IL-33 induces histidine decarboxylase in mouse tissues, especially in c-kit+ cells and mast cells, and negatively regulates eosinophilia

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Abstract

Objective and Methods

IL-33 is present in endothelial, epithelial, and fibroblast-like cells and released upon cell injury. IL-33 reportedly induces mast-cell degranulation and is involved in various diseases, including allergic diseases. So, IL-33-related diseases seem to overlap with histamine-related diseases. In addition to the release from mast cells, histamine is newly formed by the induction of histidine decarboxylase (HDC). Some inflammatory and/or hematopoietic cytokines (IL-1, IL-3, etc.) are known to induce HDC, and the histamine produced by HDC induction is released without storage. We examined the involvement of HDC and histamine in the effects of IL-33.

Results

A single intraperitoneal injection of IL-33 into mice induced HDC directly and/or via other cytokines (including IL-5) within a few hours in various tissues, particularly strongly in hematopoietic organs. The major cells exhibiting HDC-induction were mast cells and c-kit$^+$ cells in bone marrow. HDC was also induced in non-mast cells in non-hematopoietic organs. HDC, histamine, and histamine H4 receptors (H4Rs) contributed to suppression of IL-33-induced eosinophilia.

Conclusion

IL-33 directly and indirectly (via IL-5) induces HDC in various cells, particularly potently in c-kit$^+$ cells and mature mast cells, and the newly formed histamine contributes to negative regulation of IL-33-induced eosinophilia via H4Rs.

Introduction

IL-33 is a member of the IL-1 family of cytokines and is released from endothelial cells, fibroblasts, and epithelial cells by tissue/cell damage [1]. IL-33 stimulates various types of immune cells (including Th2 cells, mast cells, basophils, eosinophils, and macrophages) by binding to its receptor, ST2, leading to the production of Th2 cytokines [2–5]. IL-33 is involved in a variety of diseases, such as anaphylaxis, asthma, atopic dermatitis, sepsis, inflammatory bowel diseases, rhinitis, and cancer in both mice and humans [6–9]. Indeed, recent clinical trials have demonstrated that an anti-IL-33 antibody and anti-ST2 antibody can improve atopic dermatitis, peanut allergy, and eosinophilic asthma [10–12].

Histamine, a biogenic amine, is involved in anaphylaxis, rhinitis, atopic dermatitis, allergy, and cancer in both mice and humans [13–18]. Interestingly, these diseases overlap widely with the IL-33-related diseases (see above). Histamine is formed by the enzyme histidine decarboxylase (HDC). HDC is an inducible enzyme in various tissues or organs in response to a variety of inflammatory stimuli [including

bacterial components such as lipopolysaccharide (LPS)] and inflammatory cytokines [IL-1-family cytokines (IL-1α, IL-1β, and IL-18), TNF-α, and IL-12], but not in response to IL-2, IL-6, or IFNg [19–21]. IL-1 is the most potent HDC-inducing cytokine among the cytokines tested, and the IL-1-induced increase in HDC activity is achieved via induction of the HDC protein via HDC-mRNA [22]. Notably, hematopoietic cytokines (IL-3, G-CSF, and GM-CSF) induce HDC in hematopoietic organs (spleen and bone: with G-CSF and GM-CSF inducing HDC *only* in these hematopoietic organs) [19, 23], indicating that HDC induction is involved not only in inflammation, but also in hematopoiesis [24]. Although mast cells and basophils release histamine via degranulation of histamine-storing granules following an antigen challenge, the histamine that is newly formed via HDC induction is released without first being stored [24–31].

IL-33 reportedly induces degranulation in both mast cells and basophils under the presence of non-antigenic IgE in non-sensitized mice [32, 33] as well as in sensitized mice [34], suggesting that IL-33 may release histamine from mast cells even in the absence of an antigen challenge. Further, Schneider et al. [35] found that IL-33 activates unprimed murine basophils directly in vitro and induces expansion of basophils in vivo indirectly via productions of GM-CSF and IL-3, suggesting that IL-33 may induce HDC in hematopoietic organs via GM-CSF and IL-3, because GM-CSF and IL-3 are HDC-inducing cytokines in hematopoietic organs (see above).

On the basis of the above background, we hypothesized that in addition to the release of histamine from mast cells, IL-33, like other IL-1-family cytokines, may also induce HDC *in various organs other than hematopoietic organs*, thereby causally contributing to various diseases. Here, we examined this hypothesis.

Materials And Methods

Animals

Wild-type (WT) BALB/c, C57BL/6N, c-kit-mutated-mast-cell-deficient mice (Slc:WBB6F1-W/W^{v}) and their normal litter-mate mice (Slc:WBB6F1- +/+), TLR4-mutated-LPS-resistant mice (C3H/HeJ) and their wild-type litter-mate mice (C3H/HeN) were purchased from SLC (Shizuoka, Japan). C57BL/6 HDC-KO mice were established as previously described [36]. BALB/c IL-1-KO mice (both IL-1a and IL-1b) were established as previously described [37]. A reporter mouse model utilizing a reporter gene of a green fluorescent protein (GFP) that enables study of *in vivo* HDC induction (HDC-GFP mouse) was established as previously described [38], and Line #1 (C57BL/6) of the mouse was kindly provided by Dr. Takashi Moriguchi (Tohoku Medical and Pharmaceutical University, Japan). Females (8-10 weeks of age) were used. Mice were housed in a specific pathogen-free environment under a 12 hour-light and dark cycle, 40-60% relative humidity, and 18-23 ºC ambient temperature in open cages with plastic bedding and nesting material in groups not exceeding 6 animals. Mice had ad libitum access to tap water and standard rodent chow. Mice were randomly housed and randomly assigned to experimental groups. All animal procedures were approved by the Institutional Animal Care and Use Committee of Tohoku University (approval

number: 2018DnA-013). All experiments complied with Regulations for Animal Experiments and Related Activities at Tohoku University.

Reagents

Recombinant mouse IL-1β, IL-3, IL-5, IL-13, IL-33, stem cell factor (SCF), and GM-CSF were purchased from Biolegend (San Diego, CA, U.S.A.). *E coli* O55:B5 LPS was purchased from Sigma-Aldrich (St. Louis, MO, USA). For in vivo experiments, these reagents were dissolved in saline. All other reagents were purchased from Nacalai Tesque (Kyoto, Japan), unless otherwise indicated.

Assays of HDC activity and histamine

***In vivo* experiments**: The indicated reagents were dissolved in saline and intraperitoneally (i.p.) injected. Mice were decapitated after the period indicated in a given experiment. Indicated tissues were rapidly removed before being frozen in a box containing dry ice and stored at -80°C until assayed for HDC activity. HDC activity and histamine in tissues and plasma were determined as described previously [39, 40]. HDC activity was expressed as nmol histamine formed during 1 hour incubation by the enzyme contained in 1 g wet weight of tissue (nmol/h/g). HDC activity of bone (tibia) was also expressed as nmol/h/g, although the enzyme is mostly only in the bone marrow [40]. The amount of histamine in both soft tissues and bone was expressed as its molar amount in 1 g wet weight of each tissue (nmol/g).

***In vitro* experiments:** Cells from bone marrow and spleen of C57BL/6N mice were collected and incubated at a final concentration of 5×10^6 cells/ml with the indicated concentrations of IL-33 in RPMI 1640 supplemented with 10 % serum and antibiotics in a 96-well plate. After incubation, HDC activity in cells was measured, as was histamine in cells and supernatant.

Assays of cytokines

In *in vivo* experiments, cytokines in serum were measured. In *in vitro* experiments, cytokines in supernatant were measured. Cytokines (IL-1β, IL-4, IL-5, IL-13, GM-CSF, and IgE) were measured using mouse ELISA kits (Biolegend).

Histology and immunohistochemistry

At 4 hours after injection of mice with IL-33, tibiae were fixed in 4 % paraformaldehyde-phosphate-buffer solution and embedded in paraffin. Sections (5 μm thick) were blocked with Blocking One (Nacalai Tesque) for 1 hour at room temperature, and staining was performed with a primary antibody [rabbit polyclonal anti-HDC, 1:50, Abcam (Cambridge, UK)] overnight at 4°C, followed by staining with a secondary antibody (HRP-conjugated anti-rabbit IgG, 1:1000, Abcam) for 60 min at room temperature. Sections were then treated with DAB (Nacalai Tesque) for 5 min at RT and counterstained with hematoxylin. Images were acquired using a BZ-9000 fluorescence microscope (Keyence, Tokyo, Japan).

Blocking ST2 (IL-33 receptor), GM-CSF, and IL-5

Anti-ST2 antibody, anti-GM-CSF antibody, and anti-IL-5 antibody (Biolegend) were dissolved in saline and i.p. injected (200 μg/mouse) at 24 hours before IL-33 injection.

Depletion of c-kit$^+$ cells from bone marrow cells

Bone marrow cells were harvested from tibias and red blood cells were lysed using lysis buffer. The cells were incubated with 10 μg/ml TruStain FcX antibody (a blocking agent of non-specific binding of immunoglobulin to Fc receptors) (Biolegend) for 15 min on ice and stained with PE-labeled anti-c-kit antibody for 30 min on ice. c-Kit$^+$ cells were depleted by means of an EasySep PE selection kit, used according to the manufacturer's protocol.

Bone marrow-derived mast cells (BMMCs)

Bone marrow cells obtained from non-stimulated mice were cultured with 10 μg/ml IL-3 and SCF for 30 days in RPMI 1640 supplemented with 10 % serum and antibiotics [41]. Purity of mast cells was confirmed by flow cytometry, which revealed purity > 99 % (Fig. 6e).

Treatments with histamine and histamine-receptor (HR) antagonists

Histamine: Histamine dissolved in saline was i.p. injected (500 μg/kg). The dose was determined on the basis of our previous study [15] and this dose of histamine did not induce any toxic effects, such as bristling, weight loss, or anaphylaxis.

HR antagonists: Pyrilamine (H1R antagonist), cimetidine (H2R antagonist), and JNJ7777120 (H4R antagonist) were dissolved in dimethyl sulfoxide (DMSO) and diluted with phosphate-buffered saline (final, 10 % DMSO). These reagents were i.p. injected (each at 25 mg/kg) at 4 hours before an injection of IL-33 every other day (i.e., total, 3 times). Our previous study [42] served as the basis on which we determined the dose and administration method.

Flow cytometry

A given ear-pinna was cut into small pieces and incubated with RPMI 1640 solution containing 10 % fetal bovine serum and 1 mg/ml collagenase IV (Sigma-Aldrich) for 2 hours at 37 °C in a shaking water-bath. Bone marrow cells were obtained from tibia. The cells were washed with PBS and passed through a 70 μm cell-strainer. Red blood cells were lysed using lysis buffer and the cells were incubated with 10 μg/ml TruStain FcX antibody (Biolegend) for 15 min on ice. The cells were then stained for 30 min on ice with Pacific Blue-labeled anti-CD45 antibody, APC/Cy7-labeled anti-CD11b antibody, PE/Cy7-labeled anti-CD11c antibody, FITC-labeled anti-Gr-1 antibody, Alexa647-labeled anti-siglec F antibody, PE/Cy5-labeled lineage markers (CD3, CD11b, CD19, B220, Gr-1, and Ter119), Alexa647-labeled anti-c-kit antibody, Alexa488-labeled anti-sca-1 antibody, APC/Cy7-labeled anti-sca-1 antibody, PE-labeled anti-IgE antibody, APC-labeled anti-CD49b antibody, PE/Dazzle594-labeled anti-ST2 antibody, APC/Cy7-labeled anti-FcεR1

antibody, or PE-labeled anti-siglec F antibody (BioLegend). Dead cells were stained with DAPI (Dojindo, Kumamoto, Japan) and excluded from further analysis. Data were acquired on an LSRFortessa cell-analyzer (BD Biosciences, San Diego, CA, USA) and analyzed using FlowJo software (BD Biosciences). In the histograms comparing the distributions of various groups of cells (among which the numbers were markedly different), the scale on the vertical axis was modified for easy comparison and is shown as “Cell number (normalized to mode)”. The scale on the horizontal axis representes fluorescence intensity.

Statistical analysis

Experimental values are given as the mean ± SD. Statistical significances were analyzed using a Bonferroni multiple comparison test after ANOVA with the aid of JMP software (SAS Institute, Cary, NC, USA). Except where otherwise mentioned, the experimental results were confirmed by repeating the experiment at least once.

Results

IL-33 induces HDC activity and histamine release in various tissues, particularly in bone

A single i.p. injection of IL-33 (50 mg/kg) into C57BL/6 mice increased HDC activity in several tissues (Fig. 1a). IL-33 increased HDC activity in bone (tibia), spleen, liver, and lung, peaking 2 to 4 hours after the injection (Suppl. Fig. 1a). The increase in HDC activity was dependent on the dose of IL-33, and bone was the most sensitive, with 12.5 mg/kg IL-33 inducing a significant increase in bone HDC activity (Suppl. Fig. 1b). We confirmed HDC expression in the bone marrow regions of bone (tibia) by immunochemistry (Fig. 1b) and by using HDC-GFP mice (Fig. 1c). IL-33 increased HDC-expressing cells from 7.3% to 23.2% of live cells (Fig. 1c). These results indicate that IL-33 increases HDC activity in various tissues, particularly potently in the hematopoietic organs bone and spleen, bone being the most sensitive and greatest HDC-inducing organ.

Consistent with the increase in HDC activity, IL-33 increased histamine in plasma (Fig. 1d). However, changes in the level of histamine induced by 50 mg/kg IL-33 were variable among the organs tested (Suppl. Fig. 1c). Interestingly, histamine was reduced significantly in the bone, while it increased or tended to increase in the other tissues. It was also notable that the levels of HDC activity and histamine in non-stimulated mice (i.e., non-injected or saline-injected) were highest in bone (Figs. 1a and Suppl. Fig. 1c). These results indicate that (i) injection of IL-33 into mice induces HDC in various tissues, especially in the hematopoietic organs (bone and spleen), (ii) the histamine newly formed by such HDC-induction is largely released without storage, and (iii) in non-stimulated mice, bone marrow has the highest levels of both HDC and histamine.

Single i.p. injection of IL-33 increases IL-5, IL-13, and GM-CSF in serum, but not IgE

As described above, a single i.p. injection of IL-33 (12.5-100 mg/kg) induces HDC in various tissues. Repeated i.p. injection of IL-33 into mice (20-200 mg/kg, once a day for 7 days) reportedly induces Th2

cytokines (such as IL-4, IL-5, and IL-13) and IgE via stimulation of ST2 (the receptor for IL-33) [2]. *In vitro*, IL-33 reportedly stimulates bone-marrow-derived eosinophils to produce Th2 cytokines, including GM-CSF [43]. Interestingly, it has also been reported that (i) IL-33 induces degranulation of mast cells under the presence of IgE [32, 33] (i.e., IL-33 may induce histamine release), and (ii) aggregated IgE induces HDC *in vitro* in murine hematopoietic progenitors (but not mature cells, including mast cells) [44]. As described in Introduction, HDC is induced by various cytokines, and IL-1 (either a or b) is especially potent at inducing HDC. Hence, we measured these cytokines and IgE in the serum after a single injection of IL-33 (50 mg/kg) and examined whether these cytokines induce increases in HDC activity. As shown in Fig. 2a, a single i.p. injection of IL-33 into mice increased IL-5, IL-13, and GM-CSF (but not IL-1b or IL-4) in the serum at 4 hours after the injection (the peak time for induced HDC activity). However, IgE was not increased by such a single injection.

IL-33 exerts its effects via stimulation of its receptor ST2

In the following experiments, the spleen was used for measuring HDC activity. As shown in Fig. 2b, injection of an antibody to ST2 completely prevented the induction of HDC activity by IL-33. Anti-ST2 antibody also prevented any IL-33-induced increase in IL-5, IL-13, or GM-CSF in the serum (Suppl. Fig. 2a).

IL-33 may induce HDC activity in hematopoietic organs directly and/or via mediation by other cytokines, including IL-5

As shown in Fig. 2c, among the IL-33-induced cytokines (see Fig. 2a), injection of GM-CSF and IL-5 increased HDC activity, while IL-13 did not. IL-33 increased HDC activity in C3H/HeJ mice [TLR4-mutated/inactive mice (i.e., non-responsive to LPS)] and in mice deficient in both IL-1a and IL-1b (IL-1-KO mice) (Figs. 2d and 2e), indicating that neither IL-1 nor contaminating LPS is involved in the induction by IL-33 of HDC. Thus, it seemed possible that the HDC induction by IL-33 might be mediated by GM-CSF and/or IL-5. However, as shown in Fig. 2f, anti-GM-CSF antibody had no significant effect on the IL-33-induced HDC activity, although it did tend to reduce it. On the other hand, anti-IL-5 antibody significantly (but not completely) reduced the IL-33-induced HDC activity (Fig. 2g). The anti-GM-CSF and anti-IL-5 antibodies used here were effective at blocking the respective cytokines produced by IL-33 (Suppl. Fig. 2b and 2c). These results suggest that IL-33 may induce HDC in hematopoietic organs directly and/or via mediation by other cytokines, including IL-5.

IL-33 increases HDC in hematopoietic cells *in vitro* and releases the newly-formed histamine without it being stored

As described above, IL-33 induces HDC especially in the hematopoietic organs bone and spleen (Fig. 1a). So, we examined whether IL-33 increases HDC activity and histamine *in vitro* in cells isolated from bone marrow and spleen. As shown in Fig. 3a, bone marrow cells exhibited greater increases in HDC activity and histamine than spleen cells. Thus, the following experiments were performed using bone marrow cells. As shown in Fig. 3b, HDC activity and histamine each increased in a manner dependent on the concentration of IL-33. In the following experiments, we used 100 ng/ml IL-33. As shown in Fig. 3c, the

increase in HDC activity peaked within 24 hours, and histamine continued to accumulate in the culture supernatant throughout the 96 hours of incubation. Although histamine increased both in the cells and in the supernatant (Fig. 3d), its increase was greater in the supernatant. These results indicate that IL-33 induces HDC in hematopoietic cells and that most of the newly formed histamine is released from the cells without first being stored.

HDC-inducing cells in bone marrow in response to IL-33 are c-kit-positive cells

SCF is a cytokine essential for the proliferation of hematopoietic stem cells (HSCs), and c-kit is the receptor for SCF. Since bone marrow cells exhibited a greater ability to induce HDC than spleen cells (Fig. 3a), we compared the profiles of the cells expressing c-kit and ST2 between bone marrow and spleen. In the route underlying hematopoiesis, c-kit is expressed in HSCs and myeloid progenitor cells, while sca-1 (stem cell antigen-1) is expressed in HSCs but not in myeloid progenitor cells [45, 46]. In mice, lineage marker-negative (lin^-) (see Methods) sca-1^+ and c-kit^+ cells [i.e., lin^-sca-1^+c-kit^+ cells (called LSK cells)] are used as HSCs. LSK cells differentiate to lin^-sca-1^-c-kit^+ cells (called LK cells). Both LK cells and neutrophils ($CD45^+CD11b^+CD11c^-Gr\text{-}1^+$) were more abundant in the bone marrow than in the spleen (Fig. 4a and 4b), and ST2 was observed only in LK cells (Fig. 4c). Hence, we examined whether LK cells include HDC-inducing cells. IL-33 increases HDC-expressing cells in LK cells from 21.2% to 38.3% (Fig. 5a). Next, c-kit^+ cells were depleted from bone marrow cells (see Methods) and the c-kit^+ cell-depleted bone marrow cells were stimulated with IL-33 for 72 hours. As shown in Fig. 5b, IL-33-induced HDC activity was markedly reduced by such depletion of c-kit^+ cells. W/W^v mice are mast-cell-deficient mice due to c-kit mutation, and these mice lack LK cells, too (Fig. 5c). As shown in Fig. 5d and Suppl. Fig. 3a, IL-33-induced HDC activity in the bone and spleen was much smaller in W/W^v mice than in control +/+ mice. These results indicate that the HDC-inducing cells in bone marrow in response to IL-33 are c-kit^+ cells (i.e., cells expressing SCF receptors).

Other HDC-inducing cells in response to IL-33

Mast cells: In mice, mast cells are the main source of histamine in skin, lung, thymus, spleen [47], skeletal muscle [48], and in bone marrow-removed bone [40]. So, we examined whether IL-33 induces HDC in mast cells. As shown in Fig. 1a, HDC activity is detected at a significant level in the ear-pinnae of saline-injected (i.e., non-stimulated) mice and is augmented by IL-33. Mast cells in WT ear-pinnae express the ST2 receptor (Fig. 6a). Experiments using HDC-GFP mice demonstrated that IL-33 induced HDC in ear-pinnae without affecting the frequency of HDC-expressing cells in mast cells (Fig. 6b), indicating that IL-33 induces HDC mostly in the same mast cells. In the skin (ear-pinna) of W/W^v mice, mast cells were few (Fig. 6c), and IL-33 did not increase HDC activity at all in the ear-pinna of W/W^v mice (Fig. 6d). Mast cells purified from bone marrow (BMMCs) (see Methods) also express ST2 (Fig. 6f), and HDC activity and histamine were markedly increased by *in vitro* stimulation of BMMCs with IL-33 (Fig. 6g). These results clearly indicate that IL-33 can induce HDC in mature mast cells in various tissues, as well as skin.

Other cells: It was notable that in W/W^{v} mice, IL-33 augmented the low, but significant, HDC activity present in bone (Fig. 5d), lung, and spleen (Suppl. Fig. 3a), indicating that HDC is induced by IL-33 in cells other than mast cells and c-kit-positive cells. It has been reported that IL-33 directly activates basophils and induces degranulation [35]. So, we examined basophils. In mice depleted of their basophils by an antibody specific for mouse basophils (Ba103), the HDC activity induced by IL-33 in the spleen was essentially the same as that induced in mice not depleted of their basophils (Suppl. Figs. 3b and 3c), suggesting that basophils may be not involved in HDC induction by IL-33. Thus, it remains to be clarified which cell-types, in addition to mast cells and c-kit^{+} cells, can exhibit HDC-induction in response to IL-33.

Comparison of IL-33 with other HDC-inducing cytokines

In the present study, we found that IL-33 induced HDC activity in various tissues, but particularly potently in hematopoietic organs (bone and spleen). As described in Introduction, IL-1 and LPS are potent HDC inducers, and induce HDC in a number of tissues. So, here, we compared induced HDC activities between hematopoietic (bone and spleen) and non-hematopoietic (lung and liver) tissues in mice injected i.p. with IL-33, IL-1, and LPS. In this study, we also compared the effects of other cytokines (IL-3, IL-5, and IL-13). As shown in Suppl. Fig. 4, their abilities to induce HDC were as follows. In the bone: IL-33 ³ LPS ³ IL-3 ³ IL-1b > IL-5; and in the spleen: LPS ³ IL-1b ³ IL-3 ³ IL-33 ³ IL-5. In the lung, the ability of IL-33 was much lower than those of IL-1b and LPS. In the liver, the abilities of IL-33, IL-1b, and IL-3 were similar to each other, while LPS was much more potent. IL-5 did not induce HDC in the lung or liver, and IL-13 induced HDC in none of the tissues examined. Thus, LPS is the most potent HDC-inducer in tissues other than bone. In this study, we found that like IL-33, IL-3 can induce HDC in non-hematopoietic tissues, too.

IL-33-induced newly formed histamine negatively regulates eosinophilia partly via H4Rs

Repeated i.p. injection of IL-33 into mice (once a day for 7 days) has been reported to induce eosinophilia [2]. Such a treatment of mice with IL-33 is also reported to induce a systemic increase in IL-5, and an anti-IL-5 antibody ablates the IL-33-induced eosinophilia [49]. As described above, IL-33 increases HDC activity in various tissues (including bone marrow and spleen) and IL-5 was also shown to increase HDC activity in the spleen. Finally, therefore, we examined whether HDC/histamine is involved in IL-33-induced eosinophilia. Mice were i.p. injected with IL-33 and/or histamine every other day for a total of 3 times, and eosinophils in the bone marrow and IL-5 in the serum 24 hours after the last injection were analyzed (Fig. 7a). IL-33-induced eosinophilia was much greater in HDC-KO mice than in WT mice, and this effect was markedly reduced by the addition of histamine (Figs. 7a and c). This IL-33-induced eosinophilia in the bone marrow of WT mice was slightly but significantly augmented by the H4R antagonist JNJ7777120 (Figs. 7b and c). In addition, IL-33 increased the serum level of IL-5 (Fig. 7d). Although histamine and histamine-receptor antagonists did not influence this level in WT mice, the IL-33-induced serum IL-5 level was greater in HDC-KO mice than in WT mice, and this elevated level was markedly reduced by the addition of histamine (Fig. 7d). These results indicate that (i) IL-33-induced newly formed histamine suppresses or negatively regulates IL-33-induced IL-5-mediated eosinophilia, and (ii) H4Rs are partly involved in this effect of histamine.

Discussion

In the present study, we examined the effects of single i.p. injections of IL-33 on HDC activity and histamine in mice and obtained the following results. (i) IL-33 induces HDC activity in various tissues, especially strongly in the hematopoietic organs, and IL-33 also has the ability to release histamine. (ii) In non-stimulated mice, bone marrow has the highest levels of HDC activity and histamine. (iii) IL-33 increases IL-5, IL-13, and GM-CSF in the serum, but not IgE (a supposed factor involved in HDC induction and mast cell degranulation). (iv) IL-33 exerts its effects via stimulation of ST2 receptors. (v) IL-33 may induce HDC activity in hematopoietic organs directly and/or via other HDC-inducing cytokines, including IL-5. (vi) In vitro, IL-33 increases HDC activity in hematopoietic cells and releases the newly formed histamine without it first being stored. (vii) The HDC-inducing cells that respond to IL-33 are c-kit$^+$ hematopoietic cells (including mast cell precursors) and mature mast cells. (viii) The ability of IL-33 to induce HDC in bone is comparable to those of IL-1, LPS, and IL-3. (ix) IL-33-induced newly formed histamine may negatively regulate IL-33-induced eosinophilia partly via H4Rs. We now discuss these findings.

Involvement of IL-33-induced HDC/histamine in hematopoiesis

Histamine is a typical inflammatory mediator. However, in non-stimulated C57BL/6 mice, bone marrow is the organ highest in the levels of HDC activity and histamine, this being the case in BALB/c mice, too [50]. In the present study, IL-33 induced HDC activity in various tissues, but most strongly in bone. Although i.p. injected IL-33 or IL-1β increased HDC activity in the ear-pinnae, we detected no visible inflammation (such as swelling) in the ear-pinnae. Thus, the inflammation induced by newly formed histamine is not by itself severe, and the observations in the present study suggest that IL-33-induced HDC/histamine may play important roles in hematopoiesis.

Mechanism underlying the induction of HDC activity by IL-33

A single i.p. injection of IL-33 into mice increased IL-5, IL-13, and GM-CSF (but not IL-1β or IL-4) in the serum at 4 hours after the injection (the peak time for induced HDC activity). These effects of IL-33 were shown to be mediated via ST2. Among the cytokines induced by IL-33, IL-5 was shown to mediate the induction of HDC activity by IL-33, although its contribution is partial. Interestingly, ST2 is reportedly expressed on hematopoietic progenitor cells, and these progenitor cells have been shown to be reduced in ST2-KO mice [49, 51–53]. Thus, IL-33/ST2 may induce HDC activity directly and/or via mediation by other cytokines (including IL-5).

c-Kit$^+$ cells as main HDC-inducing cells in bone marrow in response to IL-33

Previous studies have reported that in non-stimulated mice, the main HDC-expressing cells in the bone marrow are CD11b$^+$Gr1$^+$ immature myeloid cells, although other cell populations such as c-kit$^+$ myeloid progenitor cells, bone stromal cells, T cells, and B cells also express HDC mRNA [18, 54]. In the present study, we observed that in non-stimulated mice, the IL-33 receptor ST2 was expressed mainly in c-kit$^+$

myeloid progenitor cells among bone marrow cells, while its expression was slight in $CD11b^+Gr1^+$ cells. Thus, among the various HDC-expressing cells in the bone marrow of non-stimulated mice, $c\text{-}kit^+$ cells may be the main HDC-inducing cells in this tissue's response to IL-33.

HDC induction in mast cells by IL-33

The histamine in skin is stored mostly within mast cells [47]. However, recent studies have demonstrated that in skin, HDC can be induced in cells other than mast cells (such as keratinocytes) in response to surfactant, LPS, house dust, or TNF-a [55, 56]. In the present study, IL-33 did not increase HDC activity at all in the ear-pinnae of mast-cell-deficient W/W^v mice (Fig. 6d), indicating that mast cells are the only cells in which HDC is involved in response to IL-33 in ear-pinnae, and suggesting that mast cells may be an important HDC-inducing cell-type in other non-hematopoietic tissues (such as lung and bone).

Release of the histamine newly formed in mast cells by IL-33

In the bone of non-stimulated mice, most of the histamine is present in mast cells in bone tissue (not in bone marrow), although HDC activity is present in bone marrow [40]. It is well known that mast cells store histamine and release the stored histamine upon antigen challenge to animals sensitized to the same antigen. The reduced pool of histamine remaining in mast cells following such release is replenished by HDC induction [57]. Indeed, antigen-induced HDC activity is markedly low in W/W^v mice [57]. On the other hand, a variety of inflammatory stimuli (including cytokines), induce HDC in various cells other than mast cells, and the newly formed histamine produced by the HDC induction is rapidly released without being stored, and has often been called "non-mast-cell histamine" (see Introduction). In the present study, an i.p. injection of IL-33 into mice was shown to decrease histamine in bone, although IL-33 markedly induced HDC activity (Suppl. Figure 1c). *In vitro*, IL-33 increased HDC activity in hematopoietic bone marrow cells and released the newly formed histamine without it being stored. Thus, IL-33 induces release of the histamine newly formed by HDC induction.

Release by IL-33 of the pooled histamine in mast cells

In non-stimulated mice, the histamine in bone tissues (excluding bone marrow) is largely contained in mast cells (40), and even the histamine in bone including bone marrow is reduced in IL-33-injected mice (Suppl. Figure 1c), indicating clearly that IL-33 has a potent ability to release the histamine pooled in mast cells. Notably, repeated injection of IL-33 reportedly increases IgE, and IL-33 induces degranulation of mast cells under the presence of IgE [32, 33]. In our experiment, although a single i.p. injection of IL-33 did not increase IgE, IgE was present in the serum of saline-injected mice (Fig. 2a). This IgE, therefore, may induce degranulation of mast cells in IL-33-injected mice (cooperatively with IL-33). Thus, IL-33 induces the release of two sources of histamine; namely, the histamine newly formed via HDC and the histamine pooled in granules within mast cells.

Involvement of histamine in IL-33-induced eosinophilia

We found that on IL-33-induced eosinophilia, histamine plays a negative regulatory role, with H4Rs being partially involved. In accordance with this finding, the lung eosinophilia caused by an antigen-challenge to mice sensitized to the same antigen (ovalbumin) is reduced in HDC-KO mice [58, 59]. Histamine also reportedly upregulates eosinophil migration and adhesion via H4Rs [60]. However, it has also been reported that histamine is involved in IL-4-driven eosinophilic allergic responses via *H2Rs* [61], and that histamine *reverses* IL-5-afforded human eosinophil survival by inducing apoptosis [62]. These complicated effects of histamine on eosinophils might be related to the limited effect of JNJ7777120 observed in our experiment.

Roles of histamine and H4Rs in hematopoiesis

Byron reported in 1977 that endogenous histamine may trigger (via H2Rs) the hematopoietic stem cell to pass from the G0 into the S-phase [63]. Nakaya and Tasaka reported in 1988 that histamine promotes proliferation and differentiation of granulocytic myeloid cells via H2Rs [64]. Later, Dy's group reported that IL-3-induced HDC/histamine and H2Rs are involved in IL-3-induced proliferation of hematopoietic stem cells, as evaluated by examining colony formation in the spleen of irradiated mice [65]. Although the histamine-producing cells have not been identified, neither mast cells nor their committed precursors exhibit HDC-induction in response to IL-3 [66]. The same group has also reported that histamine mediates the blockade of growth factor-induced entry of hematopoietic progenitors into the cell cycle via H4Rs [67, 68]. Importantly, Ohtsu et al. found that in HDC-KO mice, mast cells are markedly reduced, the structure of the remaining mast cells in abnormal, and the amounts of mast cell granular proteases are tremendously reduced, even though HDC-deficient mice are viable and fertile [36]. H4Rs are expressed primarily on bone marrow cells (within progenitor compartment c-kit$^+$sca-1$^+$ cells) [66] and eosinophils [69]. Recently, we clarified that histamine newly formed via HDC-induction in ear-pinnae augments inflammation via H4R stimulation when lipopolysaccharide is co-administered with a nitrogen-containing bisphosphonate [70]. Collectively, all this leads us to suppose that histamine newly formed via HDC-induction may play important roles in hematopoiesis not only in the normal state (particularly in the production of mast cells), but also in emergencies (such as infection and cell/tissue damage). Possibly, the histamine stored in mast cells may be used when histamine is urgently required, while the newly formed histamine may be used when there is a prolonged requirement. We also suppose that H4Rs may be important in mediating the actions of the histamine newly formed not only in hematopoietic organs, but also in other tissues.

HDC-inducing cytokines

In normal mice, IL-33 behaved like other IL-1-family cytokines (IL-1α, IL-1β, and IL-18) in increasing HDC activity in various organs. In mast cell-deficient W/W^v mice, IL-33 induced a low but significant level of HDC activity in lung, spleen, and bone, but none at all in the ear-pinna. These results suggest that the HDC-inducing activity of IL-33 is greater for mast cells than for non-mast cells. In contrast, IL-1 and LPS induce HDC activity similarly between W/W^v and +/+ mice [50], suggesting that the HDC-inducing activities of IL-1 and LPS are greater for non-mast cells than for mast cells. IL-12 (which shares functional properties with IL-18, including anti-tumor effects) also induces HDC in various tissues [21]. IL-

33 is a stimulator of Th2 responses, while IL-12 is a stimulator of Th1 responses, suggesting that histamine is involved in both Th1 and Th2 responses. In addition, the present study has demonstrated for the first time that IL-5, like G-CSF and GM-CSF, induces HDC only in hematopoietic organs, and that IL-3, like IL-33, induces HDC in various organs but particularly potently in bone. Thus, IL-3 may also be not only a hematopoietic cytokine, but also an inflammatory cytokine.

Summary And Conclusion

A single i.p. injection of IL-33 into mice induces HDC activity in various tissues within a few hours of the injection, particularly strongly in the bone marrow. c-Kit$^+$ cells in the bone marrow and mature mast cells are the major HDC-inducing cells in the response to IL-33. IL-33 also induces HDC in non-mast cells in non-hematopoietic tissues. The newly formed histamine produced in response to IL-33 is released without first being stored. IL-33 also releases the histamine stored in mast cells. Thus, IL-33 induces histamine release in two ways: both newly formed and mast-cell-stored histamine. IL-33-induced histamine suppresses IL-33-induced eosinophilia via histamine receptors including H4Rs (summarized in Suppl. Figure 5). Collectively, this leads us to suppose that via HDC-induction and histamine release, IL-33 may influence hematopoiesis, inflammation, and immune responses (i.e., defense responses against various harmful stimuli), and that H4Rs may be important in the effects of the newly formed histamine. It was also clarified that IL-33 induces HDC not only in hematopoietic but also in non-hematopoietic organs, while IL-5 induces HDC only in hematopoietic organs.

Declarations

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Author contributions

Study designed and conducted by KB, YT, SS, IM, and YE. Data collected by KB. Data analyzed by KB and YE. Technically assisted by YT and SW. Manuscript written by KB and YE and approved by all authors.

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Conflict of interest

Authors have no conflicts of interest in this study.

Ethics approval statement

All animal procedures were approved by the Institutional Animal Care and Use Committee of Tohoku University (approval number: 2018DnA-013). All experiments complied with Regulations for Animal Experiments and Related Activities at Tohoku University.

Consent for publication

Not applicable.

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Figures

Figure 1

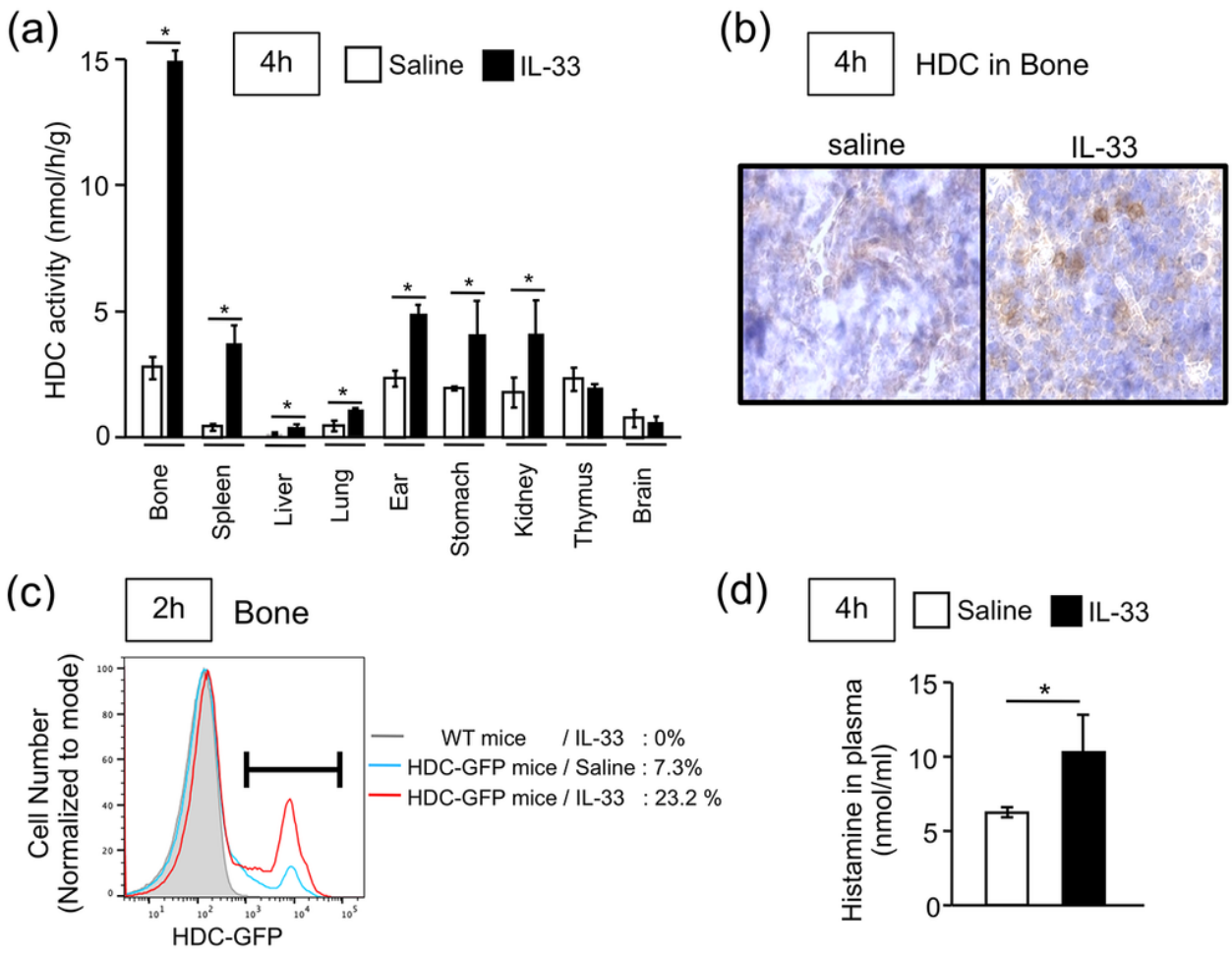

Figure 1

IL-33 and HDC/histamine may play a role in hematopoietic organs. IL-33 (50 μg/kg) or saline was i.p. injected into C57BL/6N mice. (**a**) HDC activities in various organs 4 hours after the injection of IL-33. *P < 0.01 vs saline (n = 4). (**b**) HDC-expressing cells (brown) within bone marrow region of tibia 4 hours after injection of IL-33. Results are representative of four independent experiments. (**c**) Representative flow cytometry. Values given for GFP^+ indicate the percentage of HDC^+ cells among live cells in the bone marrow of tibia 2 hours after injection of IL-33 into HDC-GFP mice. Blue-line histogram represents saline-injected HDC-GFP mice, red-line histogram represents IL-33-injected HDC-GFP mice, while gray-shaded histogram represents WT controls. Each value is the mean (n = 4). (**d**) Histamine in plasma 4 hours after injection of IL-33. *P < 0.01 vs saline (n = 4).

Figure 2

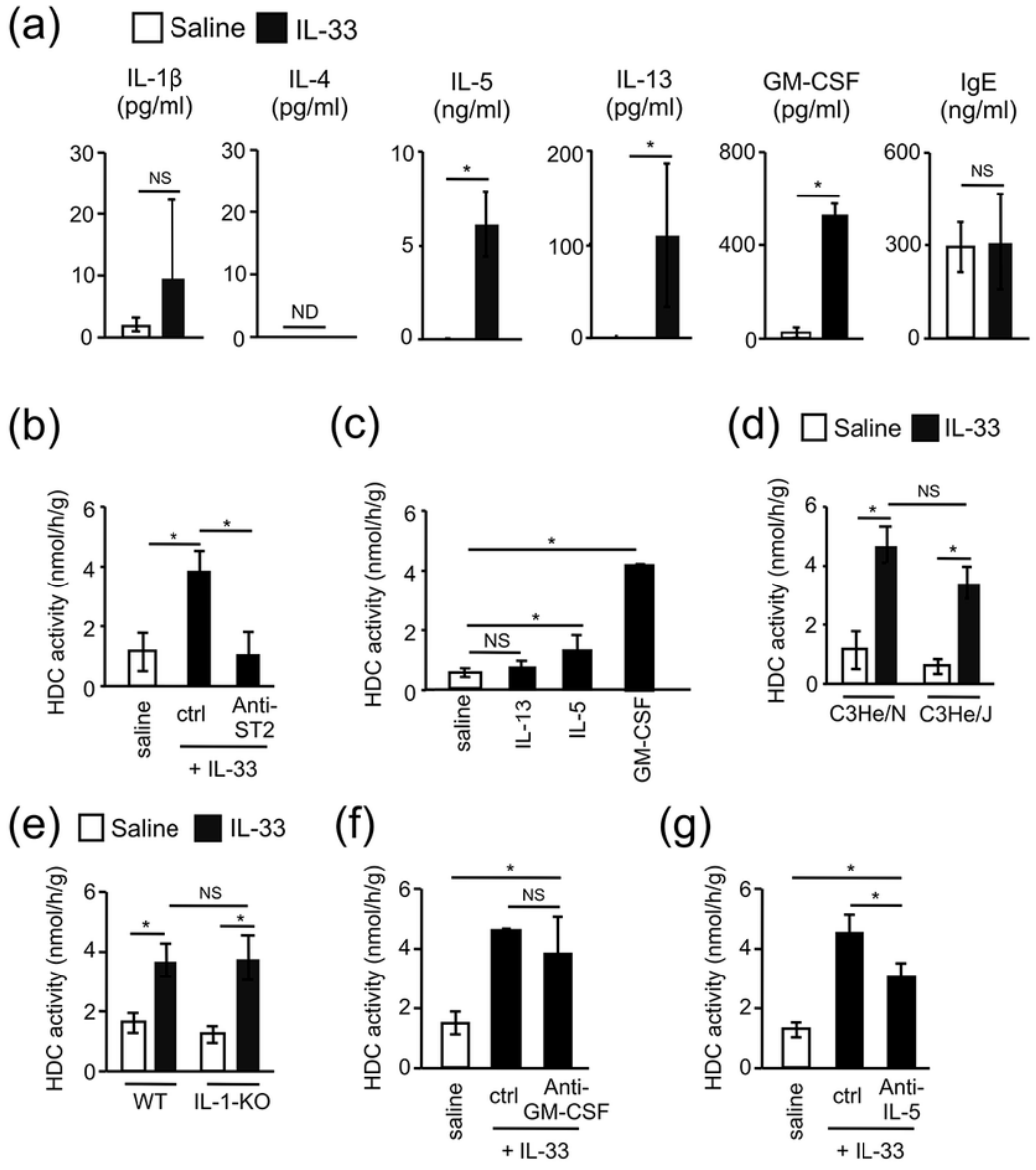

Figure 2

IL-33 induces HDC activity via ST2 receptor and partially via IL-5. In the following experiments, the dose of IL-33 was 50 μg/kg, and HDC activity in the spleen was measured 4 hours after i.p. injection of either IL-33 or the indicated substance. Unless otherwise mentioned, C57BL/6 mice were used. (**a**) Effects of IL-33 on serum levels of cytokines. IL-33 was injected into mice and the indicated cytokines in the serum were determined 4 hours after the injection. (**b**) Effects of IL-33-receptor blockade on the induction of HDC activity by IL-33. Control antibody (ctrl) or anti-ST2 antibody (200 mg/mouse) was injected into mice 24 hours before the injection of IL-33. (**c**) Abilities of cytokines to induce HDC activity. Each indicated

substance (50 μg/kg) was injected into mice, and 4 hours later, HDC activity in the spleen was analyzed. (**d**) Induction of HDC activity by IL-33 in C3H/HeJ (HeJ) mice (TLR4-mutated) and their control C3H/HeN (HeN) mice. (**e**) Induction of HDC activity by IL-33 in IL-1-KO mice. (**f-g**) Effects of anti-GM-CSF and anti-IL-5 antibodies on the induction of HDC activity by IL-33. Control antibody (ctrl) or one of the test antibodies (200 mg/mouse) was i.p. injected into mice 24 hours before the injection of IL-33. In all panels, *P < 0.01. (n = 4).

Figure 3

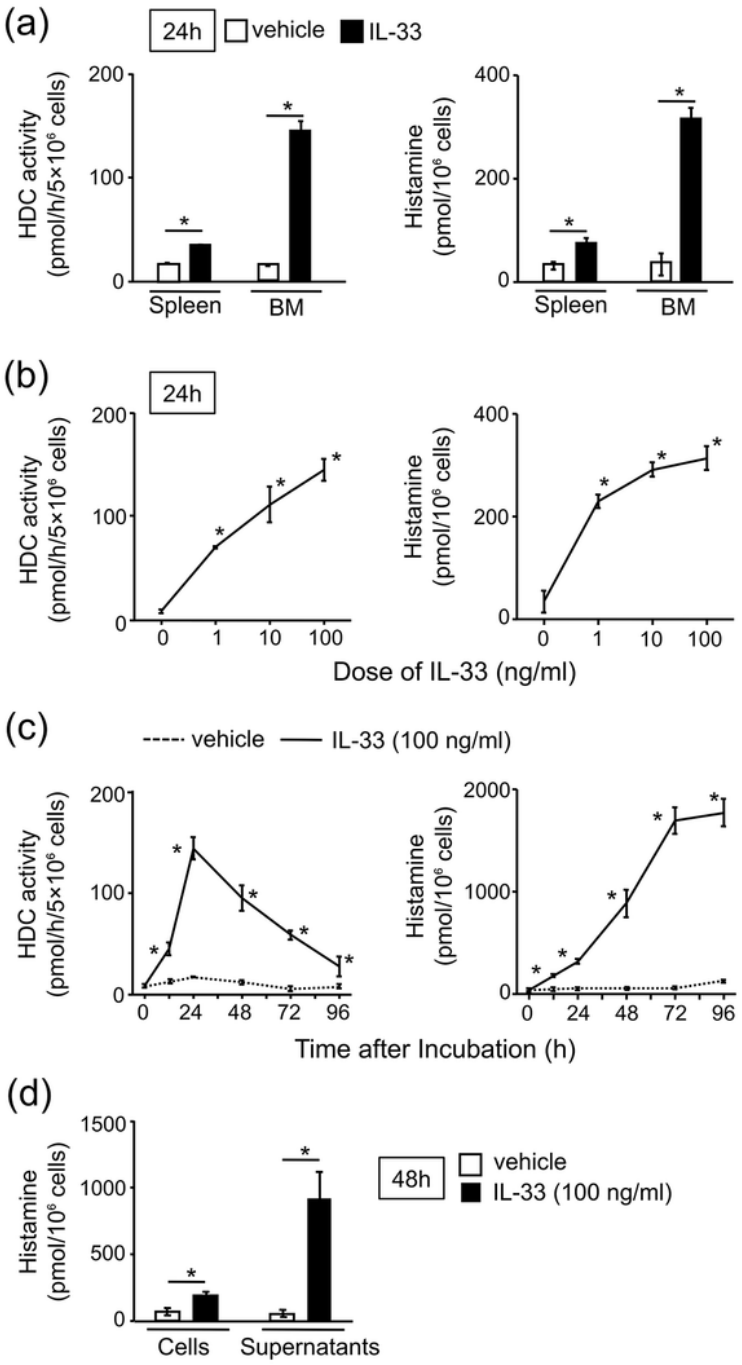

Figure 3

In vitro induction of HDC activity by IL-33 in spleen and bone marrow cells. Cells from spleen and bone marrow (BM) of C57BL/6N mice were stimulated in vitro with IL-33, and HDC activity (in these cells) and histamine (in the cells and/or the supernatant of the culture) were analyzed. (**a**) Effects of IL-33 (100 ng/ml) on HDC activity in the cells and histamine in the supernatant at 24 hours after incubation. *P < 0.01. (n = 4). (**b**) Effect of IL-33 concentration on HDC activity (cells) and histamine (supernatant) in cultures of BM cells at 24 hours after incubation. *P < 0.01 vs saline. (n = 4). (**c**) Time-course of the increases in HDC activity (cells) and histamine (supernatant) induced in cultures of BM cells by IL-33 (100 ng/ml). *P < 0.01 vs saline. (n = 4). (**d**) Histamine levels in BM cells and their culture supernatant after incubation for 48 hours with IL-33 (100 ng/ml) or vehicle. *P < 0.01. (n = 4).

Figure 4

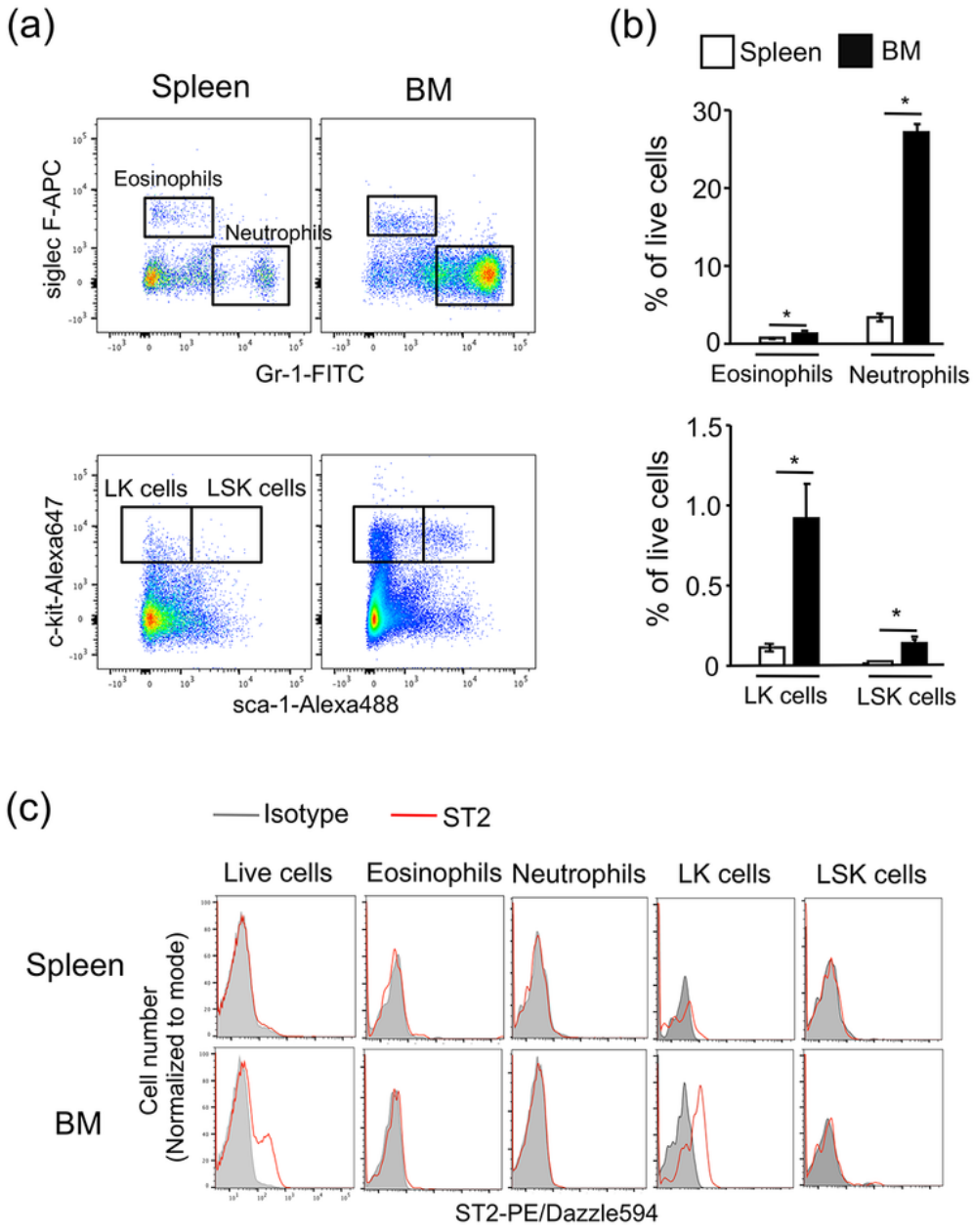

Figure 4

ST2-expressing cells in spleen and bone marrow in non-stimulated mice. Spleen and bone marrow cells from steady-state C57BL/6N mice were analyzed by flow cytometry. (**a**) Representative flow cytometry. (**b**) Percentage of eosinophils ($CD45^+CD11b^+siglecF^+$), neutrophils ($CD45^+CD11b^+Gr\text{-}1^+$), LK cells ($lineage^-$ $sca\text{-}1^-c\text{-}kit^+$), and LSK cells ($lineage^-$ $sca\text{-}1^+c\text{-}kit^+$) among live cells. *P < 0.01. (n = 4). (**c**) Flow cytometry. Expression of ST2 (IL-33 receptors) on each cell-type obtained from spleen and bone marrow.

Red-line histograms represent ST2$^+$ staining and gray-shaded histograms represent isotype controls. Results are representative of four independent experiments.

Figure 5

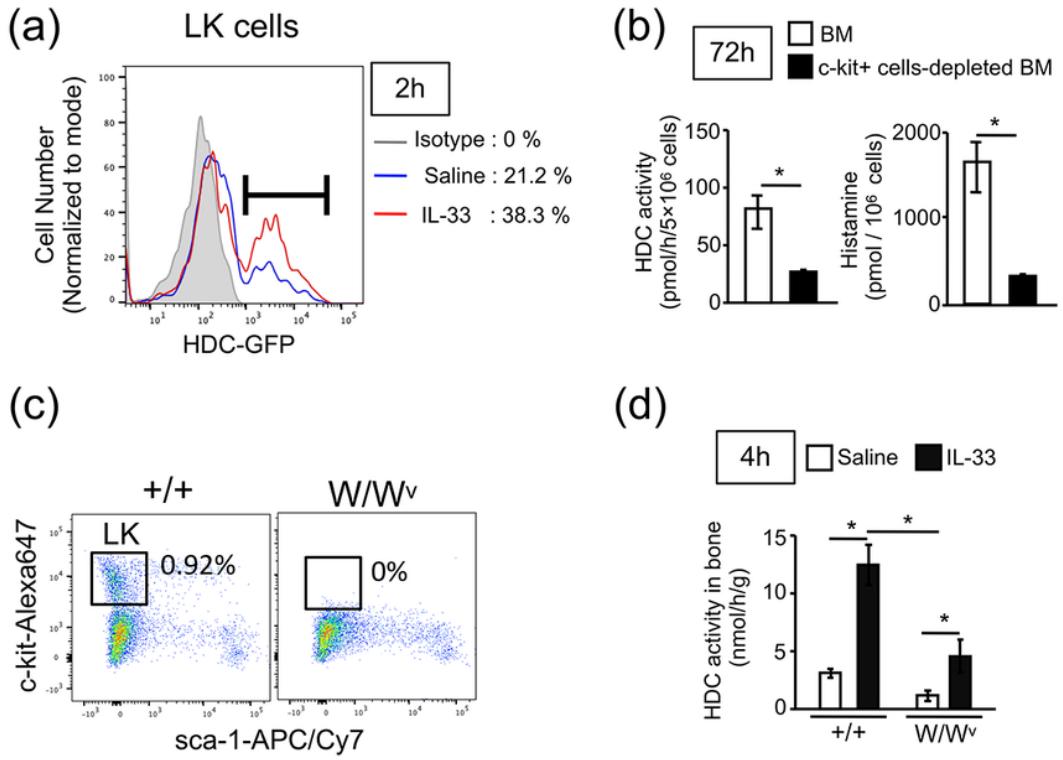

Figure 5

HDC-expressing cells in response to IL-33 in bone marrow. (**a**) Representative flow cytometry. Expression of HDC in LK cells. IL-33 was i.p. injected in WT or HDC-GFP mice, and bone marrow samples taken at 4 hours after the injection were analyzed by flow cytometry. Values indicate the percentage of HDC$^+$ in LK cells in HDC-GFP mice. Blue-line histogram represents saline-injected HDC-GFP mice, red-line histogram represents IL-33-injected HDC-GFP mice, while gray-shaded histogram represents WT control. Each value is the mean (n = 4). (**b**) Effects of depletion of c-kit$^+$ cells on HDC activity and histamine in BM cells. c-kit$^+$ cells were depleted from BM cells obtained from normal mice by means of an EasySep PE selection kit (see Methods), and the c-kit$^+$ cell-depleted BM cells were stimulated with IL-33 (100 ng/ml) for 72 hours.

(**c**) Representative flow cytometry and the percentage of LK cells among live cells in BM cells from +/+ and W/W^{v} mice. (**d**) Effects of IL-33 on HDC activity in the bones (including bone marrow) of +/+ and W/W^{v} mice. IL-33 (50 μg/kg) was i.p. injected and HDC activity in tibia was analyzed at 4 hours after the injection.

Figure 6

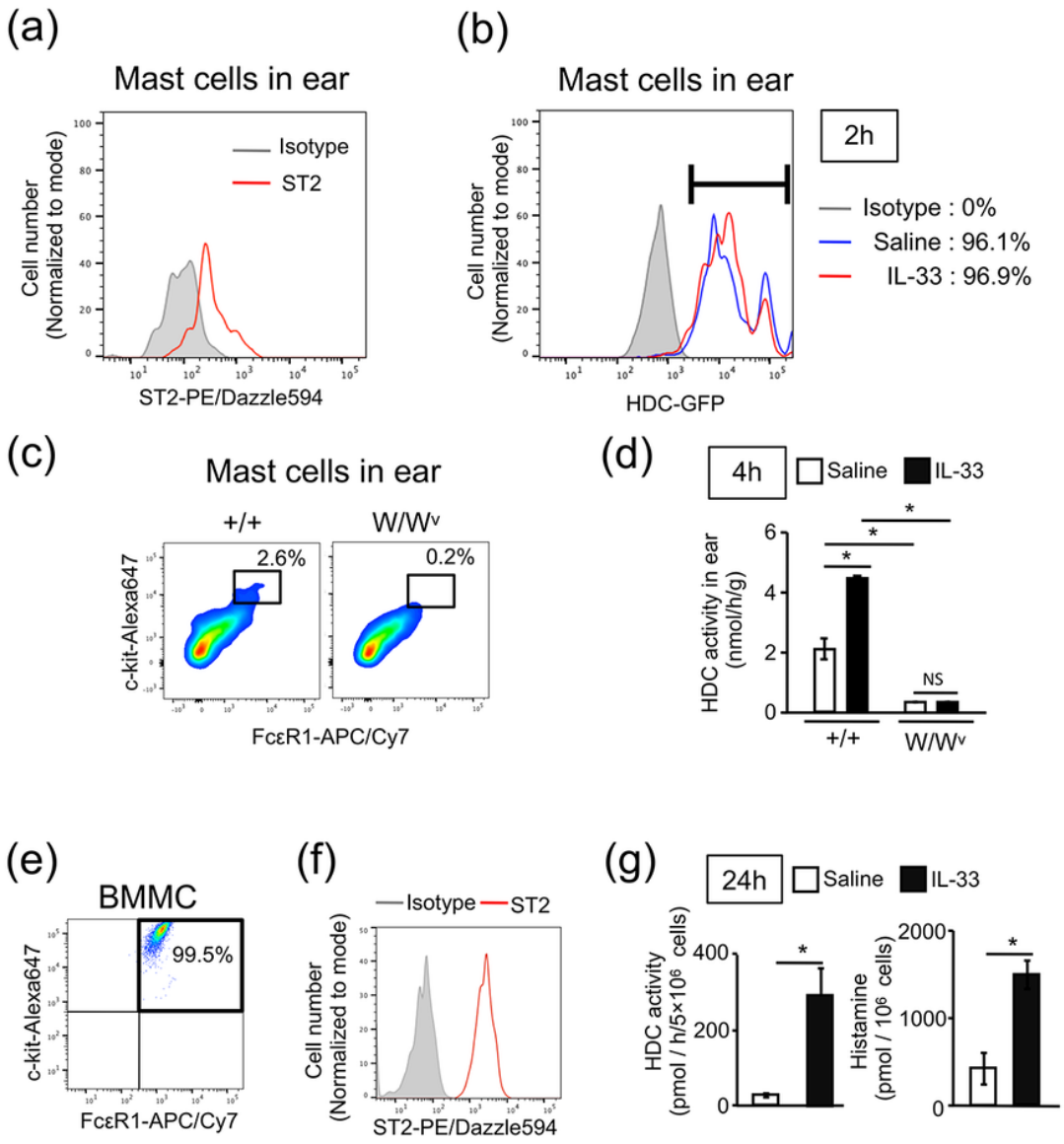

Figure 6

HDC-expressing cells in mast cells. (**a**) Representative flow cytometry. Expression of ST2 on mast cells in ear-pinnae. Red-line histogram represents ST2^{+} staining and gray-shaded histogram represents isotype

controls. Results are representative of four independent experiments. (**b**) Representative flow cytometry. Expression of HDC in mast cells. IL-33 or saline was i.p. injected into WT or HDC-GFP mice, and the ear-pinnae taken at 4 hours after the injection were analyzed by flow cytometry. Values indicate the percentage of HDC^+ in LK cells in HDC-GFP mice. Blue-line histogram represents saline-injected HDC-GFP mice, red-line histogram represents IL-33-injected HDC-GFP mice, while gray-shaded histogram represents WT control. Each value is the mean (n = 4). (**c**) Representative flow cytometry using cells prepared from the ear-pinnae of +/+ and W/W^v mice (see Methods) and the percentage of mast cells. (**d**) HDC activity in the ear-pinnae of +/+ and W/W^v mice. IL-33 (50 μg/kg) was i.p. injected, and HDC activity in the ear-pinnae was analyzed at 4 hours after the injection. Wherever shown, *P < 0.01. (n = 4). (**e**) Representative flow cytometry of BMMCs (obtained as described in Methods) and the percentage of mast cells ($CD45^+$c-$kit^+Fc\varepsilon R1^+$). (**f**) Representative flow cytometry. Expression of ST2 on mast cells in BMMCs. Red-line histograms represent $ST2^+$ staining and gray-shaded histograms represent isotype controls. Results are representative of four independent experiments. (**g**) Effects of IL-33 on HDC activity and histamine in BMMCs. BMMCs were stimulated with IL-33 (100 ng/ml) for 24 hours and HDC activity in the cells and histamine in the supernatant were analyzed.

Figure 7

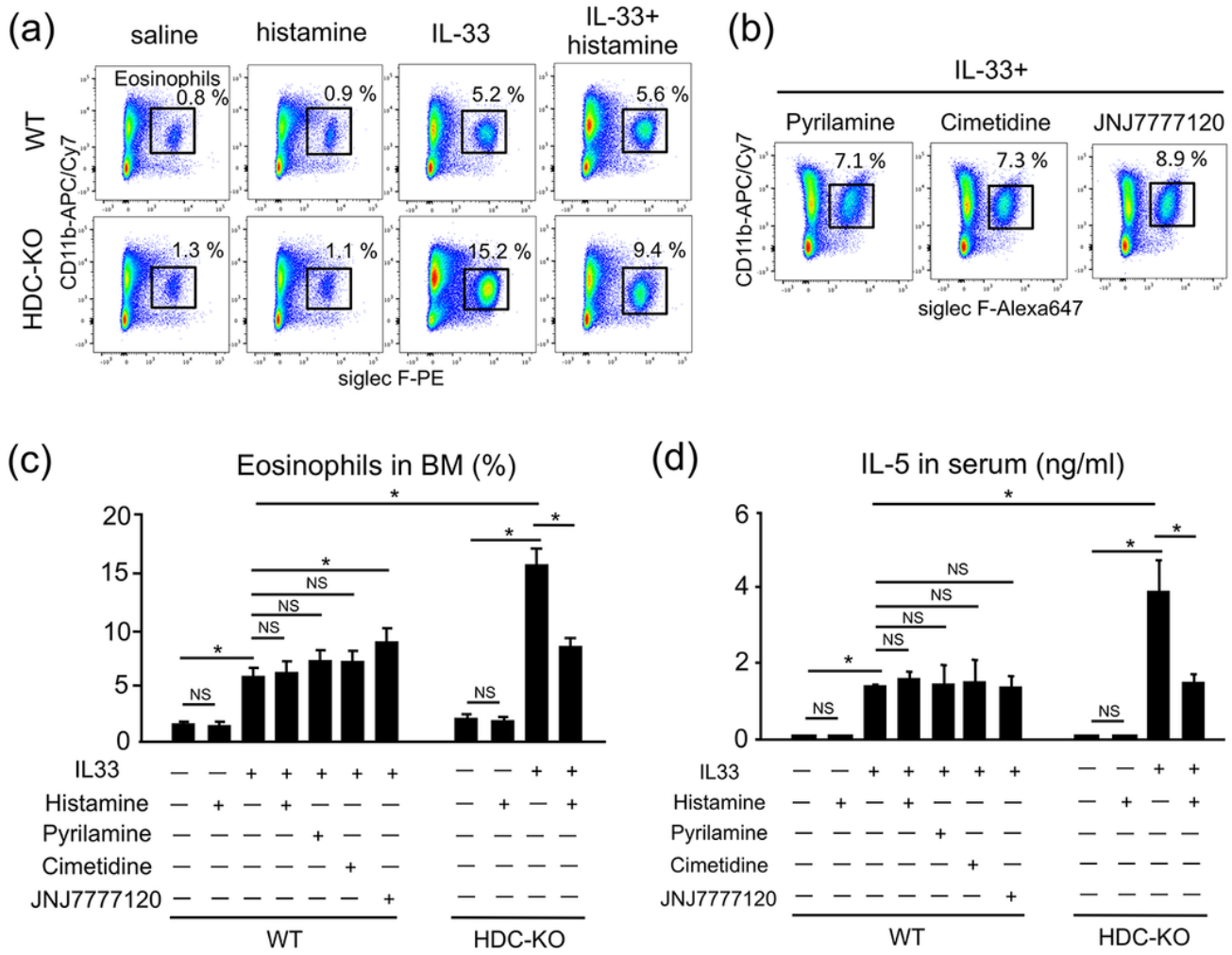

Figure 7

Roles of HDC/histamine in IL-33-induced eosinophilia. Wild type (WT) and HDC-KO mice were i.p. injected with saline, histamine (500 μg/kg), IL-33 (25 μg/kg), IL-33+histamine, or IL-33+histamine antagonists [pyrilamine (H1R antagonist), cimetidine (H2R antagonist), or JNJ7777120 (H4R antagonist) (each 25 mg/kg)] every other day (total, 3 times). One of these histamine antagonists was i.p. injected 4 hours before the injection of IL-33 (see Methods for details). Eosinophils in bone marrow (BM) and IL-5 in serum taken 24 hours after the last injection were analyzed. (**a-b**) Representative flow cytometry and the percentage of BM eosinophils ($CD45^+CD11b^+siglecF^+$) among live cells. Results are representative of four

independent experiments. (**c-d**) Effects of IL-33, histamine, and histamine-receptor antagonists on eosinophils among live cells in BM and on the serum IL-5. Wherever shown, *P < 0.01. (n = 4).

Supplementary Files

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