when mast cell-deficient mice have been engrafted (44). The induction of chronic airway inflammation did not further enhance the numbers of pulmonary mast cells in Wsh+MC mice. However, the localization of the mast cells was shown to be influenced by the OVA challenge since their localization shifted from the central airways and perivascular space toward the parenchyma. Redistribution of pulmonary mast cells has also been reported in asthmatic individuals, where these cells leave the lamina propria of blood vessels and

![Fig. 6. Cellular inflammation, as reflected in the numbers of total (A) and differentiation count (B) of leukocytes in bronchoalveolar lavage fluid (BALF). Mean values ± SE are depicted (n = 12 for each group). *P < 0.05 for comparison of the allergen-challenged groups, as analyzed by 2-way ANOVA, with the Bonferroni correction. #P < 0.05 for comparison of the control and allergen-challenged groups, as analyzed by 2-way ANOVA, with the Bonferroni correction.](image)

![Fig. 7. Inflammation, α-smooth muscle actin (α-SMA), and collagen in lung tissue. A: left panel displays representative images of pulmonary tissue from the various groups of mice stained with hematoxylin and eosin (scale bar = 200 μm). B: semiquantitative scoring of the severity of inflammatory infiltrates (median values shown; n = 12; analyzed with the χ² test for trends). Area of pulmonary tissue that stained positively for α-SMA (C) and collagen (D) by Sirius red around the airways (means ± SE; n = 12 for each group as shown; #P < 0.05 for comparison the control and allergen-challenged groups, as analyzed by 2-way ANOVA with the Bonferroni correction).](image)
accumulate instead in the mucus glands (7), epithelium (5), and smooth muscle (8) of the airways. The mechanism underlying the redistribution demonstrated here is not yet known.

In the present investigation all pulmonary mast cells stained positively for the connective tissue-type mast cell-specific proteases CPA3 and mMCP-6. Indeed, under normal, noninflamed conditions connective tissue mast cells are located predominantly in the trachea and the large airways (45). In this latter study, following a period of OVA challenges, a marked increase of lung mast cells, as a consequence of recruitment of mast cell progenitors, occurred exclusively in the intraepithelial compartment. These new mast cells were of the mucosal phenotype, not expressing CPA3. Thus the expression of CPA3 by the mast cells observed in our study, together with their...
Our hypothesis was that the mast cells influence AHR in a chronic murine model of asthma employing a sensitization procedure with adjuvant. In studies of lung resistance, the challenged wild-type and Wsh mice obtained similar AHR as demonstrated by two other studies using short protocols with adjuvant (40, 43), indicating that mast cells do not influence AHR under these particular conditions. However, when mice were engrafted with mast cells a pronounced increase in AHR was obtained, suggesting that mast cells can influence the AHR. It is possible either that the mast cells are different in phenotype or that their localization and/or number are different from wild-type mice. As discussed above the latter hypothesis seems more reasonable since we could not find any differences in the CPA3 and mMCP-6 expression whereas both the number of mast cells was increased and their localization was different compared with wild-type mice.

To further investigate whether the engrafted mast cells altered the AHR at different levels in the lung, the forced-oscillation technique was employed by using the constant-phase model (22). In the control situation without OVA challenge, the distribution of mast cells did not influence airway reactivity at any level of the lung. Allergen challenge augmented central and peripheral responses, but with marked differences between wild-type, Wsh, and Wsh+MC mice. Compared with Wsh animals, wild-type mice displayed a lower AHR in the Newtonian resistance, indicating that the proximal mast cells in wild-type mice have a dampening role. With diclofenac treatment, the Newtonian resistance in wild-type mice reached the similar level as Wsh mice. Because Newtonian resistance mainly reflects the effect on conducting airways this is probably due to a release of PGE2 relaxing the airway smooth muscle, as suggested by our earlier studies (38, 39). One reason for the lack of effect of diclofenac on Newtonian resistance in the Wsh+MC mice could be that the engraftment does not cause reconstitution of mast cells in the central airways, including trachea (44), where they would be particularly important for this process. Instead, the induced presence of mast cells in the small airways in the Wsh+MC mice may explain the further increase in Newtonian resistance due to a specific augmentation of the resistance in the distal airways. Thus mast cells can have different effects at different sites, as their phenotypes distribute distinctively, as does their COX product release (25a).

Analyzing the peripheral parameters, the increase of tissue resistance and lack of effect on tissue elastance were similar in wild-type and Wsh mice. Thus these data indicate that these parameters were independent of mast cells, since both groups lack mast cells in the lung parenchyma. In contrast, in Wsh+MC mice, OVA challenge caused a further rise of AHR in tissue resistance and was the only group in which an increase of tissue elastance was observed. In contrast to the protective effect on the Newtonian resistance, as discussed above, a COX product released by mast cells has deleterious effect for the tissue elastance. It is possible that this could be due to either PGD2 or TXA2, which are mast cell mediators known to be part of the allergic airway reaction (32).

Thus the engrafted mast cells, which were more abundant and located in the larger and smaller airways and the parenchyma, but probably absent from the trachea (44), caused increases in AHR under allergic conditions in all three parameters. However, it should be noted that parts of the increase in both tissue resistance and elastance can be overestimated due to nonuniform bronchoconstriction in the conducting airways (inhomogeneities) (36), which is not taken into account in the constant-phase model. Nevertheless, the model used in our study demonstrates the existence of a mast cell-independent AHR and a clear increased AHR after mast cell engraftment.

As a result of the sensitization protocol employed, allergen-specific IgE was present in the plasma of all groups of mice, but at markedly higher levels following challenge with OVA. The twofold higher level in Wsh+MC mice might reflect the larger population of mast cells. Spatial contact between mast cells and B cells facilitates maturation of the latter into plasma cells (15), and the Wsh+MC animals may also have elevated numbers of mast cells in their lymph nodes, as described earlier after intravenous transfer of bone marrow-derived mast cells (44). However, the use of alum as an adjuvant is thought to circumvent the contribution of mast cells to the sensitization phase in murine models of asthma (43). Moreover, the absence of mast cells from the Wsh OVA mice was not associated with an altered serum level of IgE. Thus only tentative conclusions concerning the sensitization phase can be drawn from the present model.

Our chronic model displayed an increase of the macrophages, neutrophils, lymphocytes, and eosinophils. Because chronic inflammation of the airways in asthmatic patients is primarily characterized by the presence of mixed population of leukocytes, this model closely resembled the human situation. We also found that Wsh mice had higher number of both macrophages and eosinophils, indicating that the presence of mast cells can reduce the inflammation. Furthermore, the elevated number of neutrophils and relatively low number of eosinophils, which differ from the strong eosinophilic and weak neutrophilic inflammation obtained with shorter protocols (43), may reflect the systemic allergic sensitization and subsequent chronic allergen challenges of the airways (43).

The lack of differences between the challenged groups suggests that the cellular infiltration has little or no influence on AHR as also noted in human asthmatic patients (12, 33). In the human situation, remodeling is a common phenomenon although not always linked to AHR (35). However, despite the challenge with allergen over a period of several months in this study, no increase in the depositions of α-SMA or collagen around the airways was seen in any case. This lack of remodeling, which contrasts with the results by Leigh et al. (24), who designed this protocol, is probably dependent on our use of C57BL/6 mice and their use of BALB/c mice and may limit the use of this protocol. Nevertheless, the results in this study indicate that AHR can be independent of chronic inflammation or alterations in airway architecture.

The inflammatory processes were further characterized by quantification of pulmonary cytokines and chemokines. As in mixed granulocytic types of inflammation, the levels of both Th1 (CXCL1, CXCL10) and Th2 (IL-5)-related inflammatory mediators were elevated in all three allergen-challenged groups. The OVA-induced elevations in the levels of IL-17, a pleiotropic cytokine that is suggested to have a key role in allergic asthma (30), and of IL-33, a proinflammatory cytokine produced primarily by structural cells and facilitating Th2 responses in the lung (25), were mast cell dependent. The
A higher level of IL-17 in wild-type compared with Wsh+/MC mice could be explained by the more pronounced production of IL-23 (41). Since intranasal challenge results primarily in deposition to the upper airways (28), the presence of mast cells at this site in wild-type (16), but not in Wsh+/MC mice (18), might explain why intranasal delivery favors IL-17 production. Hence, the cytokines and chemokines measured in this study could not be linked to the development of AHR in the different groups.

In conclusion, we have demonstrated that within the same protocol of a chronic mouse model of asthma, both mast cell-dependent and independent increases of AHR can be developed. Most importantly, the data indicated a specific connection between mast cell localization and development of AHR in different regions of the lung. Since elevated numbers of mast cells correlate with worsening of pulmonary function in patients (7), the animal model developed here may, both morphologically and functionally, be more similar to humans than those used previously. Since AHR is an important determinant for asthma therapy, the detailed mechanisms underlying the capacity of mast cells at different locations to modulate AHR and inflammation should be the focus of future investigations. However, the data also highlight a mast cell-independent component that induces AHR, which might also be present in people with asthma, which should also be taken into account for optimal treatment strategies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

 ENGRAFTED MAST CELLS INCREASE AIRWAY HYPERRESPONSIVENESS


