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Interleukin-33 exacerbates allergic bronchoconstriction in the mice via activation of mast cells

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Abstract

Background: Interleukin-33 (IL-33) is implicated as an epithelium-derived danger signal promoting Th2-dependent responses in asthma. We hypothesized that IL-33 might also have direct effects on mast cell-driven allergic airway obstruction.

Methods: The effects of IL-33 on allergic responses in the airways of sensitized mice were assessed both *in vivo* and *ex vivo*, as well as on cultured mast cells *in vitro*.

Results: *In vivo*, the allergen-induced increase in resistance in the conducting airways was enhanced in mice pretreated with IL-33. Also, in the isolated airways, the allergen-induced contractions were increased in preparations from animals subjected to intranasal IL-33 pretreatment. These effects *in vivo* and *ex vivo* were blocked by the 5-HT_{2A} receptor antagonist ketanserin and absent in mice without mast cells. Likewise, the IL-33-induced enhancement of the allergen response was absent in isolated airways from mice lacking the IL-33 receptor. Moreover, exposure to IL-33 increased secretion of serotonin from allergen-challenged isolated airways. In cultured mast cells, IL-33 enhanced the expression of tryptophan hydroxylase 1, serotonin synthesis, and storage, as well as the secretion of serotonin following IgE receptor cross-linking.

Conclusion: These results demonstrate that IL-33 exacerbates allergic bronchoconstriction by increasing synthesis, storage, and secretion of serotonin from the mast cell. This mechanism has implications for the development of airway obstruction in asthma.

Asthma is a chronic inflammatory airway disease, associated with episodic airflow obstructions. Allergic asthma is characterized by Th2 inflammation, allergen-specific IgE, and mast cell involvement (1). The first response in allergic asthma is the early allergic reaction in which mast cells degranulate following IgE-mediated cross-linkage of FcεRI receptors (2). Upon degranulation, mast cells secrete a host of mediators that can cause life-threatening airway narrowing through direct smooth muscle constriction.

Cells lining the airways have the capacity to release different molecules that initiate protective pro-inflammatory responses (3). Such activation of distinct cellular pathways may be triggered by allergens as well as by a variety of irritants, pathogens, and mechanical stress. One such mediator of specific interest in asthma is interleukin-33 (IL-33) (4). IL-33 belongs to the interleukin-1 family of cytokines and

signals via its receptor ST2, along with the coreceptor IL-1RAcP (5). IL-33 has been most studied in the context of a Th2 response (5), but the responses can include other cell types and cytokines than those traditionally associated with Th2 responses (6, 7). In subjects with asthma, an increased expression and release of IL-33 in the airway epithelium and smooth muscle have been correlated with disease severity (8, 9). Further indications for a key role of IL-33 in the pathogenesis of asthma are recent findings from large genome-wide association studies that strongly link IL-33, as well as its receptor ST2, to asthma development and severity (10–12). However, it is not known whether IL-33 may also directly affect airway smooth muscle responsiveness, one of the fundamental components in asthma.

We tested the hypothesis that IL-33 modulates the allergic airway obstruction in acute mouse models where its mode of

action could be assessed without confounding chronic inflammatory changes. Accordingly, the early allergic reaction was investigated by measuring lung mechanics *in vivo*. Smooth muscle contractions and secretion of mast cell granular contents were also assessed *ex vivo* in isolated airways. The direct effect of IL-33 was examined *in vitro* on cultured mast cells.

Materials and methods

Mice

C57BL/6 and BALB/c mice were purchased from Charles River (Sulzfeld, Germany). Genetically mast cell-deficient C57BL/6-Kit^{W-sh/W-sh} (sash; kindly provided by P. Besmer, Memorial Sloan-Kettering Cancer Center, New York, NY, USA) and BALB/c-T1/ST2-deficient mice (ST2^{-/-}; kindly provided by A.N.J. McKenzie, University of Cambridge, Cambridge, UK) were age- and gender-matched in each experiment. All animal handling was conducted in accordance with ethical permits approved by the Regional Committee of Animal Experimentation Ethics (Stockholm, Sweden).

In vivo allergic sensitization and IL-33 administration

Mice were sensitized with 1 µg ovalbumin (OVA) emulsified in 2.6 mg aluminum hydroxide by intraperitoneal injection on days 0, 2, and 4. Mice received recombinant mouse IL-33 (200 ng in 20 µl) via intranasal instillation on days 10–13. Experiments were performed on day 14. Ovalbumin-specific IgE levels were measured using an ELISA kit (Labor Diagnostika, Nordhorn, Germany).

In vivo measurement of pulmonary mechanics during the early allergic reaction

Mice were anesthetized with subcutaneous injection with a combination of Hypnorm (2.5 µl/g; VetaPharma Ltd, Leeds, UK) and midazolam (12.5 µg/g; Hameln Pharmaceuticals GmbH, Hameln, Germany), and lung mechanics were assessed as described earlier (13). Newtonian central airway resistance (*Rn*), tissue damping (*G*), and tissue elastance (*H*) were measured assuming the constant phase model (14). To measure the response to the early allergic reaction, OVA-sensitized mice were injected with saline followed by 200 mg/kg OVA intravenously. To block the response to secreted serotonin, mice in one of the groups were pretreated with 8 mg/kg ketanserin given intraperitoneally 30 min prior to anesthesia [modified from (15)].

Ex vivo measurements of airway smooth muscle contraction

Tracheae and main bronchi were dissected and mounted in an organ bath according to previously described methodology (16). OVA (100 µg/ml) was added followed by increasing concentrations of serotonin in the absence or presence of the 5-HT_{2A} receptor antagonist ketanserin. At the end of the

experiments, the tissues were challenged with increasing concentrations of carbachol in the presence of 3 µM indomethacin.

Ex vivo measurement of β-hexosaminidase and serotonin secretion

Tracheae were placed in buffer solution in a 96-well plate, and mast cells were activated with 100 µg/ml OVA for 30 min at 37°C. β-hexosaminidase was quantified by spectrophotometry at an absorbance of 405 nm and serotonin secretion with an ELISA kit (Labor Diagnostika).

Investigation of mast cell numbers

Mast cells were counted in histologic sections taken from evenly distributed positions throughout the length of the trachea.

IL-33 incubation and measurement of serotonin and β-hexosaminidase secretion from mast cells

Bone marrow-derived mast cells (17) were sensitized by adding 100 ng/ml anti-DNP IgE to the culture media at both 96 and 24 h prior to IgE receptor activation. Cells were also exposed to either PBS or IL-33 for four consecutive days prior to IgE receptor activation. To measure IgE receptor cross-linking-mediated serotonin secretion from mast cells, DNP albumin was added to the cell suspensions for 30 min. β-hexosaminidase and serotonin secretion were quantified as described above.

Quantitation of gene expression related to serotonin levels in mast cells

Real-time quantitative PCR was performed using TaqMan Gene Expression MasterMix (Applied Biosystems, Foster City, CA, USA) and TaqMan primers for *tryptophan hydroxylase 1* (*Tph-1*). Quantitation was calculated using the comparative *C_T* ($2^{-\Delta\Delta C_T}$) method and normalized to the expression of housekeeping genes.

Statistical analysis

Statistical analyses were performed using GRAPH PAD PRISM, version 5.01 (GraphPad Software, San Diego, CA, USA). Results are expressed as mean ± SEM, and the type of statistical analysis employed is stated in each figure legend.

Results

IL-33 increases central airway resistance during the early allergic reaction in a mast cell- and serotonin-dependent manner

To investigate whether IL-33 affects airflow obstructions *in vivo*, lung mechanics during the early allergic reaction were studied in OVA-sensitized mice. Intravenously injected OVA

caused a distinct peak in Newtonian resistance (R_n) (Fig. 1A, D), but not in tissue damping (G) or tissue elastance (H) (Fig. 1B–C,E–F). Intranasal instillations of IL-33 for four consecutive days before the OVA challenge increased the maximal response of R_n in OVA-sensitized mice compared to OVA-sensitized mice that received intranasal PBS (Fig. 1A, D). The increase in R_n in response to OVA challenge was absent in sham-sensitized mice and abolished in mast cell-deficient sash mice. The 5-HT_{2A} receptor antagonist ketanserin markedly reduced the response to OVA in mice treated with intranasal instillations of IL-33. With respect to the immune response, intranasal administration of IL-33 in OVA-sensitized mice did not result in any significant differences in OVA-specific serum IgE (PBS: 648 ± 84 ; IL-33: 533 ± 78 ng/ml; $N = 10$).

IL-33 amplifies allergen-induced smooth muscle contraction through increased release of serotonin from mast cells in the airways

The direct response of airway smooth muscle to OVA was studied *ex vivo* using isolated airway segments from OVA-sensitized mice. In tracheae, OVA induced a contraction of $13 \pm 2.3\%$ of the maximal contraction to carbachol (Fig. 2A). In control experiments, neither bovine serum albumin,

given before OVA to detect nonspecific reactions, nor tracheal segments taken from sham-sensitized mice and exposed to OVA resulted in smooth muscle contraction. Intranasal administration of IL-33 for four consecutive days before isolation of the airways led to an increased smooth muscle contraction to OVA with a more than 50% increase in response ($20 \pm 2.4\%$) at the highest dose of IL-33 (Fig. 2A). This increased contraction to OVA was observed in tracheal segments from both C57BL/6 and BALB/c mice (Fig. 2B) and in bronchial segments from BALB/c mice (Fig. 2C). In tracheal segments from mast cell-deficient sash mice, the smooth muscle contraction to OVA was completely abolished in PBS-treated as well as in IL-33-pretreated preparations (Fig. 2B). Moreover, the IL-33-induced enhancement of the response to antigen challenge was absent in tracheal segments from mice lacking ST2 (Fig. 2B). Pretreatment of the tracheal segments with ketanserin abolished the OVA-induced contraction in both PBS-treated and IL-33-pretreated preparations.

To assess whether the enhanced response was due to an action of IL-33 at the level of the smooth muscle, the contractions induced by OVA, acting indirectly, were compared to those induced by serotonin and carbachol, two agents with direct action on airway smooth muscle. In the trachea, neither potency nor maximal response to serotonin or carbachol

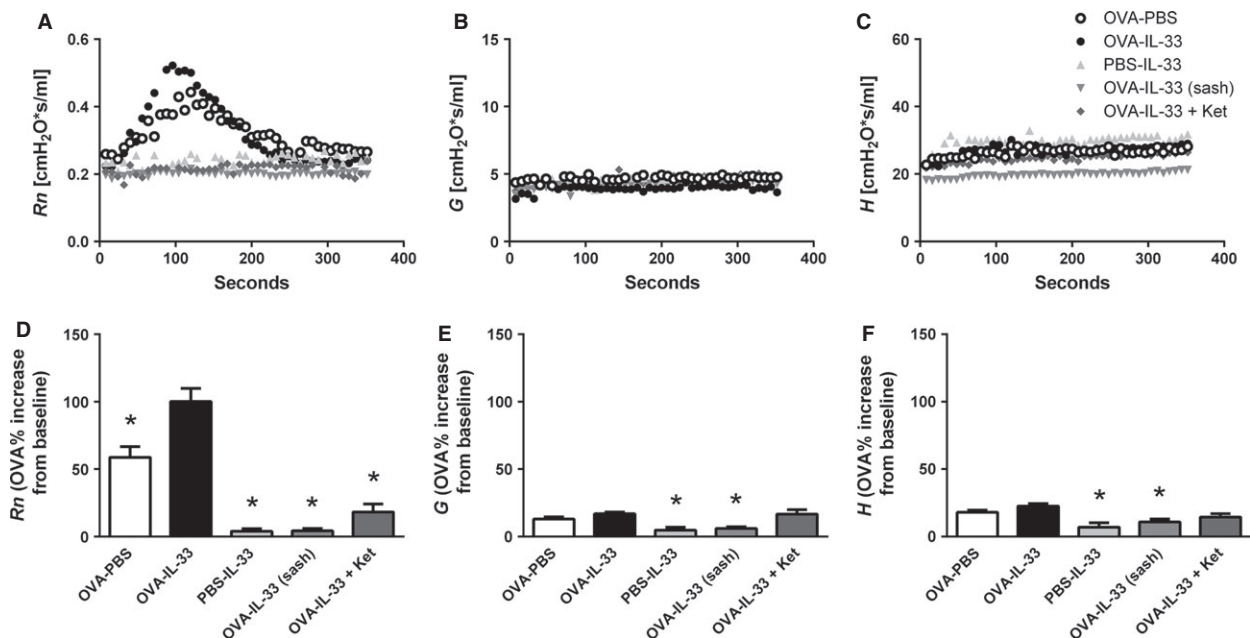


Figure 1 Interleukin-33 (IL-33) increases conducting airway resistance but does not alter peripheral lung tissue function following ovalbumin (OVA) challenge in OVA-sensitized mice (200 mg/kg). Lung mechanics measurements in OVA-sensitized C57BL/6 mice challenged with OVA. All groups were OVA-sensitized with the exception of the PBS-IL-33 group. All groups received intranasal instillations of IL-33 (200 ng) with the exception of the OVA-PBS group. OVA-PBS ($N = 12$), OVA-IL-33 ($N = 11$), PBS-IL-33 and OVA-IL-33 (sash) ($N = 5$), and OVA-IL-33 + Ket (8 mg/kg of ketanserin given 30 min intraperitoneally prior to OVA challenge) ($N = 10$).

(A–C) Representative traces from one mouse in each group are shown. The parameters measured are (A) Newtonian resistance (R_n), (B) tissue damping (G), and (C) tissue elastance (H). Maximal response of (D) R_n , (E) G , and (F) H are represented as increases in response to intravenously administered OVA and are expressed as the percentage of increase from baseline. Statistical analyses were performed using one-way ANOVA, followed by a Bonferroni posttest. * $P < 0.05$, compared to the OVA-IL-33 group.

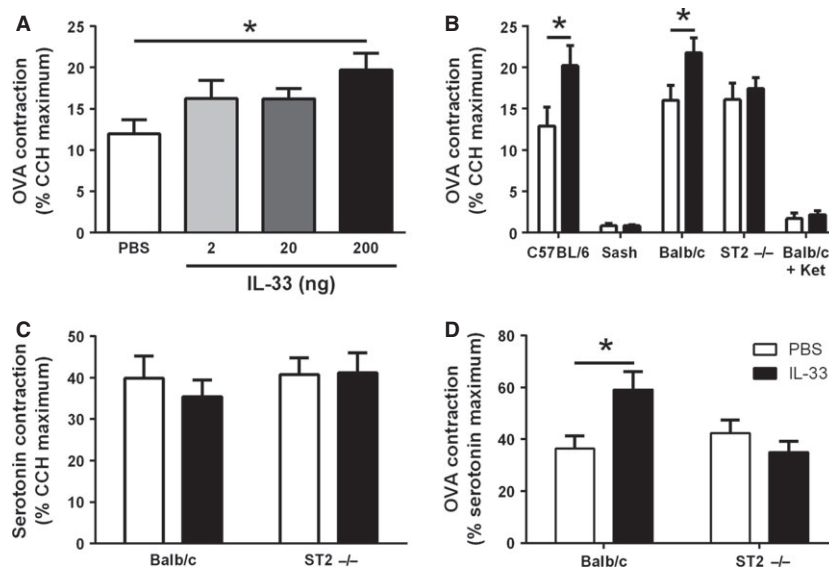


Figure 2 Interleukin-33 (IL-33) increases mast cell- and serotonin-dependent airway smooth muscle contraction during allergen challenge. Airway smooth muscle contraction during the response to ovalbumin (OVA) (100 μ g/ml) expressed as the percentage of maximal contraction to carbachol (CCH) if not otherwise stated. (A) Contraction of the trachea after treatment with IL-33 administered intranasally to C57BL/6 mice ($N = 12$; 20 ng: $N = 10$). (B) Contraction of the trachea after treatment with 200 ng of IL-33 delivered via intranasal installations in C57BL/6 (PBS: $N = 11$; IL-33: $N = 9$), sash ($N = 8$), BALB/c ($N = 20$), or ST2-deficient mice (PBS: $N = 11$; IL-33: $N = 10$). Tracheae from an additional group of IL-33 (200 ng)-

treated BALB/c mice were challenged with OVA in the presence of ketanserin (0.1 μ M; $N = 8$). (C) Contraction was measured in bronchi from BALB/c mice and was expressed as the percentage of maximal serotonin-induced contraction in BALB/c mice (PBS: $N = 8$; IL-33: $N = 10$) and ST2-deficient mice (PBS: $N = 9$; IL-33 [200 ng]: $N = 10$). (D) Maximal serotonin-induced contraction in BALB/c (PBS: $N = 8$; IL-33 [200 ng]: $N = 10$) and ST2-deficient mice. (PBS: $N = 9$; IL-33 [200 ng]: $N = 10$). Statistical analyses were performed using one-way ANOVA, followed by a Bonferroni posttest. * $P < 0.05$, compared to the PBS control.

was altered by IL-33 (Table 1). In bronchi, the maximum response to serotonin, expressed as percentage of maximal response to carbachol, was not affected by IL-33 treatment (Fig. 2C), whereas, as in the trachea, IL-33 increased the OVA response, expressed as percentage of the maximal response to serotonin, an effect that was absent in ST2-deficient mice (Fig. 2D).

The conclusion that IL-33 increased smooth muscle responsiveness via an action on mast cell mediator release, rather than by increasing the sensitivity to the released mediators, was further substantiated by the measurement of β -hexosaminidase and serotonin levels in tracheal culture supernatants. Challenge with OVA led to mast cell degranulation and the

secretion of both β -hexosaminidase (Fig. 3A) and serotonin (Fig. 3B) into the supernatant. This secretion was markedly increased in preparations from mice that had received *in vivo* treatment with IL-33 (Fig. 3A,B). Despite the enhanced mediator release, the number of mast cells present in tracheae from OVA-sensitized mice that received intranasal IL-33 was not significantly different compared to tracheae from mice that received PBS (Fig. 3C,D).

IL-33 acts directly on mast cells to enhance their production, storage, and secretion of serotonin

To further verify that IL-33 acted directly on the mast cell, we studied responses in cultured BMMCs. Following IgE receptor cross-linking, sensitized BMMCs responded with a marked secretion of serotonin. This response was further increased in BMMCs pretreated with IL-33 (Fig. 4A). The control groups, both in the absence and presence of IL-33, showed low levels of secreted serotonin (Fig. 4A). However, the total serotonin content was increased when the cells were treated with IL-33 (Fig. 4B). The sensitized BMMCs pretreated with IL-33 showed an increased gene expression of *Tph-1*, the rate-limiting enzyme in serotonin biosynthesis (18) (Fig. 4C). Additionally, BMMCs pretreated with IL-33 showed an increased β -hexosaminidase secretion upon

Table 1 Contractile responses for concentration–response curves to serotonin and carbachol in isolated tracheae from sensitized mice treated with interleukin-33 (IL-33) or PBS

Agonist	Treatment	pEC ₅₀	E _{max} (mN)
Serotonin	PBS	5.93 $\pm$ 0.06	2.5 $\pm$ 1.1
	IL-33	5.89 $\pm$ 0.08	3.6 $\pm$ 1.9
Carbachol	PBS	6.90 $\pm$ 0.08	19.0 $\pm$ 3.8
	IL-33	6.98 $\pm$ 0.06	19.8 $\pm$ 5.9

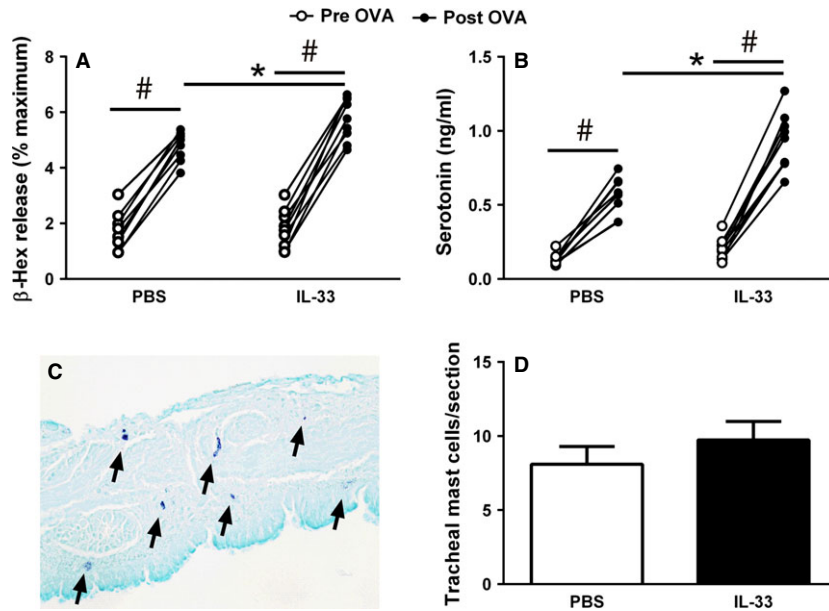


Figure 3 Interleukin-33 (IL-33) (200 ng) causes increased mast cell β -hexosaminidase and serotonin secretion during ovalbumin (OVA)-induced (100 μ g/ml) degranulation without an increase in mast cell numbers. (A) Secreted β -hexosaminidase levels expressed as the percentage of maximal secretion of β -hexosaminidase following cell lysis (PBS: $N = 8$; IL-33: $N = 9$). (B) Secreted serotonin levels (PBS: $N = 8$; IL-33: $N = 9$). (C) Representative image of mast cells apparent in the smooth muscle layer in trachea; magnification 40 $\times$. (D)

Mast cell numbers identified as dark blue (see arrows) per tracheal section stained with toluidine blue ($N = 6$). * $P < 0.05$, comparing responses between PBS and IL-33 groups pre- and post-OVA activation. Statistical analyses were performed using two-way repeated measures ANOVA, followed by Bonferroni posttest. # $P < 0.05$, comparing levels of measured substances pre- and post-OVA activation within the same group.

activation (Fig. 4D) as well as total β -hexosaminidase content measured following cell lysis (Fig. 4E).

Discussion

This study demonstrates for the first time that IL-33 can exaggerate allergen-induced airway obstruction. The airway resistance to the early allergic reaction *in vivo* was markedly increased in mice that were exposed to intranasal challenge of IL-33. This effect was absent in mice deficient of mast cells and blocked by the 5-HT_{2A} receptor antagonist ketanserin. The same pattern was seen *ex vivo* when examining the contractile responses to allergen in isolated airways. The release of serotonin was further increased from isolated airways from mice treated with IL-33. In cultured mast cells, IL-33 enhanced the expression of *Tph-1* and amplified the storage and secretion of serotonin.

Due to the difficulties associated with studying the early allergic reaction in mice, a specific protocol was used in which OVA-sensitized mice were challenged with intravenous OVA to obtain an increase in lung resistance (19). With this approach, OVA caused marked increase in resistance which in this study was shown to be restricted to the conducting airways. This region of the lung is the primary site of mast cell and smooth muscle cell distribution in the mouse lung (20) and thus intimates an IgE-mediated release of contractile mediators from mast cells. The OVA-induced increase in

resistance was further enhanced in mice that have been pretreated for 4 days intranasally with IL-33. This response was absent in mast cell-deficient mice and antagonized by ketanserin, indicating that the effect of IL-33 is mediated through mast cell secretion of serotonin which is the main contractile agonist released from mouse mast cells (19, 20).

One explanation for an enhanced OVA-mediated response could be that IL-33 caused an increase in circulating IgE, as reported for total IgE in mice given IL-33 intraperitoneally (5, 21). However, this was not found for OVA-specific IgE levels in the present study. The discrepancy from earlier studies could be due to the route of OVA administration or because specific IgE levels do not always correspond to total levels.

Because smooth muscle contractions are important for the early allergic reaction, we further investigated the direct responses to OVA in isolated airways from OVA-sensitized mice. In tracheal segments from mice, the OVA-induced contractions were markedly increased by IL-33 pretreatment. The lack of smooth muscle contraction in mast cell-deficient mice, and following ketanserin treatment, indicates that the responses were mediated by mast cell serotonin secretion and is consistent with previous reports (22, 23). As it has been shown that serotonin can mediate its effect directly on smooth muscle cell 5-HT_{2A} receptors (16) or indirectly through the activation of 5-HT_{2A} receptors on neurons or epithelium, leading to acetylcholine release (23, 24), multiple signaling pathways could have been activated as a result of

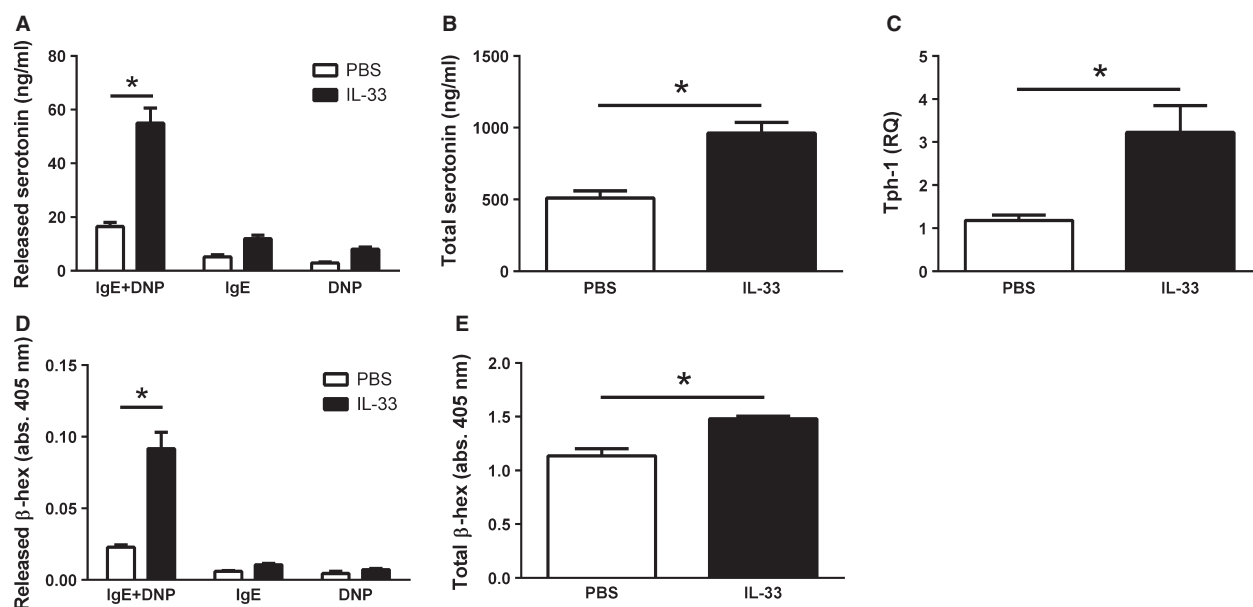


Figure 4 Mast cell production, storage, and secretion of serotonin upon activation are increased by Interleukin-33 (IL-33). BMMCs from C57BL/6 mice that were differentiated with IL-3 and stem cell factor were sensitized with anti-IgE-DNP and cultured in the presence of PBS or IL-33 (100 ng/ml) for 4 days. (A) Serotonin measured in supernatants from anti-DNP IgE-sensitized BMMCs (100 ng/ml) following activation by DNP (10 ng/ml) from sensitized BMMCs that did not receive DNP and from nonsensitized BMMCs exposed to DNP. (B) Serotonin measured in supernatants from sen-

sitized BMMCs following cell lysis with Triton X-100. (C) Relative quantitation (RQ) of mRNA levels of *Tph-1* ($N = 3$). (D) β -hexosaminidase measured in the same supernatants as in panel a. (E) β -hexosaminidase measured in supernatants, following cell lysis, from panel b. (A–B, D–E) ($N = 9$). Data are pooled from three independent experiments. (A–E) Statistical analyses were performed using two-way ANOVA, followed by a Bonferroni posttest. * $P < 0.05$, comparing results between IL-33- and PBS-treated cells.

IL-33 exposure. However, we did not observe changes in the smooth muscle response to serotonin or carbachol in OVA-sensitized mice that received IL-33, indicating that the properties of the airway smooth muscle cells are not altered.

Instead, the enhanced response could be caused by increased secretion of mast cell serotonin. An increase was indeed found when measuring serotonin secretion following allergen challenge in tracheae from sensitized mice. The similar increase observed in β -hexosaminidase secretion indicates a general increase in secretion of mast cell contents which can depend on either alteration in the number of mast cells or the mast cell properties. Although IL-33 can increase mast cell survival, maturation, and adhesion to fibronectin (25, 26), increased mast cell numbers were not found after intranasal IL-33 treatment. Therefore, these data indicate that the increased levels of serotonin are due to an effect that alters the capacity of the mast cells to release serotonin.

The direct effect of IL-33 on mast cell properties was further investigated in cultured mast cells. In sensitized mast cells exposed to IL-33, we observed an enhanced secretion of serotonin and β -hexosaminidase following IgE receptor cross-linking and an increased total storage of the same products. The demonstrated increase in total serotonin content and β -hexosaminidase is consistent with previous observations that IL-33 promoted granular accumulation of tryptase in mouse BMMCs after 7 days of treatment (27).

Also, the mRNA level of *Tph-1* was upregulated by IL-33, supporting an increase in the mast cell production of serotonin. Therefore, IL-33 seems to directly act on sensitized mast cells to increase their synthesis, storage, and secretion of granular serotonin following activation by IgE receptor cross-linking.

In conclusion, we have discovered that IL-33 enhances allergen-induced airway smooth muscle contraction through an exaggerated release of mast cell serotonin. Further investigation of the physiological relevance of this phenomenon is needed as it is possible that excessive epithelial release of IL-33 caused by viral infection (4), which is the most common cause of asthma exacerbations (28), or after tissue damage caused by allergens (29) would trigger a signal making neighboring mast cells more susceptible to activation. This feature may be of particular importance in allergic asthma where the mast cell numbers are increased in the airways smooth muscle bundles and intra-epithelial cell layers (30–32). Thus, therapeutics targeting IL-33 or associated molecules in the IL-33 signaling pathway could offer a novel strategy for asthma treatment and prevention (4).

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Author contributions

L.C.S., S.-E.D., G.P.N., and M.A. planned the conception and design of research; L.C.S. and J.A.G. performed experiments; L.C.S., S.-E.D., G.P.N., and M.A. analyzed the data;

L.C.S., S.-E.D., G.P.N., and M.A. interpreted the results of experiments; L.C.S. and M.A. prepared figures; L.C.S., G.P.N., and M.A. drafted the manuscript; L.C.S., J.A.G., S.-E.D., G.P.N., and M.A. edited and revised the manuscript.

Conflicts of interest

The authors have no conflict of interest.

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