

# Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naïve mice<sup>1</sup>

M. Komai-Koma<sup>1</sup>, F. Brombacher<sup>2</sup>, P. N. Pushparaj<sup>3</sup>, B. Arendse<sup>2</sup>, C. McSharry<sup>1</sup>, J. Alexander<sup>4</sup>, R. Chaudhuri<sup>1</sup>, N. C. Thomson<sup>1</sup>, A. N. J. McKenzie<sup>5</sup>, I. McInnes<sup>1</sup>, F. Y. Liew<sup>1,3</sup> & D. Xu<sup>1</sup>

<sup>1</sup>Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK; <sup>2</sup>Institute of Infectious Disease and Molecular Medicine, University of Cape Town and International Center for Genetic Engineering and Biotechnology, Cape Town, South Africa; <sup>3</sup>Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia; <sup>4</sup>Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK; <sup>5</sup>Medical Research Council Laboratory of Molecular Biology, Cambridge, UK

**To cite this article:** Komai-Koma M, Brombacher F, Pushparaj PN, Arendse B, McSharry C, Alexander J, Chaudhuri R, Thomson NC, McKenzie ANJ, McInnes I, Liew FY, Xu D. Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via Interleukin-4 in naïve mice. *Allergy* 2012; **67**: 1118–1126.

## Keywords

allergen independent; IgE; IL-33; IL-4 dependent; mast cell degranulation.

## Correspondence

Damo Xu, Institute of Infection, Immunity and Inflammation, 120 University Place, University of Glasgow, Glasgow G12 8TA, UK.

Tel.: +44 141 330 8415

Fax: +44 141 330 4297

E-mail: Damo.Xu@glasgow.ac.uk

<sup>1</sup>This study received financial support from Arthritis Research, UK, the Medical Research Council, UK, and the Wellcome Trust.

Accepted for publication 11 May 2012

DOI:10.1111/j.1398-9995.2012.02859.x

Edited by: Angela Haczku

## Abstract

**Background:** The regulation and function of IgE in healthy individuals and in antigen-naïve animals is not well understood. IL-33 administration increases serum IgE in mice with unknown mechanism. We tested the hypothesis that IL-33 provides an antigen-independent stimulus for IgE production and mast cell degranulation.

**Methods:** IL-33 was administered to naïve wild-type (WT), nude and ST2<sup>-/-</sup>, IL-4<sup>-/-</sup>, IL4R $\alpha$ <sup>-/-</sup> and T- or B-cell-specific IL-4R $\alpha$ <sup>-/-</sup> mice. IgE and cytokines were quantified by ELISA. T- and B-lymphocyte numbers and CD40L expression were determined by flow cytometry. Anaphylaxis was measured by temperature, mast cell degranulation and histamine release.

**Results:** IL-33 enhanced IgE production in naïve WT, T-IL-4R $\alpha$ <sup>-/-</sup> but not in ST2<sup>-/-</sup>, IL-4<sup>-/-</sup>, IL-4R $\alpha$ <sup>-/-</sup> or B-cell-specific IL-4R $\alpha$ <sup>-/-</sup> mice, demonstrating IL-33 specificity and IL-4 dependency. Moreover, IL-4 was required for IL-33-induced B-cell proliferation and T-cell CD40L expression, which promotes IgE production. IL-33-induced IL-4 production was mainly from innate cells including mast cells and eosinophils. IL-33 increased mast cell surface IgE and triggered degranulation and systemic anaphylaxis in allergen-naïve WT but not in IL-4R $\alpha$ <sup>-/-</sup> mice.

**Conclusion:** IL-33 amplifies IgE synthesis and triggers anaphylaxis in naïve mice via IL-4, independent of allergen. IL-33 may play an important role in nonatopic allergy and idiopathic anaphylaxis.

IgE antibody is induced by allergen and requires immunoglobulin heavy-chain class switching and affinity maturation in B cells in a T-cell-dependent manner (1, 2). IgE is also produced naturally (1, 3) but less is known about the selective amplification and function of natural IgE in healthy subjects and antigen-naïve animals (3). Natural IgE is secreted by B2 cells and contributes to the baseline concentrations of nonallergen-specific IgE in the serum and tissues of healthy individuals (3, 4). In contrast to allergen-induced IgE, cognate

T-helper cell via MHC class II and somatic hypermutation are not required for the generation of natural IgE (3). Instead, bystander stimulation from T-cell CD40L and low levels of IL-4 are necessary (3, 4). The functions of natural IgE are poorly understood; they are polyreactive and have a critical role in immune priming (3–7). IgE can bind the high-affinity IgE receptor (Fc $\epsilon$ RI) and prolong mast cell survival, release a spectrum of cytokines and have important roles in defence against infection and initiation of adaptive immunity (5–8).

IL-33 is a new IL-1 family member and signals via a receptor complex consisting of ST2 (IL-1LR1) and IL-1RAcP (9, 10). IL-33 is expressed by innate cells in humans and mice, primarily epithelium and endothelium, and can be released when cells sense inflammatory signals or undergo

## Abbreviations

i.p., intraperitoneal; Fc $\epsilon$ RI, IgE-Fc epsilon receptor I; AS, anaphylactic shock.

necrosis (9–11). ST2 is expressed by most innate cells but only by selected adaptive immune cells (9, 12, 13). Thus, IL-33 can directly activate eosinophils, basophils, macrophages and NK cells via ST2 (9, 14–16). In contrast, ST2 is selectively expressed on IL-5<sup>+</sup>Th, Th2 but not Th1 or Th17 cells (17–19). While enhancing mature Th2-cell function, IL-33 cannot polarize Th2 cells (17). Interestingly, IL-33 can induce IgE production in naïve mice (9). However, the effect and mechanism by which IL-33 induces IgE production in naïve mice is largely unknown.

We aim to identify the mechanism and role of IL-33 in the induction and regulation of IgE production in allergen-naïve mice. We report that IL-33 amplifies IgE synthesis via IL-4 from innate immune cells that is sufficient to trigger mast cell degranulation and anaphylaxis in the absence of allergen.

## Materials and methods

### Mice

BALB/c, BALB/c nude and C57BL/6 mice were purchased from Harlan Olac. ST2<sup>-/-</sup> mice (20), IL-4<sup>-/-</sup>, IL-4 receptor  $\alpha$  chain knockout (IL-4R $\alpha$ <sup>-/-</sup>) (21) and mice selectively deficient in IL-4R $\alpha$  on B cells (F. Brombacher, unpublished) or T cells (*Lck<sup>cre</sup>Il4ra<sup>-/lox</sup>*) (22) were on a BALB/c background. Mice were housed in specific-pathogen-free conditions at Glasgow University, UK, and Cape Town University, South Africa, in accordance with animal experimentation guidelines.

### IL-33 treatment *in vivo*

Recombinant IL-33 was prepared as described (23). Mice were injected i.p. with IL-33 (2  $\mu$ g/mouse) or PBS daily for 1–7 days unless stated otherwise. Cells were collected by peritoneal washout with 2 ml of cold PBS.

Anaphylaxis (AS): Mice were injected i.p. with IL-33 (2  $\mu$ g) or PBS daily for 3–4 days. Changes in clinical signs and temperature were monitored every 10 min after each injection, using a rectal probe (Natsume Seisakusyo Co., Tokyo, Japan). Plasma histamine was measured by ELISA (IBL, Hamburg, Germany).

### Flow cytometry

Cells (3  $\times$  10<sup>5</sup> cells/tube) were stained with fluorochrome-conjugated antibodies anti-CD3, anti-CD4, anti-CD19, anti-Fc $\epsilon$ RI, anti-CD40L, anti-CD25, anti-CD117 and Siglec-F and isotype controls (BD Bioscience, Oxford, UK). Intracellular cytokine levels in peritoneal cells were determined by staining with anti-IL-4-PE after activation (PMA/ionomycin) and permeabilization. Cells were analysed by FACSCalibur using Cell Quest software (BD Bioscience).

### Cytokine and IgE measurements

Serum concentrations of cytokines were determined by Luminex (Luminex, Biosource; Invitrogen, Life Technologies Ltd, Paisley, UK) and IgE by ELISA (Bethyl Laboratories, Inc, Montgomery, TX, USA).

### Histological analysis of mast cells

Skin samples were stained with toluidine blue to identify and count mast cells by microscopy. Degranulated mast cells were identified as those with >10% extrusion of granules (20).

### Statistical analysis

Student's *t*-test was applied to *in vitro* studies. Analysis between individuals in groups *in vivo* was carried out by ANOVA followed by Student's *t*-test.

## Results

### IL-33-induced IgE but not type-II cytokines is IL-4 dependent

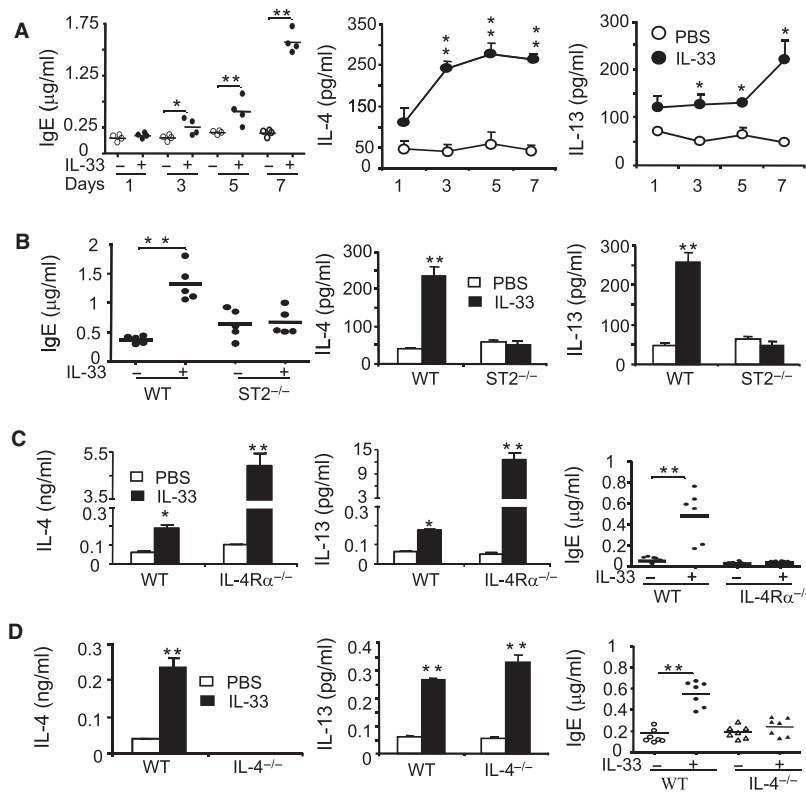
Mice had seven daily injections of IL-33 or PBS. Low basal concentrations of serum IgE, IL-4 and IL-13 were maintained in antigen-naïve or PBS-treated mice, but were significantly elevated in wild-type (WT), but not ST2<sup>-/-</sup> mice, within 3 days of IL-33 administration (Fig. 1A,B) IL-4 production preceded the increase in IgE. As reported (9), IL-33 did not induce IFN- $\gamma$  in naïve WT mice (data not shown). These results suggest that IL-33 is a powerful and rapid inducer of IgE in naïve mice that might be mediated by type-II cytokines.

IL-4 in mice and IL-4 and IL-13 in humans are key cytokines for the initiation of type-II responses and IgE isotype class switching (4, 24). We therefore determined the role of IL-4 and IL-13 in IL-33-induced IgE synthesis. WT and IL-4R $\alpha$ <sup>-/-</sup> mice were injected with IL-33 resulting in higher serum concentrations of IL-4 and IL-13 in IL-4R $\alpha$ <sup>-/-</sup> mice compared with PBS-treated IL-4R $\alpha$ <sup>-/-</sup> mice or with IL-33-treated WT mice (Fig. 1C), probably due to reduced sequestration of these cytokines in the absence of IL-4R $\alpha$  (25). More importantly, IL-33 failed to enhance IgE in IL-4R $\alpha$ <sup>-/-</sup> mice (Fig. 1C). Together, these data indicate that IL-33-mediated induction of IgE is dependent on IL-4R $\alpha$  responsiveness and that IL-4/IL-13 induction is not.

Because IL-4R $\alpha$  deficiency abolishes both IL-4 and IL-13 functions (21, 22), we assessed the requirement of IL-4 for IgE production using IL-4<sup>-/-</sup> mice. WT and IL-4<sup>-/-</sup> mice were injected with IL-33. IL-33 induced similar levels of IL-13 production in the WT and IL-4<sup>-/-</sup> mice (Fig. 1D), but IL-33 failed to enhance IgE in IL-4<sup>-/-</sup> mice (Fig. 1D). Thus, IL-4 is necessary and sufficient for the induction of IgE by IL-33 in naïve mice.

### IL-33 induces type-II cytokines mainly via innate cells in naïve mice

We next investigated whether the increased IL-4 and IL-13 levels in IL-33-treated mice were produced by T cells or innate cells *in vivo* by comparing WT and athymic nude mice (lacking T cells) injected with IL-33 or PBS as described above. Nude mice produced significantly higher levels of serum IL-4 and IL-13 than IL-33-treated WT mice, but



**Figure 1** IL-33 induces IgE but not type-II cytokine production in an IL-4-dependent manner. Groups of wild-type (A), ST2<sup>-/-</sup> (B), IL-4Rα<sup>-/-</sup> (C) and IL-4<sup>-/-</sup> (D) mice were injected i.p. with IL-33 (2 μg) or PBS daily

for 7 days. Total serum IgE, IL-4 and IL-13 concentrations were measured by ELISA. Data are representative of three experiments, n = 5–7 mice per group, \*P < 0.05, \*\*P < 0.01 compared to PBS controls.

markedly lower IgE (Fig. 2A). This suggests two points. First, T cells have minimal contribution to IL-33-induced type-II cytokine production *in vivo*. Second, while IL-33 could induce significant amounts of IgE in nude mice compared with PBS control, the levels of IgE were markedly lower than those of IL-33 in WT mice, suggesting that T cells have a modest but significant role in IL-33-mediated IgE induction, independent of enhanced type-II cytokine synthesis.

We next determined which were the IL-4-producing cells *in vivo* from IL-33- or PBS-treated WT mice by flow cytometry. IL-33 enhanced the IL-4 expression in CD117 (c-Kit)<sup>+</sup> mast cells (11% compared with 1% in PBS-treated controls) and in Siglec-F<sup>+</sup> eosinophils (31% compared with 2% in PBS controls) (Fig. 2B). However, IL-33 failed to increase the frequency of IL-4<sup>+</sup> CD4<sup>+</sup> T cells (Fig. 2B). IL-33 also increased the numbers of total cells, mast cells, eosinophils and to a lesser extent CD4<sup>+</sup> T cells in the peritoneal cavity (Fig. 2C). Thus, IL-33 mainly induces type-II cytokines via innate immune cells in naïve mice.

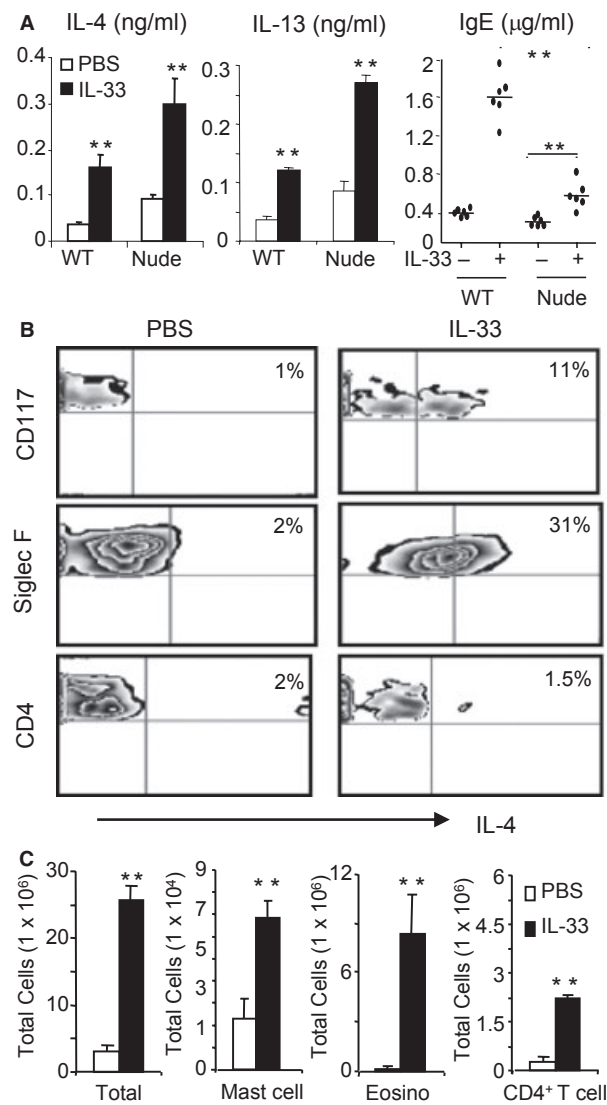
**B cells expressing the IL-4Rα are required for IL-33-induced B-cell expansion and IgE production *in vivo***

We have previously shown that conventional CD19<sup>+</sup> B cells do not express ST2 (26), suggesting that IL-33 may only induce IgE synthesis indirectly, likely via IL-4 as we have

demonstrated earlier. We therefore assessed the role of IL-4Rα signalling on B cells in IL-33-induced IgE synthesis by comparing naïve B-cell-specific IL-4Rα<sup>-/-</sup> (selectively deleted of IL-4Rα on B cells), IL-4Rα<sup>-/-</sup> (total IL-4Rα deletion) and WT mice treated with IL-33 or PBS. IL-33 induced IgE synthesis in WT but not in IL-4Rα<sup>-/-</sup> or B-IL-4Rα<sup>-/-</sup> mice (Fig. 3A). Serum IL-4 and IL-13 concentrations in B-IL-4Rα<sup>-/-</sup> and WT mice were similar but were significantly lower than those of the IL-4Rα<sup>-/-</sup> mice (Fig. 3B), suggesting that cells other than B cells may consume some of these cytokines. IL-33 increased the total cell number and the number of CD19<sup>+</sup> B cells (but not CD4<sup>+</sup> T cells) in the spleen of WT mice but not in IL-4Rα<sup>-/-</sup> or B-IL-4Rα<sup>-/-</sup> mice (Fig. 3C). Because IL-13 does not directly induce IgE in mouse B cells (24), together these results suggest that IL-33 indirectly elicits natural IgE production in naïve mice by inducing IL-4, which then directly stimulates B-cell expansion and IgE isotype class switching.

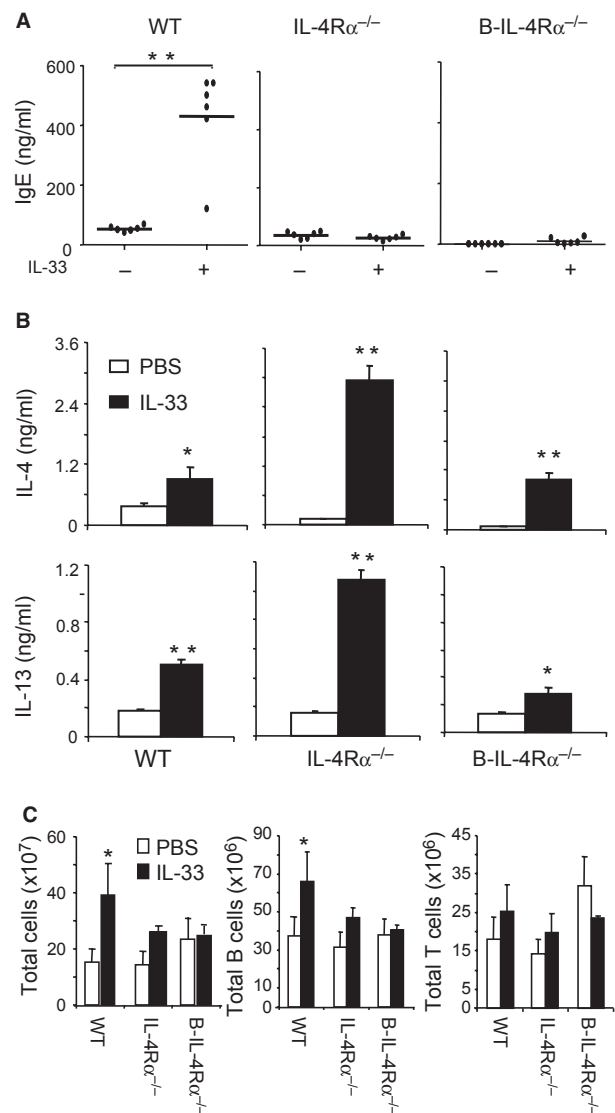
**T cells expressing IL-4Rα contribute to IL-33-induced IgE synthesis**

Our aforementioned results (Fig. 2A,B) suggest that T cells also contribute to IL-33-enhanced IgE production in naïve mice other than by providing type-II cytokines. To assess the potential role of IL-4R responses by T cells, we injected



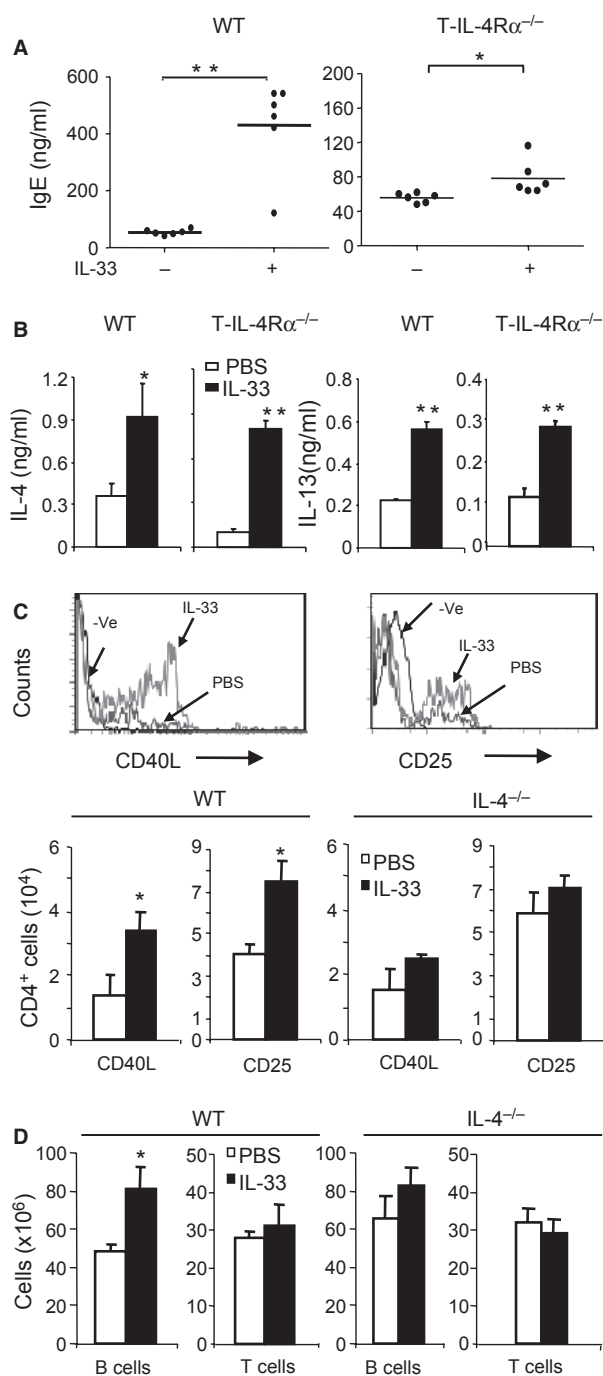
**Figure 2** IL-33 induces type-II cytokines mainly via innate cells in naive mice. Mice were treated with IL-33 for 7 days. Serum IL-4, IL-13 and IgE concentrations in (A) nude and wild-type (WT) mice were measured by ELISA. (B) The frequency of IL-4<sup>+</sup> cells and (C) total numbers of different cell populations in the peritoneum of WT mice treated with IL-33 or PBS were determined by intracellular staining using FACScan and quantified. Data are the representative of two experiments, *n* = 5 mice per group, \**P* < 0.05, \*\**P* < 0.01 compared to the PBS control or as indicated.

IL-33 or PBS into WT or T-IL4Rα<sup>-/-</sup> (*Lck<sup>cre</sup>Il4rα<sup>-lox</sup>* mice, selectively deleted of IL-4Rα on T cells) mice. IL-33 modestly but significantly increased serum IgE levels in T-IL-4Rα<sup>-/-</sup> mice (Fig. 4A). Consistent with the result from nude mice (Fig. 2A), IL-33 elicited abundantly increased level of IL-4 and IL-13 in T-IL4Rα<sup>-/-</sup> compared with WT mice (Fig. 4B), suggesting that these cytokines are unlikely to play an important role in T-cell function in IL-33-induced IgE production. An alternative contribution therefore should be considered.



**Figure 3** B cells expressing IL-4Rα are required for IL-33-induced B-cell expansion and IgE production *in vivo*. Wild-type, IL-4Rα and B-IL-4Rα mice were injected with IL-33. The levels of serum IgE (A) and IL-4/IL-13 (B) were measured by ELISA. (C) Total cell number, T cells and B cells in the spleen were determined by differential cell count. Data are the representative of two experiments, *n* = 5 mice per group, \**P* < 0.05, \*\**P* < 0.01 compared to PBS group.

CD40L expression on T cells is required for B cells to produce IgE, and the expression of CD40L on T cells can be enhanced by IL-2 receptor signals (3, 27). We therefore assessed whether IL-33 could up-regulate the expression of CD40L and CD25 (IL-2 receptor α chain) on T cells. Because murine CD4<sup>+</sup> T cells only respond to IL-4 but not IL-13 (because of the lack of IL-13 receptor) (24), we examined IL-33-mediated T-cell function in IL-4<sup>-/-</sup> mice *ex vivo*. IL-33 increased the surface expression of CD40L and



**Figure 4** IL-4R $\alpha$  T cells contribute to IL-33-induced IgE synthesis. Wild-type (WT), IL-4<sup>-/-</sup> or T-IL-4R $\alpha$ <sup>-/-</sup> mice were treated with IL-33. (A) Serum IgE and (B) IL-4 and IL-13 concentrations were measured by ELISA. (C) The levels of CD40L and CD25 on CD4<sup>+</sup> T cells and the number of CD4<sup>+</sup> CD40L<sup>+</sup>, CD4<sup>+</sup> CD25<sup>+</sup> T cells in the spleen of WT and IL-4<sup>-/-</sup> mice were determined by FACSscan and differential counting. (D) Total CD4<sup>+</sup> T and CD19<sup>+</sup> B cells in the spleen of WT and IL-4<sup>-/-</sup> mice were determined by differential cell count. Data are from two experiments,  $n = 6$  mice per group, \* $P < 0.05$ , \*\* $P < 0.01$  compared to PBS control.

CD25 on CD4<sup>+</sup> T cells in WT mice but not in IL-4<sup>-/-</sup> mice (Fig. 4C). Consistent with our finding (Fig. 3C), IL-33 also increased the total number of B cells (but not T cells) in the WT but not in IL-4<sup>-/-</sup> mice (Fig. 4D). Thus, IL-33 stimulates CD40L and CD25 expression on T cells via IL-4, which may contribute to the IL-33-induced IgE production in naïve mice.

#### IL-33 triggers mast cell degranulation in WT but not ST2<sup>-/-</sup> or IL4R $\alpha$ <sup>-/-</sup> mice

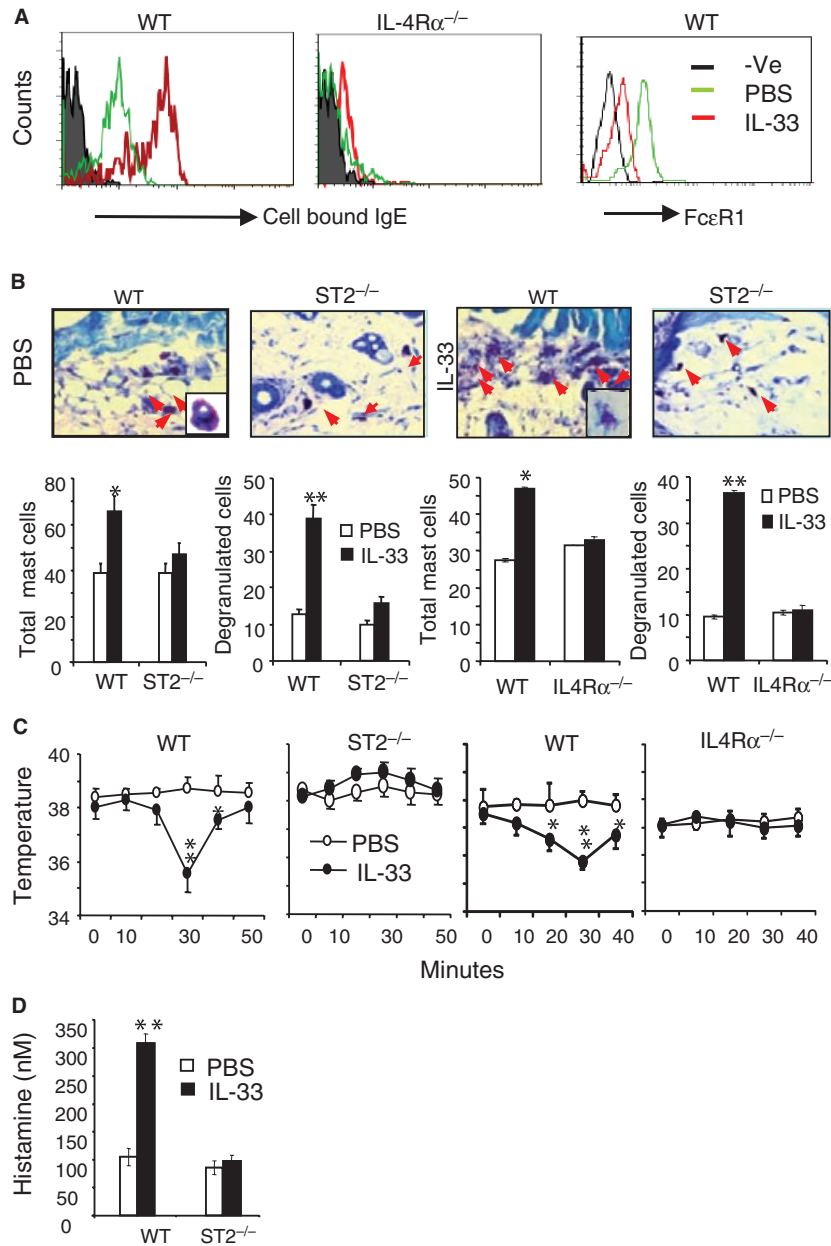
Because natural IgE can bind to mast cell Fc $\epsilon$ RI and impact mast cell functions (5–7), we sought to investigate what effect IL-33 and IL-33-derived IgE may have on mast cell function in naïve mice.

Naïve WT, ST2<sup>-/-</sup> or IL4R $\alpha$ <sup>-/-</sup> mice were injected with IL-33 or PBS daily for up to 3 days. IL-33 treatment markedly increased the mast cell surface IgE in naïve WT but not in IL4R $\alpha$ <sup>-/-</sup> mice as evidenced by enhanced levels of membrane-bound IgE and reduced levels of unoccupied Fc $\epsilon$ RI on mast cells (Fig. 5A). IL-33 treatment enhanced total and degranulated skin mast cells compared with PBS controls in WT but not ST2<sup>-/-</sup> or IL4R $\alpha$ <sup>-/-</sup> mice (Fig. 5B). More importantly, 30 min after the third IL-33 challenge, WT but not ST2<sup>-/-</sup> or IL4R $\alpha$ <sup>-/-</sup> mice showed clear signs of shock (piloerection, prostration, reduced response to stimuli), with a rapid reduction in body temperature lasting for about 15 min before returning to normal (Fig. 5C), and a substantial increase in serum histamine levels (Fig. 5D). Together, these results demonstrate that IL-33 can initiate mast cell degranulation and systemic anaphylaxis in naïve mice via IL4R $\alpha$ .

#### Discussion

Data reported in this study reveal a hitherto unrecognized effect and mechanism by which IL-33 induces IgE production and anaphylaxis in the absence of specific allergen (Fig. 6). IL-33 triggers IL-4 secretion by innate cells, which then stimulates B-cell expansion and IgE synthesis. In parallel, IL-4 enhances T-cell expression of CD40L, which interacts with CD40 on B cells to complement IgE production. IL-33 and IgE then synergistically trigger mast cell degranulation and anaphylaxis. Thus, IL-4 is the key intermediate in IL-33-mediated anaphylaxis *in vivo*. Because the mice were allergen naïve and we found that serum samples even from 7 daily injections of IL-33, which showed enhanced levels of total IgE, failed to show any detectable recombinant IL-33-specific IgE (data not shown) by ELISA (Data S1), it is likely, although not proven, that this IgE is natural IgE as described previously (3).

We demonstrated here that eosinophils and mast cells can produce IL-4 upon IL-33 treatment in naïve mice. However, it is likely that other ST2<sup>+</sup> innate cells such as basophils and NKT cells can similarly produce IL-4 (10, 14, 16). By contrast, T cells, which are the key cells expressing type-II cytokines in allergy, are not the major IL-4 producers in this innate immune context. This may be because naïve T cells do not express ST2



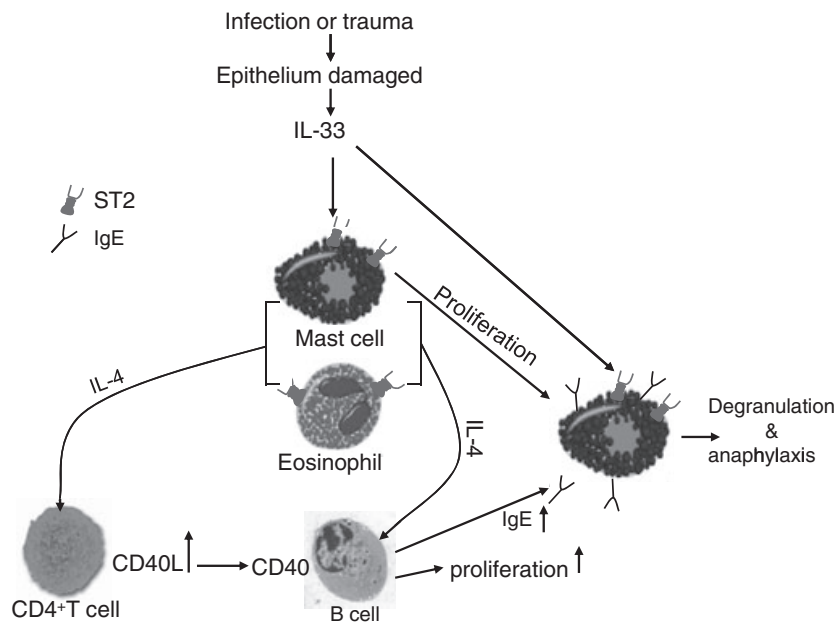
**Figure 5** IL-33 triggers anaphylaxis in naïve mice in the absence of allergen. Wild-type (WT), ST2<sup>-/-</sup> or IL-4Rα<sup>-/-</sup> mice were injected with IL-33 for 3 consecutive days. (A) The levels of peritoneal c-Kit<sup>+</sup> mast cell surface IgE and FcεR1 were determined by flow cytometry using specific antibodies. (B) Skin biopsies were stained with toluidine blue to determine the number of degranulated mast cells (indicated by arrows). (C) WT, ST2<sup>-/-</sup> or

IL-4Rα<sup>-/-</sup> mice were injected i.p. for 3 consecutive days with IL-33 or PBS, and their rectal temperature was determined for up to 50 min postinjection. (D) Mice were killed at the end of 50 min after the third IL-33 injection, and the histamine levels were determined by ELISA. Data are pooled from 3 experiments, *n* = 15 mice per group, \**P* < 0.05, \*\**P* < 0.01 compared to PBS-treated mice.

and hence are not responsive to IL-33 in the absence of T cell receptor activation (17, 18). In addition, it is known that IL-33 induces mainly IL-5 but not IL-4 in activated T cells and therefore cannot polarize Th2 cells (17).

Using nude mice and T-IL-4Rα-deficient mice, we demonstrated that T cells nevertheless play a significant role in

IL-33-derived IgE production in naïve mice. However, this process is unlikely to involve IL-4 production, because nude mice and T-IL-4Rα-deficient mice produced normal or elevated levels of IL-4 and yet had significantly less IgE compared to WT mice. Previous studies have shown that T cells expressing CD40L are required for the induction of



**Figure 6** Schematic representation of the mechanism of IL-33-induced IgE synthesis and anaphylaxis in naive mice. IL-33 binding to ST2<sup>+</sup> innate cells, such as mast cells and eosinophils, leads to mast cell proliferation and increased IL-4 synthesis. IL-4 would then activate B cells to proliferate and to produce IgE. IL-4

produced by the innate cells could also activate T cells to express CD40L that interacts with CD40 on B cells to further enhance IgE production. IgE, together with IL-33 and IL-4, would stimulate mast cells to degranulate, resulting in anaphylaxis.

natural IgE and that the CD40L expression can be enhanced by IL-2 (3, 27). Furthermore, IL-33 can induce IL-2 production in T cells (10). We show here that IL-33 can increase CD40L and CD25 expression on CD4<sup>+</sup> T cells via IL-4. It is therefore likely that IL-33-derived IL-4 and IL-2, alone or together, stimulate CD4<sup>+</sup> T cells to express surface CD40L, which subsequently interacts with B cells to promote IgE production.

Anaphylaxis (AS) is an acute, life-threatening allergic reaction in which IgE-mediated mast cell degranulation plays a critical role (28, 29). The aetiology of AS in particular idiopathic AS is not fully understood (28, 30). Our result demonstrated for the first time that IL-33/ST2 signals can trigger anaphylaxis in naïve mice in the absence of specific allergen (Fig. 5). While the detailed molecular mechanism is still unresolved, we found the IL-33-mediated allergen-independent mast cell degranulation and systemic AS described here was dependent on ST2, IL-4R $\alpha$  and required repeated IL-33 stimulation (Fig. 5C). Thus, the mechanism may comprise a combination of the following two components:

- 1 It is known that increased mast cell surface IgE expression and mast cell activation are the hallmark of mast cell degranulation and AS. We found that IL-33 can markedly enhance the levels of serum and mast cell surface IgE that is dependent on ST2 and IL-4R $\alpha$  in antigen-naïve mice. In addition, mast cells also express high levels of ST2, IL-4R $\alpha$  and IgE Fc $\epsilon$ R, and it is known that signals via these receptors play critical roles in mast cell activation and function. IL-33 can directly stimulate mast cell proliferation, cytokine production and

activation (Fig. 2B, C and ref. 19), and the signals from the interaction between IL-4/IL-13 and IgE with their receptors can activate and prolong the survival of mast cells as well as can exacerbate AS (6, 8, 31).

- 2 We also found that at least three daily IL-33 treatments were required for IL-33 to trigger mast cell degranulation and AS. Intriguingly, this was also the critical time required for IL-33 to elicit significant production of IgE and IL-4/IL-13 and mast cell proliferation (Figs 1A, 2B, C and 5).

Thus, our results suggest that while IL-33, IL-4 or IgE alone is insufficient to trigger mast cell degranulation without allergen, the accumulated number and activation status of mast cells, strong Fc $\epsilon$ R signals derived from continued and intensive interaction between IgE and its high-affinity receptor and repetitive IL-33/ST2 and IL-4R $\alpha$  signals may cumulatively or synergistically reach a signal threshold that leads to mast cell degranulation and AS.

IL-33 is clearly detected in various clinical allergic diseases including asthma and dermatitis (15, 32, 33). Given that many allergic reactions occur without a known allergen, including nonatopic asthma and idiopathic anaphylaxis (30, 34), IL-33 may play a critical role in these diseases. Supporting this hypothesis, we have measured serum IL-33 levels in atopic and nonatopic patients with severe asthma by ELISA. Our results showed that asthma subjects have a wide range of serum IL-33 levels and that there was no significant difference between atopic and nonatopic subjects (Fig. S1). This result is consistent with our finding in the animal model, as well as with a recent report that IL33

polymorphism is correlated with clinical asthma but not necessarily atopy (35).

Because IL-4 and IL-13 also play a pivotal role in antigen-specific IgE production, IL-33 may therefore have a general effect on all IgE-mediated allergic conditions. This concept suggests that IL-33 may represent a novel therapeutic target for a range of allergic diseases.

## Disclosures

The authors have no financial conflicts of interest.

## References

- Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008;**67**: 205–217.
- Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. *J Exp Med* 1989;**169**:1295–1307.
- McCoy KD, Harris NL, Diener P, Hatak S, Odermatt B, Hangartner L et al. Natural IgE production in the absence of MHC Class II cognate help. *Immunity* 2006;**24**:329–339.
- Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckmann MP, Park LS et al. Lymphokine control of in vivo immunoglobulin isotype selection. *Annu Rev Immunol* 1990;**8**:303–333.
- Asai K, Kitaura J, Kawakami Y, Yamagata N, Tsai M, Carbone DP et al. Regulation of mast cell survival by IgE. *Immunity* 2001;**14**:791–800.
- Kalesnikoff J, Huber M, Lam V, Damen JE, Zhang J, Siraganian RP et al. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 2001;**14**:801–811.
- Bryce PJ, Miller ML, Miyajima I, Tsai M, Galli SJ, Oettgen HC. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity* 2004;**20**: 381–392.
- Galli SJ, Kalesnikoff J, Grimaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as “tunable” effector and immunoregulatory cells: recent advances. *Annu Rev Immunol* 2005;**23**:749–786.
- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;**23**:479–490.
- Ali S, Huber M, Kolwe C, Bischoff SC, Falk W, Martin MU. IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proc Natl Acad Sci U S A* 2007;**104**:18660–18665.
- Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 2009;**31**:84–98.
- Trajkovic V, Sweet MJ, Xu D. T1/ST2—an IL-1 receptor-like modulator of immune responses. *Cytokine Growth Factor Rev* 2004;**15**:87–95.
- Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010;**107**:18581–18586.
- Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegel N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* 2009;**113**: 1526–1534.
- Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S et al. IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* 2009;**183**:6469–6477.
- Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int Immunol* 2008;**20**:1019–1030.
- Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC et al. IL-33 induces antigen-specific IL-5 + T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 2008;**181**:4780–4790.
- Xu D, Chan WL, Leung BP, Huang F, Wheeler R, Piedrafita D et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998;**187**:787–794.
- Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci U S A* 2008;**105**: 10913–10918.
- Xu D, Jiang HR, Li Y, Pushparaj PN, Kurowska-Stolarska M, Leung BP et al. IL-33 exacerbates autoantibody-induced arthritis. *J Immunol* 2010;**184**: 2620–2626.
- Mohrs M, Ledermann B, Kohler G, Dorfmueller A, Gessner A, Brombacher F. Differences between IL-4- and IL-4 receptor alpha-deficient mice in chronic leishmaniasis reveal a protective role for IL-13 receptor signaling. *J Immunol* 1999;**162**: 7302–7308.
- Radwanska M, Cutler AJ, Hoving JC, Magez S, Holscher C, Bohms A et al. Deletion of IL-4Ralpha on CD4 T cells renders BALB/c mice resistant to Leishmania major infection. *PLoS Pathog* 2007;**3**:e68.
- Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N et al. IL-33 reduces the development of atherosclerosis. *J Exp Med* 2008;**205**:339–346.
- Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today* 1994;**15**:19–26.
- Brewer JM, Conacher M, Hunter CA, Mohrs M, Brombacher F, Alexander J. Aluminum hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. *J Immunol* 1999;**163**:6448–6454.
- Komai-Koma M, Gilchrist DS, McKenzie AN, Goodyear CS, Xu D, Liew FY. IL-33 activates B1 cells and exacerbates contact sensitivity. *J Immunol* 2011;**186**:2584–2591.
- Yoshimoto T, Min B, Sugimoto T, Hayashi N, Ishikawa Y, Sasaki Y et al. Nonredundant roles for CD1d-restricted natural killer T cells and conventional CD4 + T cells in the induction of immunoglobulin E antibodies in response to interleukin 18 treatment of mice. *J Exp Med* 2003;**197**:997–1005.
- Golden DB. What is anaphylaxis? *Curr Opin Allergy Clin Immunol* 2007;**7**:331–336.
- Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in

## Supporting Information

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

**Figure S1.** Serum IL-33 levels are not associated with atopy.

**Data S1.** Methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.



- anaphylaxis. *J Allergy Clin Immunol* 2009;**124**: quiz 639–646.
30. Lenchner K, Grammer LC. A current review of idiopathic anaphylaxis. *Curr Opin Allergy Clin Immunol* 2003;**3**:305–311.
31. Strait RT, Morris SC, Smiley K, Urban JF Jr, Finkelman FD. IL-4 exacerbates anaphylaxis. *J Immunol* 2003;**170**: 3835–3842.
32. Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy* 2008;**38**:1875–1881.
33. Shimizu M, Matsuda A, Yanagisawa K, Hirota T, Akahoshi M, Inomata N et al. Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis. *Hum Mol Genet* 2005;**14**: 2919–2927.
34. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax* 1999;**54**:268–272.
35. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;**363**:1211–1221.