

RESEARCH ARTICLE

Mast cell-dependent IL-33/ST2 signaling is protective against the development of airway hyperresponsiveness in a house dust mite mouse model of asthma

A. M. Zoltowska Nilsson,¹ Y. Lei,¹ M. Adner,^{2,3} and G. P. Nilsson^{1,2,4}

¹Immunology and Allergy Unit, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; ²Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden; ³Unit of Experimental Asthma and Allergy Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; and ⁴Department of Medical Sciences, Uppsala University, Uppsala, Sweden

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Zoltowska Nilsson AM, Lei Y, Adner M, Nilsson GP. Mast cell-dependent IL-33/ST2 signaling is protective against the development of airway hyperresponsiveness in a house dust mite mouse model of asthma. *Am J Physiol Lung Cell Mol Physiol* 314: L484–L492, 2018. First published November 16, 2017; doi: 10.1152/ajplung.00270.2017.—Interleukin-33 (IL-33) and its receptor ST2 have been influentially associated with the pathophysiology of asthma. Due to the divergent roles of IL-33 in regulating mast cell functions, there is a need to further characterize IL-33/ST2-dependent mast cell responses and their significance in the context of asthma. This study aimed to investigate how IL-33/ST2-dependent mast cell responses contribute to the development of airway hyperresponsiveness (AHR) and airway inflammation in a mouse model of house dust mite (HDM)-induced asthma. Mast cell-deficient C57BL/6-Kit^{W-sh} (Wsh) mice engrafted with either wild-type (Wsh + MC-WT) or ST2-deficient bone marrow-derived mast cells (Wsh + MC-ST2KO) were exposed to HDM delivered intranasally. An exacerbated development of AHR in response to HDM was seen in Wsh + MC-ST2KO compared with Wsh + MC-WT mice. The contribution of this IL-33/ST2-dependent mast cell response to AHR seems to reside within the smaller airways in the peripheral parts of the lung, as suggested by the isolated yet marked effect on tissue resistance. Considering the absence of a parallel increase in cellular inflammation in bronchoalveolar lavage fluid (BALF) and lung, the aggravated AHR in Wsh + MC-ST2KO mice seems to be independent of cellular inflammation. We observed an association between the elevated AHR and reduced PGE₂ levels in BALF. Due to the protective properties of PGE₂ in airway responses, it is conceivable that IL-33/ST2-dependent mast cell induction of PGE₂ could be responsible for the dampening effect on AHR. In conclusion, we reveal that IL-33/ST2-dependent mast cell responses can have a protective, rather than causative role, in the development of AHR.

AHR; airway hyperresponsiveness; HDM mouse model; IL-33; mast cells; ST2

remodeling, and airway hyperresponsiveness (AHR). The airway epithelium is now recognized as central in the pathogenesis of asthma. Accordingly, it has been proposed that aberrant interactions between epithelial cells and underlying resident cells are fundamental for the development of asthma (15). Several studies support this concept identifying the release of key regulatory cytokines, including IL-18, IL-25, IL-33, and thymic stromal lymphopoietin, from epithelial cells following exposure to several common allergens including house dust mite (HDM), *Aspergillus fumigatus*, and *Alternaria alternate* (1, 9, 13, 20, 21, 27).

Several large-scale genome-wide association studies link polymorphism in the genes for IL-33 (IL33), its receptor (IL1RL1), and the downstream signaling with asthma susceptibility and have advanced considerable interest for IL-33 as a central instigator and attractive candidate for therapeutic intervention of asthma and allergic disease (7, 25, 37). Released as one of the earliest signaling molecules following epithelial damage or stress, IL-33 can orchestrate the recruitment and activation of inflammatory cells involved in asthma. Dysregulated IL-33 activity leads to activation of cells including Th2 cells, ILC2s, mast cells, dendritic cells, and eosinophils that express the IL-33 receptor ST2, subsequently leading to increased expression of cytokines and cellular recruitment that define the disease (6, 23).

Mast cells are recognized as tissue-resident cells that are involved in allergic disorders including asthma (8, 10, 38). IL-33 is a crucial regulator of mast cell functions (2, 9, 16, 19, 31), being able to activate mast cells to release several cytokines associated with asthma, including IL-5, IL-13, and CXCL8 (2, 16). In addition, IL-33 promotes survival, maturation, and adhesion to fibronectin of mast cells (16), increases tryptase expression (19), modulates degranulation (17), and increases synthesis, storage, and secretion of serotonin leading to exacerbations of the early allergic bronchoconstriction (33).

We have previously shown that ST2-deficient BALB/c mice are protected against the development of peripheral AHR and lung inflammation in a HDM model of allergic asthma (42). Based on these findings and previous studies implicating mast cells in the ST2/IL-33 axis (9), we sought to investigate the role of mast cells in this setting and hypothesized that IL-33/ST2-dependent mast cell responses would contribute to the development of AHR and inflammation. In this study we reveal

INTRODUCTION

Allergic asthma is a complex, multifactorial airway disorder that is characterized by chronic inflammation, airway wall

Address for reprint requests and other correspondence: G. P. Nilsson, Dept. of Medicine, Solna, Immunology and Allergy Unit, Karolinska Institutet, KS L2:04, 171 76 Stockholm, Sweden (e-mail: gunnar.p.nilsson@ki.se).

a protective, rather than a causative, role for IL-33/ST2-dependent mast cell responses on AHR that originates from the peripheral lung compartment, which seems to be independent of cellular inflammation yet associated with elevated PGE₂ levels.

MATERIALS AND METHODS

Allergens and reagents. HDM extract (*Dermatophagoides pteronyssinus*; kindly provided by ALK, Copenhagen, Denmark) was reconstituted in sterile PBS. The endotoxin level in the HDM extract was 2 EU/mg protein as determined by the *Limulus* Amoebocyte Lysate Assay (Charles River Endosafe, Charleston, SC). Acetyl- β -methacholine (Sigma-Aldrich) was dissolved in sterile PBS.

Mice. Female mast cell-deficient C57BL/6-Kit^{W-sh} (Wsh) mice at 6–12 wk of age were housed with a 12-h light-dark cycle and were given food and water ad libitum. All animal handling and experimentation were conducted in accordance with ethical permits approved by the Regional Committee of Animal Experimentation Ethics (Stockholm, Sweden).

Mast cell engraftment and intranasal exposure to HDM. Age-matched Wsh mice were engrafted by intravenous injection of congenic mast cells ($5 \times 10^6/100 \mu\text{l}$) derived from the bone-marrow of 6-wk-old wild-type C57BL/6 mice (Wsh + WT MC) or ST2^{-/-} (Wsh + ST2KO MC) mice on the C57BL/6 background, as previously described (11). Preceding the engraftment, bone marrow-derived mast cells (BMMCs) were confirmed by positive tryptase staining and a dual staining for KIT/CD117 and Fc ϵ RI by flow cytometry. The presence and absence of the ST2 receptor were evaluated by flow cytometry in the wild-type and ST2^{-/-} BMMC populations. After 12 wk were allowed for lung engraftment, these mice were then subjected to a 43-day protocol designed to produce chronic lung inflammation through repeated intranasal exposure to HDM (25 $\mu\text{g}/20 \mu\text{l}$ PBS) or equivalent volume of PBS (Fig. 1A) while mice were under light anesthesia with isoflurane (3.5% isoflurane, Forene; Abbot Scandinavia, Solna, Sweden). Two days following the final HDM administration, airway responses were measured and bronchoalveolar lavage fluid (BALF) and lung tissue were collected.

Pulmonary mechanics. Mice were anesthetized by intraperitoneal injections of pentobarbital (70–90 mg/kg body wt; Apoteket Produktion Laboratorier, Stockholm, Sweden), tracheotomized with a blunted 18-gauge needle, and ventilated (Scireq, Montreal, Canada). Ventilation was performed at 2.5 Hz in a quasisinusoidal fashion to generate a sinusoidal pressure waveform during lung inflation. The tidal volume was set at 12 ml/kg body wt with a positive end-expiratory pressure of 3 cmH₂O. A bilateral thoracotomy was performed to equalize pleural pressure and to eliminate the possibility of spontaneous chest wall movements from interfering with lung mechanics measurements. Airway responses were then measured to increasing doses (0, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg body wt) of methacholine delivered intravenously. Respiratory impedance was measured using the forced oscillation technique, and the obtained data were fitted to the constant phase model where Newtonian resistance R_N reflecting the resistance of the conducting airways, G characterizing tissue dampening, and H characterizing tissue stiffness or tissue elastance were determined (5).

Tissue collection and preparation. BALF was collected following pulmonary mechanics. The left lung lobe was removed and fixed in 4% formaldehyde overnight (Histolab, Stockholm, Sweden). The superior and middle lobes of the right lungs were snap-frozen on dry ice and stored at -80°C awaiting further analysis. Protein was extracted from the snap-frozen samples of lung tissue with a cell lysis kit (Bio-PlexTM Cell Lysis Kit; Bio-Rad, Hercules, CA) and supplementary proteinase inhibitors (Sigma-Aldrich, Stockholm, Sweden). Right and left lung lobes were collected for flow cytometric analysis.

Characterization of inflammation in BALF. The total number of inflammatory cells present in BALF was determined following Turk's solution staining (Histolab Products, Gothenburg, Sweden) using a hemocytometer (FastRead102; Immune Systems, Paignton, UK). Cytospin centrifugation was performed by plating $\sim 50,000$ cells/sample on a glass slide. Cells were then stained by May-Grunwald and Giemsa (Sigma-Aldrich), and differential cell counts were done by counting 300 cells/sample (34).

Mast cell counts in lung sections. Following formaldehyde fixation of the left lung lobe as stated in *Tissue collection and preparation*, the lobe was dehydrated and embedded in paraffin and 5- μm -thick paraffin section were made in the coronal plane to visualize the airway tree. The sections were mounted on glass slides and deparaffinized. Mast cells were visualized by staining with 1% toluidine blue (Sigma-Aldrich) followed by fast green counterstaining (Polysciences, Warrington, PA) and counted in three to six sections per lung, derived from three cross-sectional locations.

ELISA. The cytokines IL-5, IL-13, and IL-33 were quantified in lung homogenates and mast cell protease mMCP-1 in BALF by ELISA kits (eBioscience, San Diego, CA) according to the instructions of the manufacturer. PGE₂ was measured in BALF by an ELISA kit (Cayman Chemical).

Surface staining and flow cytometric analysis. Single-cell suspensions of lung tissue were prepared from right and left lung lobes by manually disrupting the tissue with scalpels and incubating the lung segments in RPMI medium containing collagenase D (0.75 mg/ml working concentration; Roche, Mannheim, Germany) at 37°C for 30 min on shaker. After this initial incubation, detached cells were isolated from the supernatant while the undegraded tissue was again subjected to digestion as stated above. The remaining tissue was then homogenized manually over a 70- μm cell strainer and, the cell fractions were pooled and red blood cells lysed using ACK buffer. Cells were surface stained with fluorescently labeled antibodies to CD3, CD4, CD8a, CD11b, Gr-1, B220, CD11c, Siglec-F, NK1.1, F4/80, CD19, ST2, and ICOS as described in *Antibodies*. FMO controls including matching isotype controls for ST2 and ICOS were used. Samples were analyzed using LSRFortessa cytometer (BD Biosciences, Franklin Lakes, NJ) Flow cytometric data were analyzed using the FlowJo software (Tree Star Inc., Ashland, OR). ILC2 cells were characterized as SSC^{low}FSC^{low}Lineage⁻ICOS⁺ST2⁺ cells (24). Markers in the lineage cocktail consisted of CD3, CD4, CD8a, CD11b, Gr-1, B220, CD11c, Siglec-F, NK1.1, F4/80, and CD19. Alveolar macrophages were identified as SiglecF⁺CD11c⁺, eosinophils as SiglecF⁺CD11c⁻CD11b⁻Gr-1^{lo}, neutrophils as SiglecF⁻CD11c⁻CD11b^{hi}Gr-1^{hi}, and lymphocytes (B/T cells) as CD19⁺CD3⁺ (24).

Antibodies. Cells were pretreated with anti-CD16/32 (BD PharMingen) before staining to prevent unspecific Fc-binding of antibodies. ILC2 cells were identified by the following antibodies: PE-conjugated anti-lineage antibodies CD3 (clone 17A2), CD4 (clone RM4-4), CD8a (clone 53-6.7), CD11b (clone M1/70), Gr-1 (clone RB6-8C5), CD11c (clone N418), Siglec-F (E50-2440); APC-Cy7-conjugated lineage antibodies CD19 (clone 6D5), NK1.1 (PK136), and F4/80 (T45-2342); ICOS conjugated to Alexa Fluor 647 (clone C398.4A); and FITC-conjugated T1/ST2 (clone DJ8). Macrophages, eosinophils, neutrophils, and lymphocytes were characterized using PE-conjugated Siglec F (clone E50-2440), PB-conjugated CD11c (N418), FITC-conjugated Gr-1 (RB6-8C5), APC-Cy7-conjugated F4/80 (T45-2342), APC-conjugated CD11b (clone M1/70), and PECy7-conjugated CD19 (clone 6D5) and CD3 (17A2). The antibodies were purchased from Biolegend (San Diego, CA), BD Biosciences, eBiosciences (Hatfield, UK), or MD Bioproducts (Zurich, Switzerland).

Statistical analysis. Statistical analyses were performed using GraphPad Prism, version 5.0 (GraphPad Software, San Diego, CA). Results are expressed as means $\pm$ SE. ANOVA followed by Bonfer-

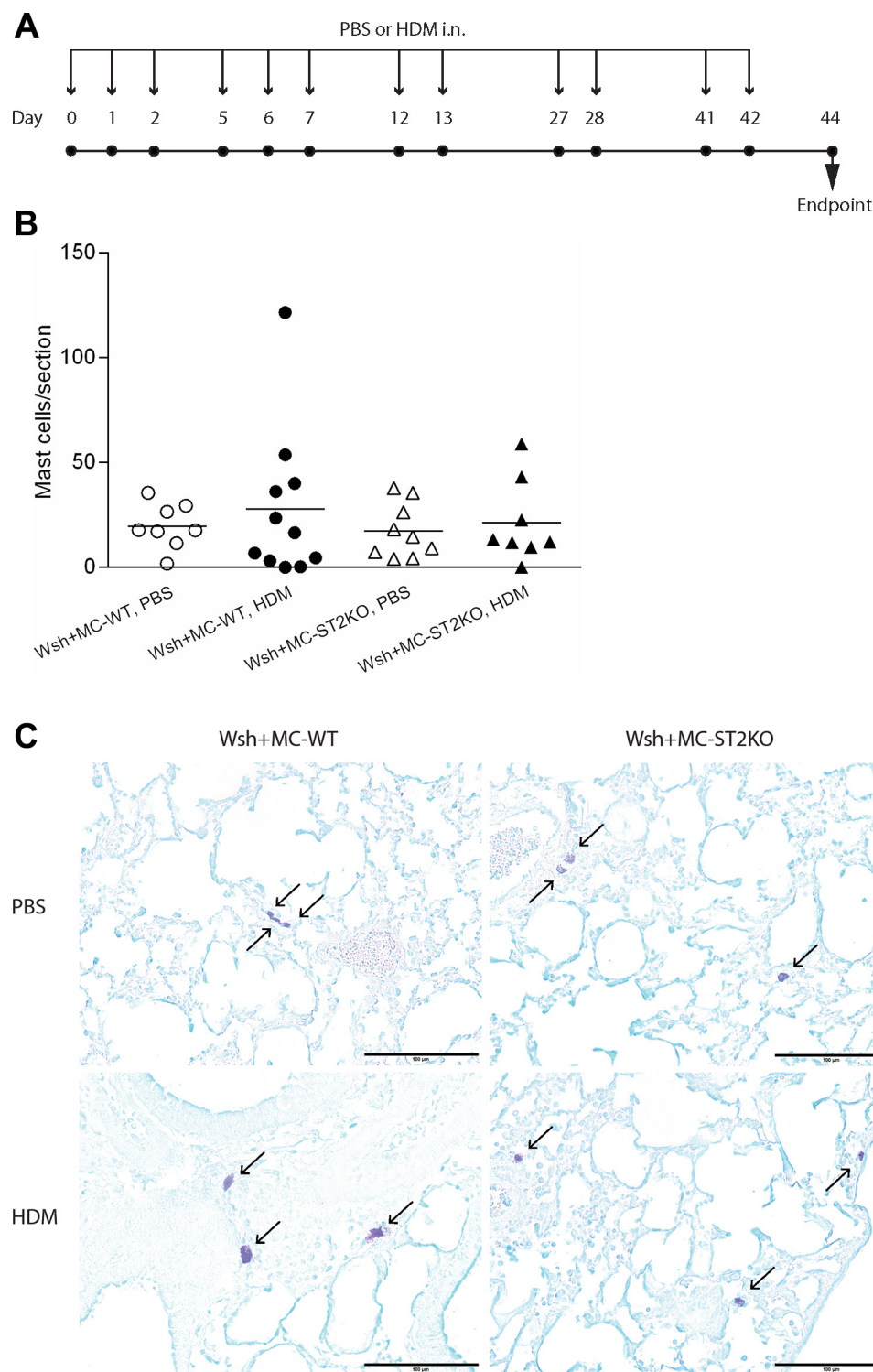


Fig. 1. Mast cell-deficient Wsh-mice are engrafted with wild-type (WT) and ST2KO bone marrow-derived mast cells (BMMCs). **A**: schematic representation of the exposure protocol. Wsh-mice were engrafted with congenic WT (Wsh + MC-WT) or ST2-deficient (Wsh + MC-ST2KO) bone marrow-derived mast cells (BMMCs). Following 12 wk of engraftment, mice were exposed to intranasal house dust mite (HDM) extract (25 μ g/20 μ l) or equivalent volume of PBS. **B**: mast cell engraftment of lung tissue evaluated at day 44. Each point represents the mean of mast cell counting from 3 to 6 lung sections. Results are expressed as means of $n = 8$ –11 mice in each group. **C**: representative toluidine blue-staining of lung sections from the different groups of mice showing mast cell distribution within the tissue. Mast cells are mainly located within the alveolar parenchyma. Arrows indicate mast cells, and the scale bar = 100 μ m.

roni's post correction was used to compare differences between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Mast cell ST2 expression protects against HDM-induced development of airway hyperresponsiveness in the peripheral lung. To investigate the role of IL-33/ST2-dependent mast cell responses on AHR and inflammation, mast cell-deficient

C57BL/6-Kit^{W-sh} (Wsh) mice were reconstituted with either wild-type (Wsh + MC-WT) or ST2-deficient (Wsh + MC-ST2KO) mast cells. Allowing 12 wk for lung engraftment, the mice were subsequently subjected to a HDM protocol (Fig. 1A). Mast cell engraftment of the lung compartment was confirmed and limited to the peripheral lung, predominantly to the alveolar parenchyma and small airways, while mast cells were absent around the central airways (Fig. 1, B

and C). The engraftment was similar in all the groups investigated with respect to numbers as well as tissue location.

AHR was assessed using a forced-oscillation technique, and the obtained data were fitted to the constant-phase model, allowing a separation of events taking place in the conducting airways and peripheral lung. Mast cell-engrafted Wsh-mice with either wild-type (Wsh + MC-WT) or ST2-deficient mast cells (Wsh + MC-ST2KO) subjected to intranasal treatment with PBS exhibited the same conducting airway resistance (Newtonian resistance R_N), tissue resistance (G), and tissue elastance (H) upon intravenous exposure to increasing doses of methacholine (Fig. 2). Intranasal exposure to HDM increased the Newtonian resistance (R_N) in Wsh + MC-WT as well as Wsh + MC-ST2KO mice (Fig. 2A). However, HDM exposure did not induce an increase in tissue resistance (G) in Wsh + MC-WT mice, which is in contrast to the significant development of tissue resistance in Wsh + MC-ST2KO mice (Fig. 2B). There is a tendency to increased tissue elastance (H) in the HDM-exposed Wsh + MC-ST2KO group compared with Wsh + MC-WT group (Fig. 2C), although not statistically significant ($P = 0.07$).

Similar cellular inflammation in BALF and lung tissue of Wsh mice engrafted with either ST2-expressing or ST2-deficient mast cells. We next investigated the presence of inflammatory cells in BALF and lung tissue. HDM exposure led to a similar induction of inflammatory cells in BALF in Wsh + MC-WT and Wsh + MC-ST2KO mice compared with their respective PBS control (Fig. 3A). The inflammatory cells that were predominantly increased were eosinophils and lymphocytes (Fig. 3B). However, there was no difference in cellular composition in BALF between HDM exposed Wsh + MC-WT and Wsh + MC-ST2KO (Fig. 3B). Flow cytometry was used to assess the inflammation in lung tissue where alveolar macrophages were identified as SiglecF⁺CD11c⁺, eosinophils as SiglecF⁺CD11c⁻CD11b⁻Gr-1^{lo}, neutrophils as SiglecF⁻CD11c⁻CD11b^{hi}Gr-1^{hi}, and lymphocytes (B/T cells) as CD19⁺CD3⁺ (Fig. 4A). The pulmonary inflammation was characterized by an increased frequency of eosinophils in both Wsh + MC-ST2KO and Wsh + MC-WT mice exposed to HDM compared with their respective PBS controls (Fig. 4B). ILC2s were identified as SSC^{low}FSC^{low}Lineage⁻ICOS⁺ST2⁺ cells (Fig. 4C). Examination of ILC2 frequency in the lung did not reveal any significant differences between any of the experimental groups (Fig. 4D).

ST2 deficiency in mast cells has limited effect on the levels of IL-5, IL-13, and IL-33 or mast cell protease mMCP-1. The levels of IL-5 and IL-33 in lung homogenates were significantly elevated following HDM exposure in both groups, although no difference was observed between the HDM treated Wsh + MC-ST2KO and Wsh + MC-WT mice (Fig. 5A). IL-13 increased in the lung homogenates of Wsh mice engrafted with mast cells with a functional ST2 receptor (Wsh + MC-WT) but did not reach significance in Wsh mice engrafted with ST2-deficient mast cells (Wsh + MC-ST2KO) following HDM administration (Fig. 5A). The levels of mast cell mediator mMCP-1 were not affected by HDM in any group (Fig. 5A).

Mast cell ST2 expression is important for PGE₂ generation in response to HDM. Previous studies have implicated PGE₂ to have a protective role in the development of AHR (28, 29, 34), which led us to conceive the idea that PGE₂ could contribute to the attenuated development of tissue resistance through mast

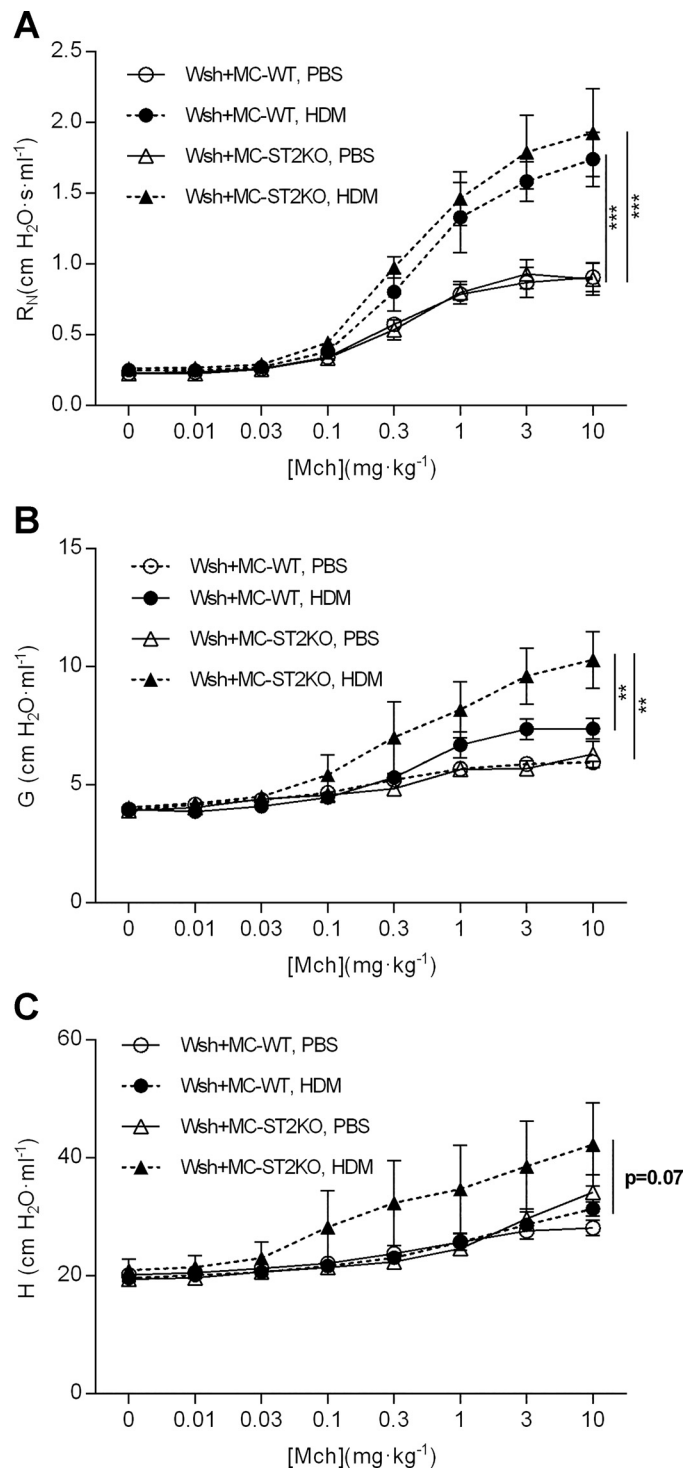


Fig. 2. Mast cell dependent IL-33/ST2 signaling protects against the development of airway hyperresponsiveness. Airway hyperresponsiveness (AHR) in response to intravenously delivered methacholine (Mch) was assessed in intubated and ventilated Wsh mice (flexiVent ventilator) engrafted with either wild-type (Wsh + MC-WT) or ST2-deficient (Wsh + MC-ST2KO) mast cells and exposed to HDM extract or PBS. A: Newtonian resistance (R_N) of the central airways, tissue damping (G) (B), and tissue elastance (H) (C). ** $P < 0.01$, *** $P < 0.001$ (ANOVA, Bonferroni). Results are expressed as means $\pm$ SE of $n = 8$ –11 mice in each group.

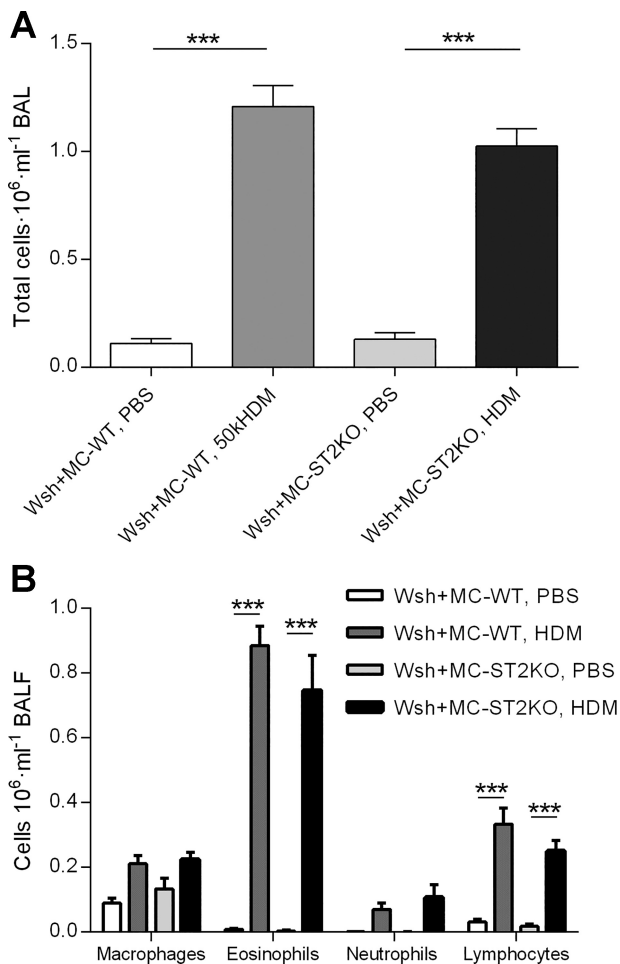


Fig. 3. Cellular inflammation in bronchoalveolar lavage fluid (BALF). Total cell count (A) and differential count of leukocytes (B) in BALF in Wsh mice engrafted with either wild-type (Wsh + MC-WT) or ST2-deficient (Wsh + MC-ST2KO) mast cells and exposed to HDM extract or PBS. *** $P < 0.001$ (ANOVA, Bonferroni). Results are expressed as means $\pm$ SE of $n = 8$ –11 mice in each group.

cell-dependent IL-33/ST2 signaling that we observed in our model. Indeed, there was an association between attenuated tissue resistance G (Fig. 2B) and elevated PGE_2 BALF levels in Wsh mice engrafted with mast cells expressing the ST2 receptor (Wsh + MC-WT) (Fig. 5B).

DISCUSSION

This study reveals a protective role for IL-33/ST2-dependent mast cell responses in the development of AHR that appears to be independent of cellular inflammation. Instead, the attenuated AHR could be mediated by other mechanisms including the generation of bronchoprotective PGE_2 .

The exacerbated development of peripheral AHR in response to HDM exposure in Wsh + MC-ST2KO compared with Wsh + MC-WT suggests an important protective role for IL-33/ST2-dependent mast cell responses in our model. The absence of an effect on AHR in the conductive airways could possibly be explained by that engraftment does not cause reconstitution of mast cells at this location where they could take part in this process. This lack of mast cells in the conductive airways after engraftment of Wsh mice is in accor-

dance with previous studies (11, 39). Instead, AHR of the peripheral lung is affected in this model, consistent with the increase of mast cells in the lung and location in the small airways and parenchyma after engraftment (11). This is an altered mast cell distribution compared with wild-type mice that primarily have mast cells in the larger airways and the trachea while mast cells are extremely rare in the peripheral lung (12, 22, 40). Thus, the distribution of mast cells in mouse differ both in wild type and after mast cell engraftment to the human lung, where mast cells are more widely distributed and can be found throughout the conductive airways and in the peripheral lung including the lung parenchyma (3, 22).

While IL-33 activation of mast cells has mainly been implicated in enhancing airway and inflammatory responses by inducing the release of proinflammatory mediators and enhancing the capacity of antigen to elicit this response (9, 16, 33), some studies suggest IL-33 mediated mast cell involvement to be of protective nature (4, 18, 26). One study showed that mast cells can be rendered hyporesponsive by long-term exposure to IL-33 and proposed that elevated levels of IL-33 in asthmatic lungs rather than contributing to the disease may indeed be protective by dampening mast cell responses (18). Prolonged exposure to IL-33 is also characteristic of the current study where repetitive exposure to HDM induced significant IL-33 levels in the lungs of Wsh + MC-WT as well as Wsh + MC-ST2KO mice. Thus the protective effect on AHR could be explained by repetitive IL-33 exposure rendering mast cells with a functional IL-33 receptor less responsive.

We did not observe any significant reduction in cellular infiltration into the BALF and lung accompanying the diminished peripheral AHR in HDM-exposed Wsh + MC-WT and Wsh + MC-ST2KO mice, which suggests a mechanism independent of the accumulation of inflammatory cells in these compartments. Dissociation between airway inflammation and AHR has been reported previously in a study where cyclooxygenase-1 inhibition during ovalbumin challenge enhanced AHR without affecting the inflammatory cell response, suggesting that AHR and inflammatory response can occur independently (35). The enhancing effect of cyclooxygenase-1 inhibition on AHR in that study was associated with attenuated levels of PGE_2 . The exacerbated AHR that we observed in the present study in Wsh mice engrafted with ST2-deficient mast cells and exposed to HDM could thus be explained by a reduction in PGE_2 . Indeed, PGE_2 levels were markedly lower in the BALF of the mice showing enhanced AHR. A protective role of PGE_2 has also been reported in asthma patients and inhalation of PGE_2 prevents allergen-induced early and late airway responses after allergen challenge (14). The protective response on AHR mediated by PGE_2 could be explained both in terms of inhibition of the airway smooth muscle constriction and suppression of mast cell mediators (14) or by a yet not identified mechanism. The question remains as to what mechanism mediates the elevated PGE_2 levels in our model. One possibility is that IL-33 induces PGE_2 synthesis and release directly from mast cells or through an intermediate mast cell mediator acting on a PGE_2 -releasing target cell. Elevated PGE_2 levels could in turn have a direct relaxing effect on the airway smooth muscle and/or an inhibitory effect on mast cell activity through the PGE_2 -mast cell axis subsequently diminishing AHR (30, 32). Based on in vitro studies where we stimulated BMMCs from wild-type animals with IL-33 before IgE cross

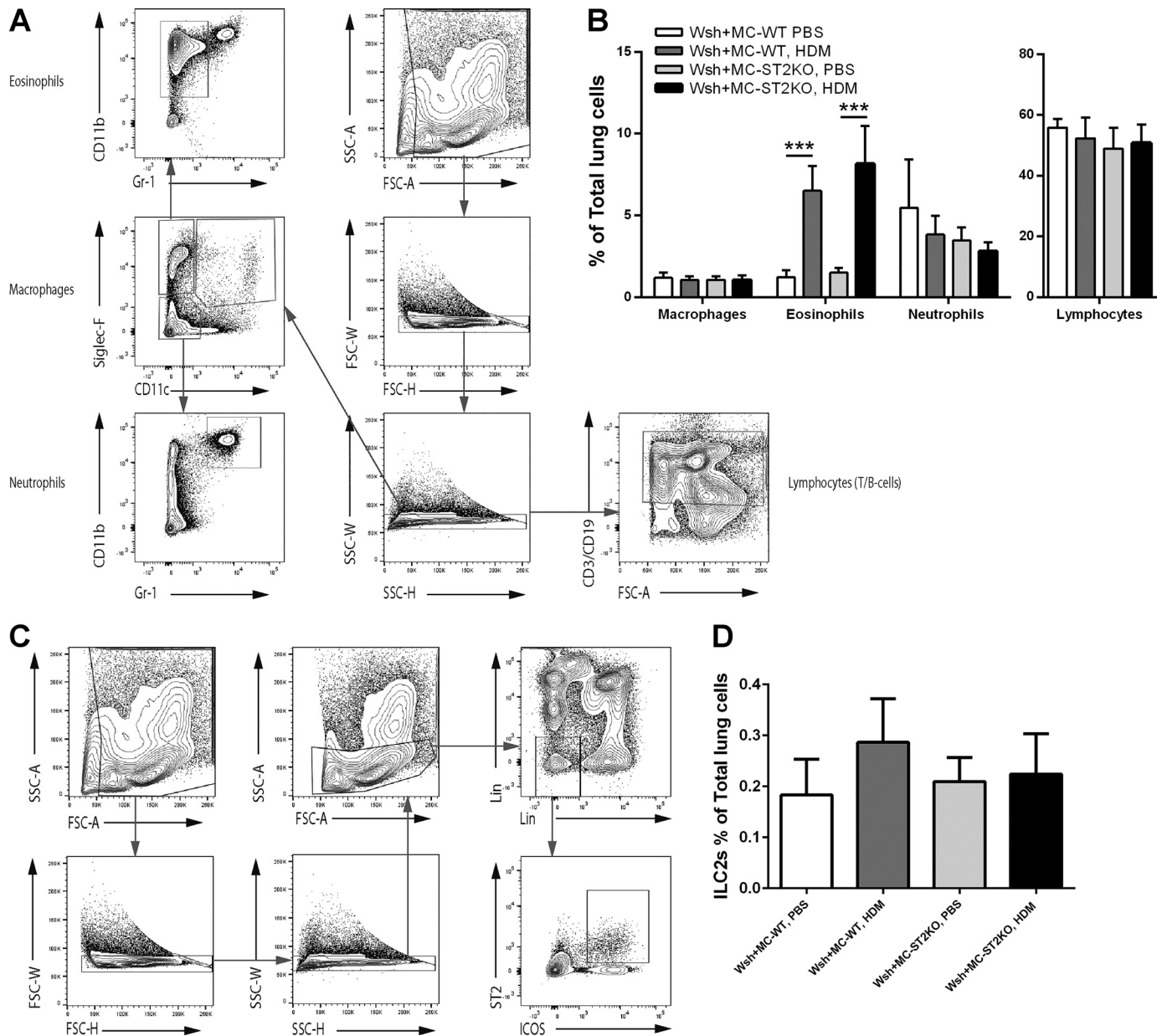


Fig. 4. Inflammatory cells in the lung. Cellular inflammation in lung tissue was evaluated using flow cytometry of Wsh mice engrafted with either wild-type (Wsh + MC-WT) or ST2-deficient (Wsh + MC-ST2KO) mast cells and exposed to HDM extract or PBS. **A**: gating strategy for alveolar macrophages, eosinophils, neutrophils and lymphocytes. Alveolar macrophages were identified as SiglecF⁺CD11c⁺, eosinophils as SiglecF⁺CD11c⁺CD11b⁺Gr-1⁺, neutrophils as SiglecF⁺CD11c⁺CD11b⁺Gr-1⁺, and lymphocytes (B/T cells) as CD19⁺CD3⁺. **B**: alveolar macrophages, eosinophils, neutrophils, and lymphocytes expressed as frequencies of total lung cells. **C**: gating strategy for identification of ILC2s. ILC2s were identified as SSC^{low}FSC^{low}Lineage⁻ICOS⁺ST2⁺ cells. **D**: ILC2s expressed as frequency of total lung cells. ****P* < 0.001 (ANOVA, Bonferroni). Results are expressed as means ± SE of *n* = 5 mice in each group.

linking and subsequently measured PGE₂ in the cell supernatants, mast cells do not seem to constitute a significant source of PGE₂ in this setting (data not shown). Nevertheless, this did not exclude the possibility that IL-33 together with other costimulatory signals could induce PGE₂ release from mast cells in vivo. We therefore engrafted Wsh mice with BMMCs derived from prostaglandin E synthase 1 (mPGES-1)-deficient mice and subjected the Wsh mice to the HDM protocol. The results showed that mPGES-1 deficiency in mast cell did not affect the PGE₂ levels in BALF after HDM exposure suggesting, in line with the in vitro studies, that mast cells are not the

primary source of PGE₂ (data not shown). Thus it remains elusive as to which cell or cells are the PGE₂ producer following IL-33 exposure.

The levels of the mast cell mediator mMCP-1, indicating mast cell degranulation, were not altered in any of the groups investigated in this study suggesting that the ST2-dependent protective mechanism does not require mast cell degranulation to exert its effector function. HDM did increase the IL-13 level in Wsh + MC-WT while this effect did not reach significance in Wsh + MC-ST2KO mice. Although IL-13 is usually associated with increased AHR (36), IL-13

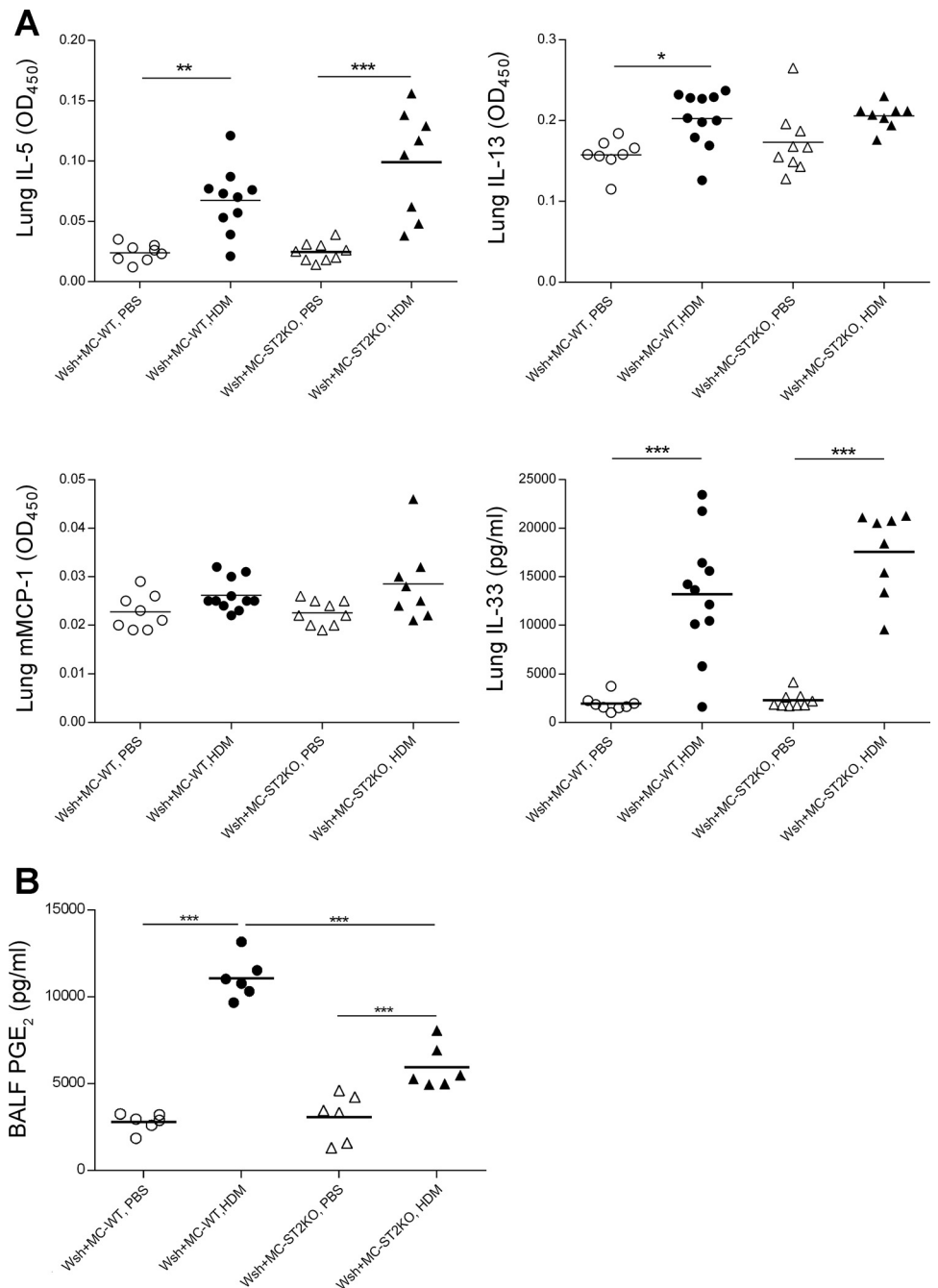


Fig. 5. Inflammatory mediators. Inflammatory mediators were measured in BALF or lung homogenates of Wsh mice engrafted with either wild-type (Wsh + MC-WT) or ST2-deficient (Wsh + MC-ST2KO) mast cells and exposed to HDM extract or PBS. A: levels of the cytokines IL-5, IL-13, IL-33 and mast cell mediator mMCP-1 were measured in lung homogenates. B: PGE₂ levels were determined in BALF. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (ANOVA, Bonferroni). Results are expressed as means $\pm$ SE of $n = 6$ –11 mice in each group.

has also been shown to induce PGE₂ levels in neutrophils (41) and may provide a potential source for the elevated PGE₂ levels mediated by IL-33/ST2-dependent mast cell responses in our model.

In conclusion, we have revealed a protective role for IL-33/ST2-dependent mast cell responses in the development of AHR in the peripheral lung that seems to be independent of cellular inflammation yet is associated with elevated PGE₂ levels. More studies are needed to understand the mechanisms underlying the observed effects.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.A. and G.P.N. conceived and designed research; A.M.Z.N. and Y.L. performed experiments; A.M.Z.N. and Y.L. analyzed data; A.M.Z.N., Y.L., M.A., and G.P.N. interpreted results of experiments; A.M.Z.N. and Y.L. prepared figures; A.M.Z.N. drafted manuscript; A.M.Z.N., Y.L., M.A., and G.P.N. edited and revised manuscript; A.M.Z.N., Y.L., M.A., and G.P.N. approved final version of manuscript.

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