

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

IL-33 is a mediator rather than a trigger of the acute allergic response in humans

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To cite this article: Fux M, Pecaric-Petkovic T, Odermatt A, Hausmann OV, Lorentz A, Bischoff SC, Virchow JC, Dahinden CA. IL-33 is a mediator rather than a trigger of the acute allergic response in humans. *Allergy* 2013; DOI: 10.1111/all.12309.

Keywords

asthma; basophil/mast cell degranulation; bronchoalveolar lavage fluids; IgE; IL-33.

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Accepted for publication 30 September 2013

DOI:10.1111/all.12309

Edited by: Thomas Bieber

Abstract

Background: IL-33 enhances FcεRI-induced mediator release in human basophils without inducing degranulation itself. In contrast, studies in mice suggested that in the presence of high IgE levels, IL-33 triggers degranulation and anaphylaxis of similar severity as specific allergen. Consistent with this view, sera of atopic patients contain elevated levels of IL-33 after anaphylaxis. In this study, we determined whether IL-33 is potentially anaphylactogenic in humans with high IgE levels by regulating exocytosis independent of FcεRI cross-linking. Furthermore, we investigated whether IL-33 is released upon allergen provocation *in vivo*.

Methods: In subjects with high serum IgE levels, we measured IL-33-induced histamine/LTC₄ *in vitro*, CD63 translocation *ex vivo*, and responsiveness of mast cells *in vivo* by skin prick test (SPT). In asthma patients, release of IL-33 and its correlation with early (tryptase)- and late-phase markers (IL-13 levels, eosinophil numbers) of the allergic response were assessed in bronchoalveolar lavage fluids (BALFs) after allergen challenge.

Results: IL-33 itself does not trigger basophil degranulation *in vitro* and *ex vivo*, even in subjects with high serum IgE levels, and negative SPTs demonstrate that skin mast cells do not degranulate in response to IL-33. However, in response to allergen challenge, IL-33 is rapidly released into BALFs at levels that do not correlate with other immediate- and late-phase parameters.

Conclusion: IL-33 is unlikely an independent trigger of anaphylaxis even in subjects with high IgE levels. However, the rapid release of IL-33 upon allergen provocation *in vivo* supports its role as a mediator of immediate allergic responses.

IL-33 is a member of the IL-1 cytokine family (1), which includes among others IL-1α, IL-1β, and IL-18. There are both an intracellular and extracellular form of IL-33. Intracellular IL-33 is expressed broadly and constitutively in the nuclei of many different human tissue cells (1, 2) and is enhanced under inflammatory conditions such as asthma

(3, 4). Extracellular IL-33 acts through a receptor complex consisting of transmembrane ST2 (ST2L) and interleukin-1 receptor accessory protein (IL-1RAcP).

We have previously shown that IL-33 targets human basophils resulting in the activation of NF-κB and phosphorylation of p38 MAP kinase. In addition, we have demonstrated that IL-33 promotes the secretion of Th2 cytokines and chemokines (IL-4, IL-13, IL-8) from human basophils in synergy with IL-3 and enhances FcεRI-induced histamine and leukotriene C₄ (LTC₄) release (5). However, unlike IL-3, IL-33 does not prime human basophils for C5a-dependent histamine and LTC₄ release. Mouse models have supported

Abbreviations

BALFs, bronchoalveolar lavage fluids; IL-1RAcP, interleukin-1 receptor accessory protein; LTC₄, leukotriene C₄; SAC, segmental allergen challenge; SPT, skin prick test; ST2L, transmembrane ST2.

the role of IL-33 to initiate allergen-driven Th2-type immune responses (6, 7). Thus, IL-33 is implicated in Th2-type immune responses in both humans and mice. However, contradictory data have been published regarding the capacity of IL-33 to induce exocytosis and/or generation of LTC₄ by itself. Namely, in human basophils purified from unselected donors, IL-33 never induced the release of histamine and LTC₄ formation directly, as published by us and others (5, 8, 9). This is in contrast to two studies in mice demonstrating that IL-33 can trigger histamine release and systemic anaphylaxis independent of allergen provocation. Important to note is that high IgE levels seem to determine whether IL-33 initiates exocytosis itself or not (10, 11). A set of publications by Pushparaj et al. have been retracted due to double publication (12), but their main conclusion that IL-33 is a trigger of anaphylaxis has been maintained.

IL-33 has been detected in bronchoalveolar lavage fluids (BALFs) of asthma patients (4), indicating that IL-33 is released during allergic inflammation. This is consistent with the observation of elevated IL-33 levels in sera after anaphylaxis (10). It has been shown that IL-33 is, on the one hand, released upon cellular necrosis and, on the other hand, through the stimulation of purinergic receptors and independent of cell death (13). Furthermore, the work of Kouzaki et al. demonstrates that in mice IL-33 is released upon intranasal administration of allergenic *Alternaria* extract (14), suggesting that IL-33 might be released through an active process in this species. However, whether FcεRI cross-linking is implicated in IL-33 release during allergic responses *in vivo* has not been investigated so far.

In the present study, we aimed at a better understanding of the role that IL-33 plays in allergic responses in humans. Our data show that IL-33 is rapidly released upon allergen challenge and can thus act as a mediator of IgE-dependent responses. However, we found no indication that IL-33 can directly induce basophil and mast cell degranulation and the generation of lipid mediators even in patients with high IgE levels. Taken together, our data make it very unlikely that in humans IL-33 acts as a direct trigger of anaphylaxis mimicking allergen-induced immediate-type hypersensitivity.

Methods

Studies with humans and human specimens were performed according to the Declaration of Helsinki and were approved by the local ethics committee. Informed consent was obtained from all patients.

Stimulation of purified basophils *in vitro* and measurement of histamine and LTC₄

Basophils from peripheral blood of subjects with high serum IgE levels (523–1737 kU/l, mean 1085 kU/l) were purified as described (15), resulting in a purity of >95%. Basophils were cultured in 96-well U-bottom plates in RPMI 1640 supplemented with 10% heat-inactivated FCS, 10 mM HEPES, 100 µg/ml streptomycin, and 100 U/ml penicillin (all from Gibco Invitrogen, Paisley, UK) at a density of 1×10^6 cells/ml

(100 µl/well) with or without 10 ng/ml IL-3 (Novartis, Basel, Switzerland) for 18 h at 37°C, 5% CO₂. Thereafter, basophils were exposed to buffer control (Nil), 100 ng/ml anti-FcεRIα antibody 29C6 (Roche, Basel, Switzerland), or 50 ng/ml IL-33 (Alexis, Lausen, Switzerland) for 30 min, and supernatants were harvested. LTC₄ was measured by the CAST ELISA (Bühlmann, Schönenbuch, Switzerland), and histamine was determined by fluorometry using AutoAnalyzer3 (Bran+Luebbe, Bio-Rad, Reinach, Switzerland) as described (16).

Basophil activation test *ex vivo*

As a means to examine basophil degranulation in unfractionated blood, the Flow2CAST basophil activation test was performed (Bühlmann, Schönenbuch, Switzerland). Briefly, fresh whole-blood samples from patients with high serum IgE levels were diluted 1 : 1 in buffer with or without IL-3, incubated for 10 min at 37°C, followed by the addition of buffer control (Nil), 100 ng/ml anti-FcεRIα antibody 29C6 or 150 ng/ml IL-33 (Alexis, Lausen, Switzerland) all together with staining reagent (anti-CD63 FITC and anti-CCR3 PE) for 25 min. After lysis of erythrocytes, the cells were analyzed by flow cytometry using a FACSCalibur or FACSCanto (both BD Biosciences, Allschwil, Switzerland) and FLOWJO software (TreeStar Inc., USA).

Assessment of p38 and Erk1/2 phosphorylation in basophils *ex vivo*

Phosphorylation of p38 and Erk1/2 in basophils from subjects with high serum IgE levels and normal donors was assessed as follows: Fresh whole-blood samples were stained with anti-CRTH2 Alexa647 (15 min, RT), warmed up (10 min, 37°C), incubated in buffer control (Nil) or 150 ng/ml IL-33 (Alexis, Lausen, Switzerland) (15 min, 37°C), lysed and fixed (10 min, 37°C) using Lyse/Fix buffer, permeabilized (10 min, RT) using Perm/Wash buffer, and stained with anti-P-p38 Alexa488 or anti-P-Erk1/2 Alexa488 and anti-CD3 PE (30 min, RT). All reagents were from BD Biosciences, Allschwil, Switzerland. Basophils were defined as CRTH2^{pos}/CD3^{neg} within a scatter gate set around the lymphocytes including the lymphocyte/monocyte overlapping region. Phosphorylation of p38 and Erk1/2 was determined using FACSCalibur (BD Biosciences, Allschwil, Switzerland) and FLOWJO software (TreeStar Inc., USA).

Skin prick test

To study the potential of IL-33 to induce degranulation of human skin mast cells, a skin prick test (SPT) was performed in patients with high IgE levels. Antihistamines and topical corticosteroids treatment were withheld for at least 7 days prior to the test. Recombinant IL-33 at 0.1 mg/ml (Alexis, Lausen, Switzerland) dissolved in phenol-containing SPT solution (Allergopharma, Germany) was applied to the volar forearm using a sterile prepacked plastic lancet (Stallerpoint, Stallergenes, Antony, France). Histamine and SPT solution (both Allergopharma AG, Germany) served as positive and

negative controls, respectively. After 15 min, the test reaction was considered as positive if wheal size exceeded 3 mm in diameter and was accompanied by a surrounding flare reaction without reaction to the negative control.

Analysis of bronchoalveolar lavage fluids for IL-33

Bronchoalveolar lavage fluids (BALFs) were obtained from segmental allergen challenge (SAC) experiments performed in a previous study (15). Briefly, SAC was performed in 13 asthma patients who had a positive skin prick test and specific IgE (Kabi Pharmacia CAP System, Uppsala, Sweden) to either house dust mite, birch pollen, or rye pollen (Allergopharma, Reinbek, Germany) and had a history of reversible bronchoconstriction after inhalation of the relevant allergen. There was no history of respiratory tract infection prior to or at the time of SAC. SAC was conducted by instilling individual allergen or saline into separate lung segments in the same patient during bronchoscopy. A first lavage was performed 10 min after challenge in allergen- and saline-challenged segments, and a second lavage after 18 h. Prior to determination of IL-33 by ELISA (Apotech; Axxora Platform, Lausen, Switzerland), collected BALF was processed as described (15).

Results

IL-33 never induces release of histamine and LTC₄ in purified human basophils

Because we intended to study the influence of high IgE levels on the capacity of IL-33 to induce mediator release, we purified basophils from 11 blood donors with high serum IgE levels (523–1737 kU/l, mean 1085 kU/l). Prolonged exposure of basophils to IL-3 not only results in a particularly high responsiveness to all known IgE-dependent and IgE-independent stimuli (15), but also in an upregulation of the IL-33 receptor ST2L (5). We therefore investigated the capacity of IL-33 to induce mediator release in basophils cultured either with or without IL-3. Fig. 1 demonstrates that IL-33 did not promote the release of the granule marker histamine and the formation of the lipid mediator LTC₄ in basophils from donors with high IgE levels, regardless of whether IL-3 was present or not during culture. As expected, FcεRI cross-linking used as a positive control induced the release of both mediators, and these responses were enhanced by IL-3.

IL-33 does not trigger basophil degranulation *ex vivo*

To assess the triggering capacity of IL-33 for basophil degranulation in a setting as close as possible to an *in vivo* condition, we studied basophil degranulation *ex vivo* by using freshly drawn whole blood from the donors with high IgE levels and CD63 surface exposure as a marker of degranulation. As shown in Fig. 2, IL-33 did not induce degranulation of basophils in unfractionated whole blood, neither in IL-3-primed nor in nonprimed cells. To verify cellular responsiveness to IL-33 in basophils from the donors with high IgE

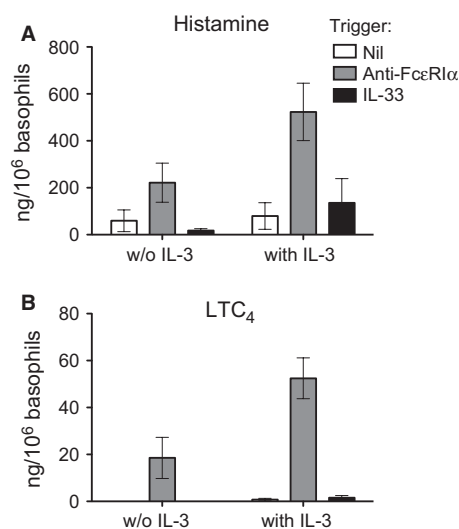


Figure 1 IL-33 does not induce histamine release and LTC₄ formation in human basophils purified from patients with high IgE levels. Basophils were cultured in medium alone (w/o IL-3) or with 10 ng/ml IL-3 (with IL-3) for 18 h and subsequently exposed for 30 min to buffer control (Nil, white bars), anti-FcεRIα antibody 29C6 (100 ng/ml, gray bars), or IL-33 (50 ng/ml, black bars). Released histamine (A) and newly generated LTC₄ (B) were measured in cell supernatants. Data show mean ± SEM of 7 independent experiments.

levels, we determined phosphorylation of MAP kinases p38 and Erk1/2. As comparison, we included fresh whole blood from healthy donors in our experimental settings. The data in Fig. 3 indeed demonstrate that under the *ex vivo* conditions used to study degranulation, basophils from both patients and normal donors responded to the addition of IL-33 with a prominent phosphorylation of p38 and a weak phosphorylation of Erk1/2. This result is comparable to the previously published *in vitro* data using PBMC from unselected donors (5).

IL-33 does not induce a wheal and flare response in subjects with high IgE levels

In 7 of 11 subjects with high IgE levels, we also performed skin prick tests (SPTs) to evaluate whether skin mast cells might release early mediators *in vivo* in response to IL-33. However, no wheal and flare reaction was observed in response to IL-33 in any of these subjects.

IL-33 is released during the early phase of segmental allergen challenge

To evaluate whether allergen provocation induces the release of IL-33 *in vivo*, we analyzed bronchoalveolar lavage fluids (BALFs) collected after segmental allergen challenge (SAC) that had been performed in a previous study (15). Fig. 4A shows IL-33 levels in BALFs 10 min and 18 h after instillation of allergen or vehicle in different lung segments of asthmatic patients. Significantly elevated IL-33 levels were

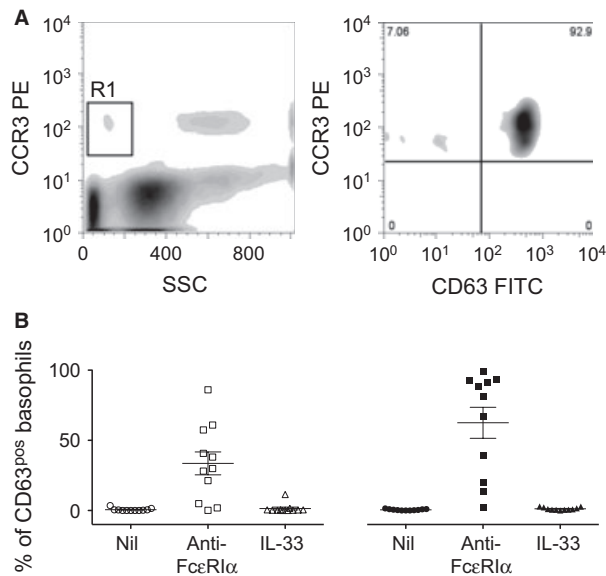


Figure 2 IL-33 does not induce CD63 surface translocation *ex vivo* in basophils of subjects with high IgE levels. Unfractionated fresh whole-blood samples were diluted 1 : 1 in buffer without (open symbols) or with 10 ng/ml IL-3 (closed symbols). Cells were subsequently exposed to buffer control (Nil), 100 ng/ml anti-FcεRIα antibody 29C6, or 150 ng/ml IL-33. (A) Gating strategy. Basophils were defined as CCR3^{high} cells with low SSC (R1). Within R1, percentage of CD63^{pos} basophils was determined. (B) Shown are percentages of CD63^{pos} basophils of individual experiments including mean ± SEM.

only found early (at 10 min) in the allergen-challenged, but not in the sham-challenged, segments. Interestingly, IL-33 levels were not significantly elevated in the allergic late-phase reaction (18 h). However, early IL-33 did not correlate with IL-13 levels (Fig. 4B) that are observed in the allergic late-phase reaction (15). Because IL-33 is known to act on eosinophils, we wondered whether early IL-33 might correlate with the extent of eosinophil migration into the lung at 18 h post-challenge (Fig. S1), but no such correlation was found (Fig. 4C).

We next wondered whether the early increase in IL-33 might correlate with the mast cell degranulation marker tryptase. However, no such correlation was found, suggesting that IL-33 originates from a cellular source other than mast cells (Fig. 4D). To further ask whether mast cells might produce IL-33 upon FcεRI cross-linking, we used purified cultured human intestinal mast cells as a model (17). Because IL-4 has been shown to augment FcεRI expression and to enhance the release of Th2-type cytokines, some of these mast cells were cultured with the survival factor SCF in combination with IL-4 (18). However, although these mast cells readily released the granule constituent β-hexosaminidase upon FcεRI cross-linking used as a mast cell activation control (Fig. S2), no IL-33 was detectable in the supernatants. Furthermore, no IL-33 mRNA was detectable in mast cells with or without FcεRI cross-linking (data not shown).

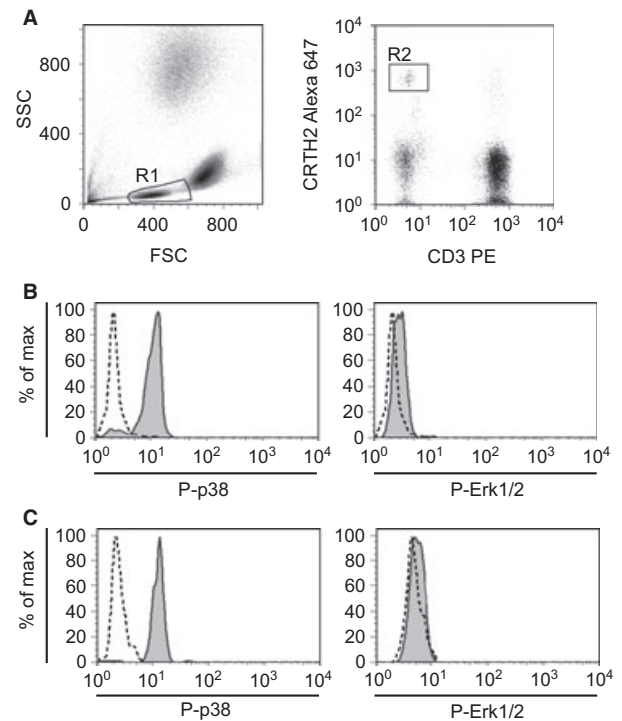


Figure 3 Serum IgE levels do not determine the capacity of IL-33 to activate MAP kinases in basophils *ex vivo*. Unfractionated fresh whole-blood samples were exposed to buffer control (dotted lines) or 150 ng/ml IL-33 (gray areas), and P-p38 and P-Erk1/2 were analyzed in basophils. (A) Gating strategy. Gating region R1 was set around lymphocytes including cells of lymphocyte/monocyte overlapping region. Within R1, basophils were defined as CRTH2^{pos}/CD3^{neg} cells (R2). (B, C) Representative overlays of P-p38 (left) and P-Erk1/2 (right) in basophils from a subject with high serum IgE level (B) and a healthy blood donor (C) are shown.

Discussion

In this study, we analyzed whether in the presence of high IgE levels IL-33 might be capable of triggering exocytosis in basophils and mast cells independently of FcεRI cross-linking. Our study was initiated by findings in mice which indicated that IL-33 promotes systemic anaphylaxis and degranulation of mast cells as efficiently as allergen as long as IgE was present at high concentrations (10). The possibility that high IgE levels may prime cells for IL-33-induced degranulation could be in line with several studies demonstrating that monomeric IgE bound to the receptor can result in a sustained cellular responsiveness (19). We further hypothesized that human basophils may be particularly prone to be influenced by IgE levels because in these cells FcεRI receptor density and turnover are regulated by the concentration of IgE (20). However, the present study with subjects with high IgE levels clearly shows that IL-33 cannot induce histamine release and *de novo* formation of LTC₄ *in vitro* in purified human basophils. Furthermore, we show that IL-33 can neither promote CD63 surface translocation

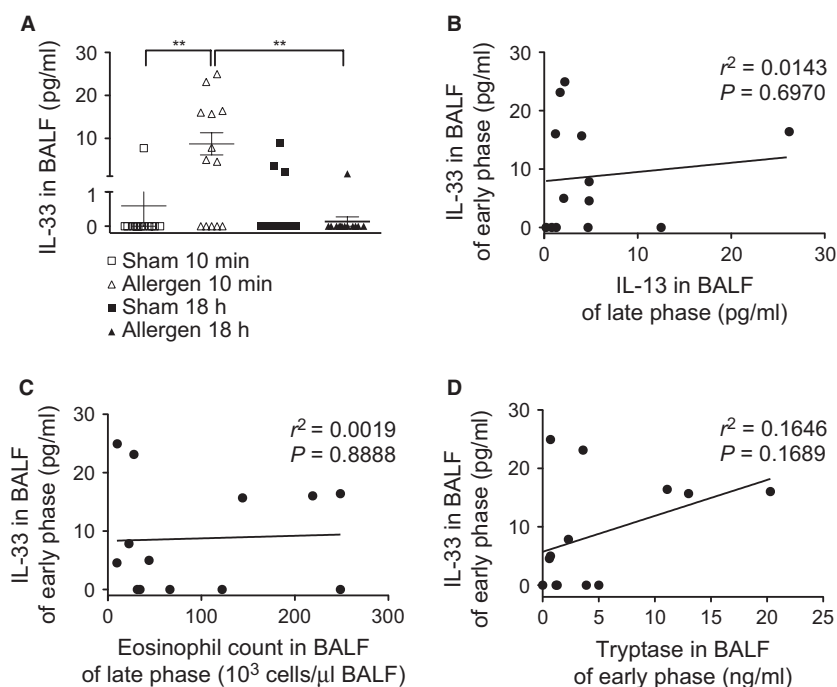


Figure 4 IL-33 is released rapidly into BALF of asthma patients upon allergen challenge, but does not correlate with other parameters of the early and late allergic responses. BALFs of sham- or allergen-challenged lung segments were collected at 10 min and at 18 h after challenge. (A) IL-33 levels in BALFs. Each data point shows IL-33 levels of individual patient's BALF including mean $\pm$

SEM. $**P < 0.01$ by one-way ANOVA, Bonferroni's multiple comparison test. (B–D) Linear regression of IL-33 of early phase (10 min) with IL-13 levels (B), eosinophil numbers (C) of late phase (18 h), and mast cell degranulation marker tryptase of early phase (10 min) (D) is shown.

ex vivo in human basophils from fresh whole-blood samples nor provoke a wheal and flare reaction *in vivo* in skin prick test (SPT) using IL-33 as a trigger. Taken together, our data make it very unlikely that IL-33 is capable of inducing anaphylaxis in subjects with high IgE levels *sui generis*. This conclusion has major clinical implications. In fact, it is not uncommon that systemic immediate-type-like symptoms of variable severity, such as skin rash, urticaria, bronchoconstriction, or drop in blood pressure, can occur during operations under general anesthesia. Furthermore, systemic reactions can accompany major tissue injury due to trauma or ischemia. Thus, if IL-33 would have had the potential to induce degranulation in cells of human subjects with high IgE levels, they would have been at risk for anaphylaxis, because IL-33 is a widely expressed nuclear protein that can be released by necrotic cells (13).

A major finding of this study is the rapid release of IL-33 during the early phase of allergic responses in allergen-challenged, but not in sham-challenged, lung segments. This observation is consistent with recent data demonstrating the presence of IL-33 in BALFs of patients with moderate asthma (4). It is interesting to note that IL-33 is as yet the only cytokine that has been detected in the early phase of experimental challenge models in humans because a large number of cytokines, including the IL-1 family member IL-1 β , are only increased during the allergic late-phase reaction

but never detected early after allergen challenge (21). The fact that IL-33 is present in BALFs early after allergen challenge of the lung segments suggests that IL-33 is released by an active process rather than by cellular necrosis that may occur in chronic allergic inflammation. Recently published data suggest vesicular transportation of IL-33 as a mechanism of active IL-33 release (22).

Although the release of IL-33 is allergen dependent, mast cells are probably not the source of IL-33 because there was no correlation between IL-33 and tryptase levels in BALFs. This is supported by our observation that IL-33 mRNA was not present in cultured human mast cells and IL-33 protein was not detectable in supernatants of cells stimulated by Fc ϵ RI cross-linking. This may be different from the murine system, because mouse bone marrow-derived mast cells have been shown to express IL-33 upon Fc ϵ RI cross-linking (23, 24). Airway smooth muscle cells, which constitutively express intracellular IL-33 (3) and which display Fc ϵ RI on the surface (25), could be a source of IL-33 in BALFs. Recent studies suggest alveolar macrophages or type-2 pneumocytes as a source of IL-33 upon induction of airway inflammation in mice (26–28). The cellular source of IL-33 upon Fc ϵ RI cross-linking in humans needs further investigations. Furthermore, it remains unknown whether IL-33 is released by Fc ϵ RI receptor-expressing cells or indirectly through the action of a mediator released by Fc ϵ RI cross-linking.

In addition to basophils, human eosinophils are also an important cellular target of IL-33 (5, 29–31). We therefore wondered whether IL-33 levels found early after allergen challenge might correlate with eosinophil numbers during the allergic late-phase reaction. However, there was no such correlation. Thus, although many data support a major role of IL-33 in allergic inflammation (32), the regulation of eosinophilic inflammation is not solely IL-33 dependent and clearly more complex and most likely dependent on other factors such as IL-5 and eotaxins.

In essence, our data show that IL-33 does not induce an immediate release of basophil and mast cell mediators in allergic subjects with high IgE levels and is thus unlikely anaphylactogenic in humans. However, IL-33 is released rapidly upon allergen provocation *in vivo* probably through an active process rather than simple necrosis.

Author contributions

M.F. acquired, analyzed, and interpreted data and wrote the manuscript. T.P., A.K., and O.H. acquired data. A.L. acquired and analyzed data. S.B. was supervisor and revised

the manuscript. C.W. acquired data and supervised research and revised the manuscript. C.D. was supervisor and approved final version of the manuscript.

Acknowledgment

This work was supported by the Swiss National Foundation Nr. 310030_1273.

Conflicts of interest

There are no conflicts of interest related to this study.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Figure S1. Eosinophils migrate to the lung during the late allergic response.

Figure S2. Measurement of β -hexosaminidase as activation marker of purified human intestinal mast cells.

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Supporting Information

TITLE PAGE

Original Article

IL-33 is a mediator rather than a trigger of the acute allergic response in humans

Short title: IL-33 does not trigger basophil and mast cell degranulation in humans.

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This work was supported by the Swiss National Foundation Nr. 310030_1273

METHODS

Determination of absolute eosinophil numbers in bronchoalveolar lavage fluids

Eosinophil numbers from 13 asthma patients were determined in bronchoalveolar lavage fluids (BALFs) collected 18h after sham- or allergen-challenge in separate lung segments. SSC^{high}/CCR3^{pos} eosinophils were quantified by flow cytometry using TruCOUNT tubes (BD Biosciences, Allschwil, Switzerland).

Isolation, culture and stimulation of human intestinal mast cells

Mature human mast cells originated from surgical tissue specimens (macroscopically normal tissue) derived from patients who underwent bowel resection because of cancer. Mast cell isolation combined mechanical and enzymatic tissue dispersion and is described in detail elsewhere (1). Isolated mast cells were incubated overnight in culture medium (RPMI 1640 supplemented with 10% heat-inactivated FCS, 25mM HEPES, 2mM glutamine and antibiotics (100µg/ml streptomycin, 100U/ml penicillin, 100µg/ml gentamycin and 0.5µg/ml amphotericin). After culturing mast cells overnight, they were enriched by positive selection using MACS CD117 microbead kit with prior usage of a dead cell removal kit (all reagents from Miltenyi Biotec, Bergisch-Gladbach, Germany). The fraction containing the CD117 positive cells was cultured at a density of 2×10^5 cells/ml in culture medium supplemented with 25ng/ml recombinant human SCF (Peprotech, Rocky Hill, NJ) alone or in combination with 2ng/ml IL-4 (Peprotech, Rocky Hill, NJ) for at least 10 days. Cultured mast cells were fed once a week by exchanging half of the culture medium supplemented with cytokines.

To examine whether IL-33 is released upon FcεRI cross-linking, cells were sensitized with 400ng/ml myeloma IgE for 1h and subsequently stimulated with 1µg/ml anti-

human IgE antibody for 1h. IL-33 was measured by ELISA (Apotech; Axxora Platform, Lausen, Switzerland). As a control for cellular activation mast cell degranulation was assessed by measuring β -hexosaminidase activity (2). Released β -hexosaminidase is presented as percentage of total cellular β -hexosaminidase content.

Measurement of IL-33 mRNA in human mast cells

Total RNA was prepared from 1×10^5 mast cells and real-time PCR was performed as described (3). The following specific sense and anti-sense primers for IL-33 and GAPDH mRNA were used: GAPDH: 5'-TGGTCTCCTCTGACTTCAAC-3', 5'-CCTGTTGCTGTAGCCAAATT-3', IL-33: 5'-GTGGAAGAACACAGCAAGCA-3', 5'-ACAGGCAGCGAGTACCAGAT-3'. To quantify IL-33 mRNA expression real-time PCR reactions containing 1.5 μ l of cDNA template (sample or standard), 8 μ l of H₂O, 10 μ l of SYBR®Green PCR Master Mix and 0.25 μ l of 20 μ M sense and anti-sense primer were performed in optical tubes (Applied Biosystems). Reaction mixture without cDNA was used as negative control in each run. All reactions were performed using the ICycler (Bio-Rad Laboratories, Munich, Germany). Specificity of the reaction was controlled by melt curve and separation of amplified fragments on 1 % agarose gel.

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Figure Legends

Figure S1: **Eosinophils migrate to the lung during the late allergic response.**

BALFs of sham- or allergen-challenged lung segments collected at 18h after challenge were analyzed for the numbers of eosinophils. Data show individual absolute eosinophil numbers of different BALFs (10^3 cells/ml BALF) including mean $\pm$ SEM. ** $P < 0.01$ by paired two-tailed t test.

Figure S2: **Measurement of β -hexosaminidase as activation marker of purified human intestinal mast cells.**

Mast cells were cultured with 25ng/ml SCF alone or in combination with 2ng/ml IL-4 for at least 10 days. Cells were subsequently sensitized with 400ng/ml myeloma IgE and triggered with 1 μ g/ml anti-IgE antibody (gray bars) for 1h each. Non-triggered cells served as control (white bars). Data show mean $\pm$ SEM of released β -hexosaminidase as percentage of total cellular β -hexosaminidase content.

Figure S1

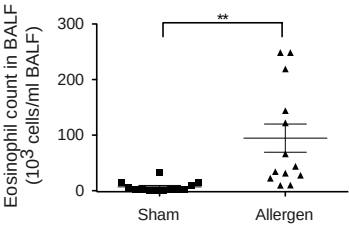


Figure S2

