

REVIEW ARTICLE

Interleukin-33 in allergy

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Abstract

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family, which includes IL-1 and IL-18, and is considered to be important for host defense against nematodes by inducing Th2 cytokine production via the IL-33 receptor. IL-33 receptor is a heterodimer of IL-1 receptor-like 1 (IL-1RL1; also called ST2, T1, Der4, and fit-1) and IL-1 receptor accessory protein (IL-1RAcP). On the other hand, excessive and/or inappropriate production of IL-33 is considered to be involved in the development of various disorders, such as allergic and autoimmune diseases. Unlike IL-1 β and IL-18, IL-33 does not seem to be secreted through the activation of inflammasomes in events such as apoptosis. However, IL-33 is localized in the nucleus of cells and is released during tissue injury associated with necrosis. This suggests that it acts as an alarmin, like IL-1 α and high-mobility group box chromosomal protein-1 (HMGB-1). This review summarizes current knowledge regarding the roles of IL-33 in the functions of various cell types and the pathogenesis of allergy.

IL-33 [also identical to DVS27 and NF-HEV (1)] has been identified as a ligand for IL-1RL1 (also called ST2, T1, Der4, and fit-1), which is a member of the Toll/IL-1 receptor superfamily (2), and is produced/expressed by various types of immune and nonimmune cells (1). In addition, IL-33 is localized in the nucleus because of its association with heterochromatin via a helix–turn–helix motif within the N-terminal part, acting as a transcriptional repressor, at least *in vitro* (3, 4). Thus, like IL-1 α and high-mobility group box 1 (HMGB1) (5, 6), IL-33 is considered to act not only as a proinflammatory cytokine but also a nuclear factor. However, the pathophysiological roles of IL-33 as a nuclear factor remain unclear.

Several members of the IL-1 family of cytokines, such as IL-1 β and IL-18, are initially synthesized as precursor forms,

which lack the amino acid sequences of signal peptides and fail to induce cell activation, in the cytosol (7, 8). After certain stimuli in cells, the precursor is proteolytically cleaved by caspase-1 through the activation of inflammasomes, after which the cleaved form becomes biologically active and is secreted (7, 8). However, full-length IL-33—which does not have a typical caspase-1 cleavage site—is bioactive even without caspase-1-dependent cleavage, while its caspase cleavage product does not show biological activities (9–11). Thus, the caspase-mediated proteolytic cleavage during apoptosis associated with the activation of inflammasomes is not necessary for activation and secretion/release of IL-33. Conversely, IL-33 released by necrotic cells without cleavage by caspases has biological activity (11, 12).

The IL-33 receptor (IL-33R) is a heterodimer consisting of ST2 and IL-1 receptor accessory protein (IL-1RAcP) (13–15). Transmembrane-form ST2 (ST2 or ST2L) and soluble-form ST2 (sST2) are two major products of ST2 genes as a result of alternative splicing under the control of two distinct promoters (1, 16). ST2L is considered to be a functional component of IL-33R. On the other hand, like soluble IL-1R for IL-1 α and IL-1 β , sST2 is regarded as a decoy receptor for IL-33 (1, 16). Signal transduction downstream of IL-33R is mediated in a similar manner with other IL-1R family members such as IL-1R and IL-18R (2, 17).

Target cells of IL-33

T cells

Recently, in addition to Th1 cells and Th2 cells, distinct Th cell subsets have been newly identified as being differentiated from naïve CD4⁺ T cells in the distinct cytokine milieu existing during antigen presentation (Fig. 1). ST2 is preferentially expressed on *in vitro* skewed Th2 cells, but not naïve T cells, Th1 cells, Th17 cells, or Treg cells in CD4⁺ T cells (18–22). ST2-expressing Th cells are detected even in IL-4-, IL-5-, or IL-10-deficient mice (18, 23). In addition, two distinct Th2

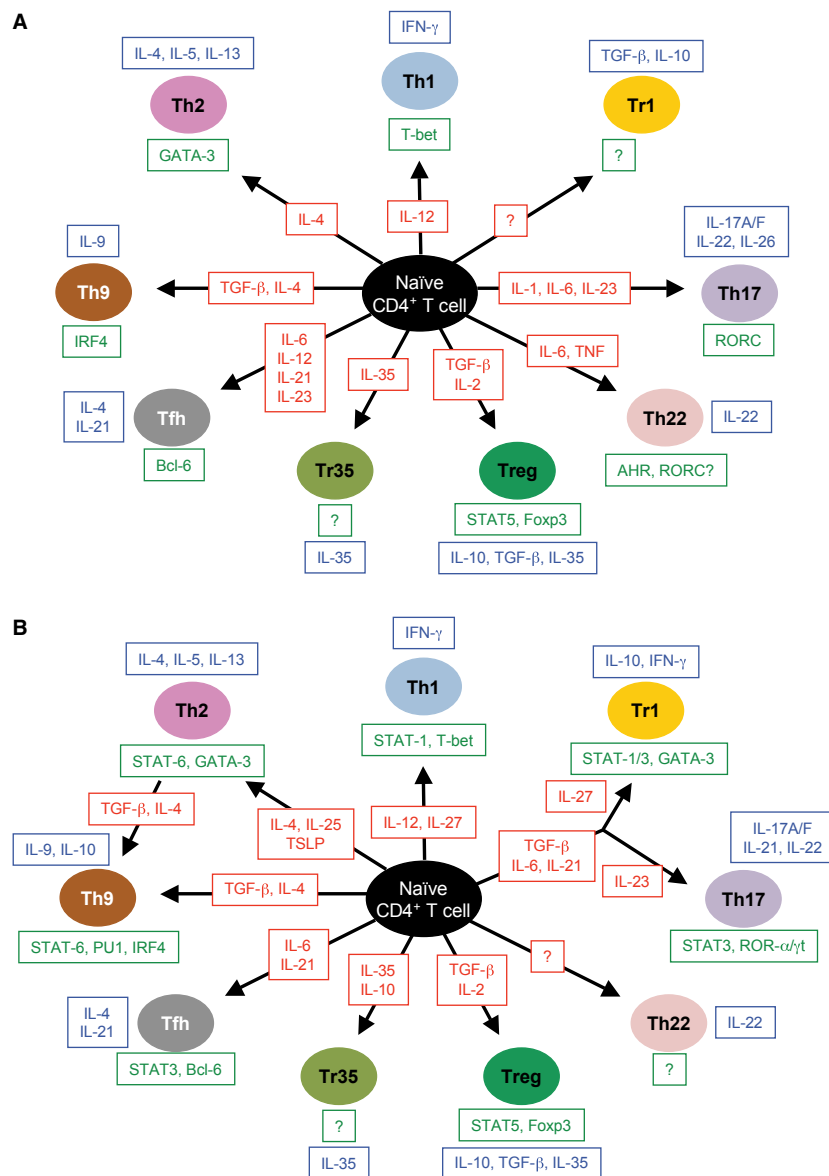


Figure 1 Helper T-cell subsets in humans and mouse. Naïve CD4⁺ cells can differentiate into various types of effector and regulatory T cells in the distinct cytokine milieu existing during antigen presentation in humans (A) and mouse (B). Red and green boxes = master

cytokine(s) and transcription factor(s) for differentiation, respectively, and blue boxes = major products. Th = helper T cell, Treg = CD4⁺ CD25⁺ Foxp3⁺ regulatory T cell, Tr = CD4⁺ CD25-negative Foxp3-negative regulatory T cell, and Tfh = follicular helper T cell.

cells—IL-4- and IL-10-producing IL-5⁺ ST2⁺ Th2 cells and IL-4- and IL-10-producing IL-5⁻ ST2⁻ Th2 cells—were identified in spleen and/or lymph node cells from mice infected with *Leishmania major* (24). Crosslinking of ST2 by anti-ST2 mAb or exogenous IL-33 enhanced Th2 cytokine secretion and chemotaxis in *in vitro* skewed Th2 cells (2, 18, 21, 25–27), but did not induce the differentiation of Th2 cells from naïve T cells (25). Supportively, normal Th2 cell differentiation was observed in ST2-deficient and IL-33-deficient mice (28–30). Moreover, IL-33 polarized naïve CD4⁺ T cells into T cells (hereinafter, called atypical Th2 cells) that produce IL-5 and IL-13, but not IL-4, independently of GATA-3, STAT-6, and IL-4, which are important factors for typical Th2 cell differentiation in humans and mice (31). *In vitro*, IL-33 induced IL-9 production by Th2 cells in humans (32) and by non-Th2 cells—probably Th9 cells—in mice (33). Moreover, IL-10-producing Tr1 cells expressed ST2 (34), but the roles of IL-33 in those ST2-expressing Tr1 cells and in IL-33-stimulated Tr1 cells in immune responses remain unclear.

As in the case of Th2 cells, ST2 is predominantly expressed on *in vitro* skewed Tc2 cells rather than *in vitro* skewed Tc1 cells in CD8⁺ T cells from human PBMCs (35). In contrast to humans, ST2 expression is observed on *in vitro* skewed Tc0, Tc1, Tc2, and Tc17 cells, but not naïve CD8⁺ T cells, in mice, and the ST2 expression levels on Tc1 cells are highest in the other Tc subsets (36). In addition, T-bet, which is a master transcriptional factor for Th1 and Tc1 cell differentiation, is required for the expression of ST2 on Tc1 cells (36). IL-33 enhanced IFN- γ production by Tc1 cells in the presence of IL-12 and following stimulation via T-cell receptors (36) (Fig. 2).

NKT cells

Human and mouse iNKT cells, which express ST2, produce a variety of cytokines—including both Th1 and Th2 cytokines such as IFN- γ , IL-2, IL-4, IL-5, IL-13, and TNF—in response to IL-33 in the presence of α -galactosylceramide-dependent TCR stimulation (37, 38) (Fig. 2). IL-12 enhanced ST2 expression in iNKT cells (37) and induced IFN- γ production by them that was enhanced by IL-33 (37, 38). IL-33 increases the number of mouse iNKT cells *in vitro* and in the spleen and liver *in vivo* (38). J α 18-deficient mice, which lack iNKT cells, developed much severer IL-33-induced airway inflammation than wild-type mice, suggesting that iNKT cells play a regulatory role in the induction of IL-33-mediated inflammation (39).

B cells

B-1 cells, which are divided into CD5⁺ B-1a cells and CD5⁻ B-1b cells, are localized in the peritoneal and pleural cavities and respond to T cell-independent antigens such as carbohydrate antigens, suggesting that they contribute to the induction of innate immunity (40, 41). B-2 cells, which are a major B-cell population in the spleen and lymph nodes, respond efficiently to protein antigens and undergo immunoglobulin heavy-chain class switching and affinity maturation dependent on the function of follicular helper T cells (Tfh cells). ST2 expression on B-1 cells is detected and increased by stimulation with IL-33, but not on B-2 cells (42). IL-33 can enhance—in *vitro* and/or *in vivo*—proliferation and IgM and Th2 cytokine (such as IL-5 and IL-13) production by B-1 cells, but not B-2 cells (42) (Fig. 2). IL-33-mediated

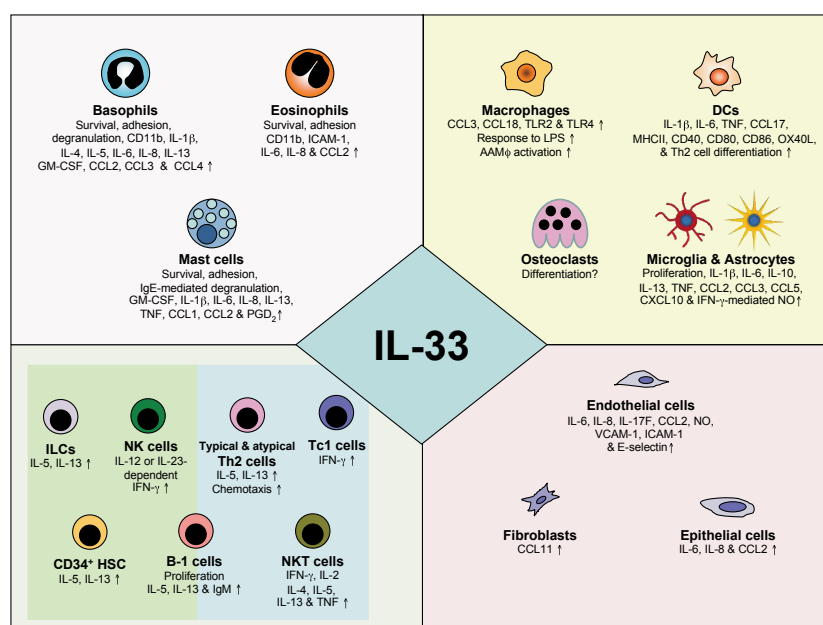


Figure 2 Bioactivities of IL-33. IL-33 promotes the expression and/or production of various mediators and functions (indicated as $\uparrow$) in vari-

ous types of cells. DCs = dendritic cells, ILCs = innate lymphoid cells, NKs = natural killer cells, and HSC = hematopoietic stem cells.

peritoneal B-1 cell proliferation and IgM production are highly dependent on IL-5, which is a crucial cytokine for the development of B-1 cells (43), and partially dependent on T cells in mice (42).

Mast cells

ST2 is constitutively expressed on human mast cells (MCs) derived from peripheral blood or cord blood progenitor cells and on mouse peritoneal and bone marrow-derived cultured mast cells (BMCMCs) (13, 15, 44–47). IL-33 can induce the production of various cytokines and chemokines (Fig. 2) and expression of mouse mast cell protease-6, prolong survival, and promote the adhesion of naïve human and murine MCs without inducing degranulation (44–46, 48, 49). It can also enhance degranulation as well as cytokine and chemokine production by IgE-sensitized MCs with and without FcεRI crosslinking in humans and/or mice (25, 44–46, 48, 50) (Fig. 2). IL-33 also enhances C5a-, stem cell factor (SCF)-, or nerve growth factor (NGF)-mediated cytokine production in a human mast cell line, HMC-1 (51). Intradermal injection of IL-33 to mice caused the development of skin inflammation dependent on dermal mast cell activation via ST2 (52).

Basophils

Human and mouse basophils constitutively express ST2, but at lower levels than Th2 cells and mast cells (37, 53–55). IL-33 can induce the secretion of such cytokines and chemokines (Fig. 2) and adhesion by promoting CD11b expression, without inducing degranulation or migration, in human and/or mouse naïve basophils (37, 53, 54, 56). It can also enhance IgE-mediated degranulation and migration, as well as IgE- and IL-3-mediated cytokine and chemokine production in human and/or mouse basophils (37, 51, 53, 54, 56) (Fig. 2). Otherwise, IL-33 enhances the receptor for leptin—which is an adipokine and a member of the IL-6 family of cytokines—on human basophils, suggesting that IL-33 is involved in metabolic abnormalities associated with inflammation (54).

Eosinophils

Human eosinophils also express ST2 (57, 58), and IL-33 activates eosinophils to enhance their survival, cell adhesion accompanied by increased expression of CD11b and ICAM-1, and production of cytokines, chemokines, and superoxide (53, 57–60) (Fig. 2). The role of IL-33 in degranulation of human eosinophils is controversial: IL-33 failed to induce degranulation as assessed by the release of eosinophil-derived neurotoxin (EDN) and leukotriene C4 (57), but others conversely found it to induce degranulation as assessed by EDN release (58).

NK cells

As in the case of iNKT cells, IL-33 can promote IFN-γ production by human and/or mouse NK cells in the presence of IL-12, which enhances ST2 expression in human NK cells, or

IL-23 (37, 38) (Fig. 2). However, the pathophysiological role of IL-33-stimulated NK cells in immune responses remains unclear.

Dendritic cells

IL-33 induces GM-CSF production by mouse bone marrow cells and promotes their differentiation into DCs (61). It can also activate GM-CSF-induced mouse bone marrow cell-derived cultured DCs (BMDCs), which express intracellular ST2 as well as ST2 mRNA, but no/little surface ST2 (61, 62), to produce IL-1β, IL-6, TNF, and CCL17, but not IL-12, and it can enhance their expression of MHC class II and costimulatory molecules such as CD40, CD80, CD86, and/or OX40 ligand (62, 63) (Fig. 2). When IL-33-pulsed BMDCs are cultured with naïve DO11.10 or OTII CD4⁺ T cells, which constitutively express OVA (ovalbumin)-specific T-cell receptors, the BMDCs promote robust production of IL-5 and IL-13—but not IL-4, IL-10, or IFN-γ—by atypical Th2 cells in the presence of OVA peptides (62, 63); notably, IL-33-pulsed BMDC-mediated IL-5 and IL-13 production by T cells is observed even in the absence of antigens (62). In accordance with this, OVA-induced airway inflammation is exacerbated in mice injected with IL-33-pulsed BMDCs compared with nontreated BMDCs (63). Administration of recombinant IL-33 to mice enhances the accumulation of DCs in the lung, suggesting that IL-33 is involved in DC recruitment in that organ (63). In support of this, ST2-deficient mice show reduced CD11c⁺ DC counts in the lymph nodes, resulting in attenuated OVA-induced airway inflammation (63), although ST2's role in the development of OVA-induced airway inflammation is controversial, as discussed below [also see the review (1)]. On the other hand, dermal DCs and Langerhans cells in IL-33-deficient mice exhibit normal levels of migration activity from the skin to draining lymph nodes during contact hypersensitivity (30). Because various DC subtypes have been identified (64), the roles of IL-33 and ST2 may differ with the DC subtype.

Macrophages

Mouse macrophages such as bone marrow cell-derived cultured, peritoneal, and alveolar macrophages constitutively express ST2 mRNA and/or proteins (65–67). In addition, soluble ST2 expression is increased in macrophages after treatment with LPS, TNF, IL-1, and IL-6 (65, 68, 69). Macrophages are considered to be divisible into two distinct major populations, M1 and M2 [also called alternatively activated macrophages (AAMφ)]. IL-33 induces the expression of CCL3, which is expressed in M1, in human bone marrow cell-derived macrophages, and expression of CCL18 and mannose receptor in human M2/AAMφ (70) (Fig. 2). Moreover, IL-33 amplifies IL-13-mediated polarization of M2/AAMφ from mouse alveolar-, bone marrow cell-derived cultured, or adipose tissue macrophages *in vitro* or *in vivo* (71, 72), suggesting that IL-33 contributes to the development of allergic responses by enhancing polarization and activation of M2/AAMφ.

Although IL-33 alone cannot induce cytokine production by thioglycolate (TGC)-induced mouse peritoneal macrophages, it can promote their expression of TLR2, TLR4, MD2, CD14, and MyD88, resulting in increased production of IL-6 and/or TNF by macrophages in response to LPS (a TLR4 ligand) and LTA (a TLR2 ligand) dependent on MyD88, but not poly(I:C) (a TLR3 ligand) independently of MyD88 (67) (Fig. 2). TGC-induced mouse peritoneal macrophages produce IL-33 after stimulation with LPS, and the IL-33 subsequently auto-/paracrine enhances IL-6 and TNF production by the cells (12, 73). Supportively, LPS-induced cytokine production is impaired in TGC-induced mouse peritoneal macrophages from IL-33-deficient mice (30, 73) and in bone marrow cell-derived cultured macrophages in the presence of soluble ST2 (66). In contrast, cytokine production by TGC-induced mouse peritoneal macrophages from ST2-deficient mice is enhanced (74) or normal (67) in comparison with macrophages from wild-type mice. The exact reason for the apparent discrepancy between these reports is unclear, but it is speculated that ST2 deficiency results in increased formation of the other IL-1R family members, such as IL-1R (IL-1R1 and IL-1RAcP) and IL-36 (IL-1RAcP and IL-1RAcP2) because of the lost formation of IL-33R (ST2 and IL-1RAcP), causing cytokine hyperproduction by ST2-deficient macrophages in response to IL-1 or IL-36 [see review Reference (1)]. Indeed, cytokine hyperproduction in response to IL-1 is observed in ST2-deficient macrophages compared with wild-type macrophages (74).

Osteoclasts

Expression of ST2 mRNA is detected in both human and mouse bone tissues, human bone marrow stromal cells and osteoblast-like MC3T3-E1 cells (75, 76). IL-33 mRNA is also expressed in osteoblasts from humans and mice (76, 77), suggesting a contribution of IL-33 to bone homeostasis. However, the role of IL-33 in osteoclastogenesis is controversial. IL-33 enhances the expression of TRAF-6, NF-AT, cytoplasmic 1, c-Fos, c-Src, cathepsin K, and calcitonin receptor, which are involved in osteoclast differentiation, and induces M-CSF-mediated differentiation of osteoclasts from human CD14⁺ monocytes and bone resorption by osteoclasts through ST2 independently of RANKL, which is a key cytokine for osteoclast differentiation (78). In contrast, IL-33 inhibits M-CSF- and RANKL-dependent and 1,25-dihydroxyvitamin D3-dependent differentiation of osteoclasts from bone marrow cells and also inhibits their bone resorptive activity (75, 77, 79). IL-33 has no effect on M-CSF-mediated osteoclast differentiation from human CD14⁺ monocytes with or without RANKL (76).

Microglia and astrocytes

High levels of IL-33 mRNA can be detected in the spinal cord and brain of mice (2). ST2 and IL-33 are expressed in mouse microglia and astrocytes with and without stimuli such as LPS (80–82). IL-33 induces the proliferation and production of cytokines and chemokines (Fig. 2) and enhances

IFN- γ -mediated NO production in microglia *in vitro* (81) (Fig. 2). Although increased IL-33 expression was observed in astrocytes in inflamed spinal cord lesions of mice with experimental autoimmune encephalomyelitis (81), development of this disease model in IL-33-deficient mice was similar to in wild-type mice (30), indicating that IL-33 is not essential for its development. IL-33 expression is increased in brains of mice infected with Theiler's murine encephalomyelitis virus (82), but the role of IL-33 in this setting is unclear.

CD34⁺ hematopoietic progenitor cells

CD34⁺ hematopoietic progenitor cells from human umbilical cord blood cells express ST2 and produce various cytokines and chemokines in response to IL-33 (83) (Fig. 2). The number of CD34⁺ hematopoietic progenitor cells is increased in peripheral blood from allergic patients. In particular, IL-5- and IL-13-producing CD34⁺ hematopoietic progenitor cells are detected in sputum from patients with asthma (83), suggesting that CD34⁺ hematopoietic progenitor cells stimulated with IL-33 contribute to the pathogenesis of certain allergic disorders.

Innate lymphoid cells

A number of IL-5- and IL-13-producing Lin[−] c-kit⁺ Sca-1^{−/+} innate lymphoid cells (ILCs), such as natural helper (NH) cells, multipotent progenitor type 2 cells (MPP^{type2} cells), nuocytes, and innate helper type 2 (Ih2) cells—populations that are distinct from lymphoid progenitors and lymphoid tissue inducer cells—were identified (84–90) (Fig. 2). Lin[−] c-kit⁺ Sca-1⁺ NH cells, found in fat-associated lymphoid clusters in visceral adipose tissue, constitutively express ST2 and produce larger amounts of IL-5, IL-6, and IL-13, but not IL-4, than basophils and mast cells in response to IL-33 or IL-2+ IL-25, but not IL-25 alone. They are involved in host defense against helminths such as *Nippostrongylus brasiliensis* (*N. brasiliensis*) via IL-5- and IL-13-dependent eosinophilia and goblet cell hyperplasia (84, 91). ST2-expressing Lin[−] c-kit⁺ Sca-1⁺ nuocytes and ST2-expressing Lin[−] c-kit⁺ Sca-1-negative Ih2 cells accumulate in the spleen and mesenteric lymph nodes, spleen, or liver in mice injected with IL-25 or IL-33 and infected with *N. brasiliensis*. Both nuocyte- and Ih2 cell-derived IL-13 as well as NH cells are known to be crucial for host defense against *N. brasiliensis* (86, 88, 92) and the development of allergic airway inflammation (93, 94). Expansion of Lin[−] c-kit⁺ Sca-1⁺ MPP^{type2} cells, which do not express ST2, is observed in mesenteric lymph nodes and gut-associated lymphoid tissues including Peyer's patches of mice injected with IL-25 or infected with helminths such as *N. brasiliensis* and *Trichuris muris*, contributing to host defense against such pathogens (87). Although IL-33 and/or IL-25 are key cytokines for expansion of IL-5- and IL-13-producing ILCs such as NH cells, nuocytes, Ih2 cells, and MPP^{type2} cells in mice, except for NH cells, it is unclear whether these cells produce IL-5 and/or IL-13 in response to IL-33 or IL-25. IL-33 is produced by alveolar macrophages stimulated with influenza virus, resulting in expansion of ILCs in the lung. Subsequently, ILC-derived IL-13 is crucial

for the pathogenesis of influenza-mediated airway inflammation in mice (95, 96). As in mice, resident ST2-expressing Lin⁻ CD127⁺ CD25⁺ ILCs have been identified in lung parenchymal tissue from healthy humans (96), suggesting the importance of resident ILCs for immune responses in the human lung. In addition, ST2-expressing Lin⁻ CD127⁺ CD161⁺ CRTH2⁺ ILCs, which produce IL-13 in response to IL-33 or IL-25 in the presence of IL-2, have been identified in the human fetal gut and observed to have accumulated in inflamed, but not noninflamed, nasal polyps from adult patients with chronic rhinosinusitis (97).

Endothelial and epithelial cells

In response to IL-33, human endothelial cells also express ST2 (68, 98, 99) and produce and express such cytokines and chemokines, as well as nitric oxide and such adhesion molecules (98–101) (Fig. 2). Moreover, IL-33 induces angiogenesis—by enhancing proliferation, migration, and morphologic differentiation of human vascular endothelial cells—and vascular permeability in mice (98).

Epithelial cells, which also express ST2 (65, 99, 102), produce cytokines and chemokines in response to IL-33 (99, 103) (Fig. 2). Moreover, the IL-33-mediated cytokine and chemokine production by human epithelial cells is enhanced by IL-4 stimulation (99). Activation of ERK (extracellular regulated kinase), but not p38 MAPK, is required for IL-33-mediated IL-8 production by human epithelial cells, while the activation of p38 MAPK, but not ERK, is important for IL-33-mediated IL-8 production by human endothelial cells (99), suggesting distinct regulation of IL-33-mediated cell activation in different cell types.

Fibroblasts

Fibroblasts express ST2 and IL-33 (1, 104, 105), and they produce CCL11 in response to IL-33 (Fig. 2). In particular, increased ST2 expression is observed in mouse lung fibroblasts during OVA-induced allergic airway inflammation (105), suggesting that IL-33-induced CCL11 production by fibroblasts contributes to recruitment of eosinophils in such inflammation.

IL-33 in allergic diseases

Asthma/Airway inflammation

A genetic polymorphism in IL-33 or ST2 is identified in patients with asthma and is suspected of contributing to susceptibility to the development of asthma (106). The soluble ST2 level has been shown to be increased in adult and child asthmatic patients and corresponds to the severity of asthma exacerbation (107, 108). The levels of IL-33 are higher in lung smooth muscle or epithelial cells from asthmatic patients than from healthy subjects (109, 110). Likewise, the levels of soluble ST2 and IL-33 are increased in the serum and/or lung of mice during OVA-induced airway inflammation (111, 112). In the lungs, IL-33 is expressed in alveolar

macrophages (95, 113, 114), CD11c⁺ cells, and epithelial cells (105). However, the role of ST2 in airway inflammation in mice remains controversial [also see (1)]. OVA-induced airway inflammation or hyper-reactivity develops normally in ST2-deficient mice sensitized twice with OVA emulsified with alum (28, 31, 115), but is attenuated (31) or exacerbated (95) in the case of a single sensitization. In the former case, the IL-33-ST2 pathway enhances the differentiation of IL-5-, but not IL-4-, producing atypical Th2 cells (31) and AAMφ dependent on IL-13 (71) (Fig. 3). Thus, ST2 deficiency aborts the differentiation of those cells and IL-33-induced AAMφ-derived CCL17 and CCL24 production, leading to attenuated airway inflammation in response to OVA (31, 71). Different experimental protocols have demonstrated distinct phenotypes in mice during OVA-induced airway inflammation, that is, the use of alum in the antigen sensitization [see review (116)]. Mast cells, B cells, IgE, TNF, IL-1, and CCR8 are essential for the development of OVA-induced airway inflammation in mice sensitized with OVA alone, but not with OVA emulsified with alum (116, 117). In sensitization without alum, the development of OVA-induced airway inflammation is reduced in ST2-deficient mice because of the failure of IL-33-mediated lung DC activation, that is, migration and expression of costimulatory molecules (63) (Fig. 3). As described above, IL-33 can activate ST2-expressing Th2 cells to secrete Th2 cytokines, suggesting that IL-33-stimulated Th2 cells may enhance the induction of OVA-induced airway inflammation (Fig. 3). Unexpectedly, development of OVA-induced airway inflammation was aggravated in wild-type or Rag-1-deficient mice that had undergone adoptive transfer of ST2-deficient DO11.10 Th2 cells (115), suggesting that ST2 on Th2 cells has a regulatory role in the setting. Distinct phenotypes were also reported in mice treated with anti-ST2 Ab or soluble ST2 from ST2-deficient mice, as described elsewhere [see review (1)].

Administration of α -galactosylceramide or *Sphingomonas*-derived glycolipid, which can stimulate iNKT cells via TCR, to mice results in the development of airway inflammation associated with neutrophils rather than eosinophils, and increased IL-33 production and ILCs in the lung (113). Development of that airway inflammation is impaired in ST2-deficient mice (113). In that development, TCR-dependent iNKT cell activation leads to IL-33 production by alveolar macrophages and DCs (113) (Fig. 3). IL-33 then activates lung ILCs as well as iNKT cells to secrete IL-13, contributing to the development of iNKT cell-mediated airway inflammation (113) (Fig. 3).

On the other hand, regardless of whether or not alum was used in OVA sensitization, OVA-induced airway inflammation was significantly impaired in IL-33-deficient mice (30, 118), without affecting Th2 cell induction after OVA sensitization or postchallenge IgE production (30). In addition, IL-33-deficient mice also did not develop airway inflammation in response to an extract derived from house dust mites (30), which are considered to be a major source of allergens in allergic patients and can provoke allergic inflammation resembling human asthma in mice by potentiating airway epithelial barrier disruption, inflammation, and allergen

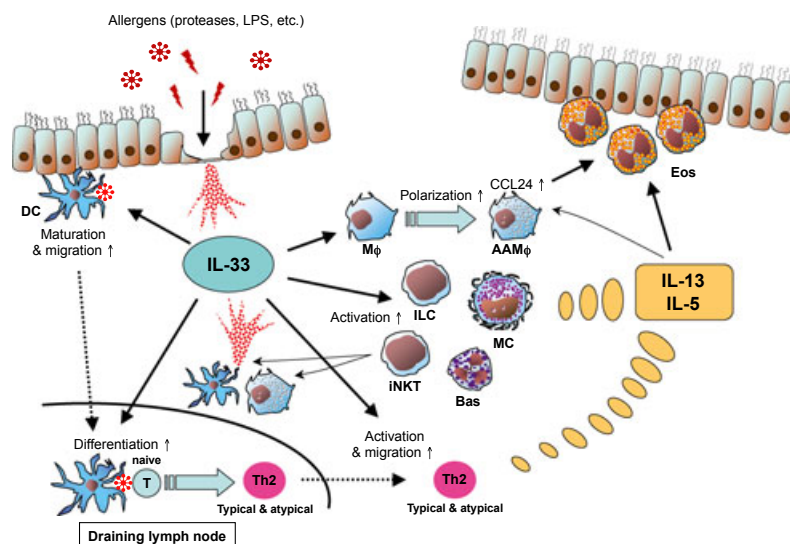


Figure 3 The roles of IL-33 in airway inflammation. IL-33, which is released by damaged cells in response to allergens (proteases, LPS, etc.), enhances the differentiation of IL-5- and IL-13-, but not IL-4-, producing Th2 cells by promoting maturation and migration of

lung DCs, polarization of alternatively activated macrophages (AAMφ), and IL-5 and/or IL-13 secretion by innate lymphoid cells (ILCs), iNKT cells, mast cells (MCs), and basophils (Bas). IL-33-mediated IL-5 and/or IL-13 induces airway eosinophilia.

sensitization of the airways through TLR4-dependent innate and acquired immunity (119–121). These observations suggest that in allergen-induced airway inflammation, IL-33 is important for the induction of local inflammation in the elicitation phase, rather than for the induction of Th2 cells in the sensitization phase. In support of this notion, IL-33 induces IL-13- and STAT6-dependent airway inflammation even in the absence of such acquired immune cells as T, B and iNKT cells, NK cells, and mast cells (2, 25). As described above, in particular, iNKT cells play a regulatory role in the development of IL-33-induced airway inflammation (39). As in the case of administration of recombinant IL-33, inhalation of papain, which is a cysteine protease, as well as Der f1 and Der p1 derived from house dust mites and regarded as a cause of occupational asthma (122), by naïve mice (i.e., without prior sensitization to papain), results in IL-13- and STAT6-dependent airway inflammation associated with increased IL-33 mRNA expression in the lung even in the absence of acquired immune cells (30). It was recently reported that lung ILCs, rather than acquired immune cells, are crucial for that event (123). Inhalation of chitin, β -(1-4)-poly-*N*-acetyl-D-glucosamine, which is a component of the cell wall of such pathogens as bacteria and fungi, the exoskeleton of house dust mites, insects, and crustaceans (crabs and shrimp), and the sheath of parasitic nematodes, by mice also causes airway inflammation independently of STAT-6 and acquired immune cells (124); notably, IL-33-deficient mice fail to develop STAT6-dependent and STAT6-independent airway inflammation in response to papain and chitin, respectively (30, 125). Thus, IL-33 is a crucial cytokine for the induction of various types of airway inflammation.

Rhinitis, rhinosinusitis, and conjunctivitis

Genetic polymorphism in ST2 or IL-33 was found in patients with rhinitis, conjunctivitis, and rhinosinusitis (126, 127). The serum IL-33 levels were significantly higher in patients with rhinitis and/or conjunctivitis caused by Japanese cedar pollens than in healthy subjects (126, 128). It is noteworthy that the number of IL-33-responding ILCs was also increased in nasal polyps of patients with rhinosinusitis (97).

Mice treated with anti-IL-33-neutralizing Ab show reduced eosinophilia during OVA-induced allergic rhinitis (129), suggesting an important role of IL-33 in the disease development.

Expression of IL-33 is observed in vascular endothelial cells, epithelial cells, and fibroblasts of giant papillae in patients with allergic conjunctivitis (130). Expression of IL-33 is constitutive in epithelial cells of the conjunctiva in naïve mice and increased in mice during ragweed-induced allergic conjunctivitis (131). In addition, IL-33 exacerbates eosinophilic inflammation during ragweed-induced allergic conjunctivitis (131), suggesting that IL-33 contributes to the pathogenesis of allergic conjunctivitis.

Dermatitis

Genetic polymorphism in ST2 and increased expression of IL-33 in inflamed skin are also found in patients with atopic dermatitis (50, 130, 132), suggesting the involvement of IL-33 in the pathogenesis of atopic dermatitis. However, the precise role of IL-33 in that setting remains unclear.

Expression of ST2 mRNA is increased in the inflamed skin of rats during contact hypersensitivity (CHS) induced by 2,

4-dinitrofluorobenzene (DNFB). Development of CHS induced by 2-phenyl-4-(ethoxymethylene) oxazol-5-one (oxazolone) is attenuated in ST2-deficient mice because of the impairment of IL-33/ST2-dependent activation of B-1 cells (42), which are important for CHS induction (133, 134). In contrast, IL-33-deficient mice show normal development of CHS in response to DNFB and fluorescein isothiocyanate (FITC) as well as normal levels of migration of DCs from the skin to draining lymph nodes, FITC-specific proliferation and IL-4 production in draining lymph node cells and FITC-specific serum Ig levels during CHS (30). Because the molecular mechanism for the induction of CHS is known to differ as a function of the genetic background and/or the chemical compounds used (116), the apparent discrepancy between these reports may be due to their different protocols for CHS.

Anaphylaxis

IL-33 serum levels are significantly increased in atopic patients during anaphylaxis (50), suggesting a contribution of IL-33 to the induction of anaphylaxis. IL-33 induces degranulation of IgE-sensitized, but not non-IgE-sensitized, BMCMCs without specific antigens *in vitro*, as described above. In accordance with this, IL-33 can induce systemic and passive cutaneous anaphylaxis in IgE-sensitized, but not non-IgE-sensitized, mice by provoking mast cell degranulation via ST2 even in the absence of specific antigens for IgE (50, 135). On the other hand, in regard to passive cutaneous anaphylaxis after the administration of specific antigens for IgE in ST2-deficient mice and mice treated with anti-IL-33-neutralizing Ab, the immediate reaction mediated by mast

cell-derived mediators such as histamine develops normally, but the inflammation mediated by mast cell-derived cytokines such as TNF in the late phase is significantly suppressed (136). This suggests that the IL-33-ST2 pathway is important for inflammation in the late phase, but not the immediate phase, of passive cutaneous anaphylaxis in mice.

Conclusion

IL-33 is now considered to be crucial for the development of allergic disorders through the activation of not only acquired immunity but also innate immunity. In particular, IL-33 is a key cytokine for inducing and activating ILCs in allergic disorders. In addition to allergic disorders, IL-33 plays key roles in the development of non-Th2-type immune responses such as host defense against a variety of pathogens and the development of autoimmune disorders (1, 30). Thus, IL-33 neutralization may provide a potential target for novel therapeutics for such disorders.

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Conflicts of interest

The authors have no conflicts of interest to declare.

References

- Oboki K, Ohno T, Kajiwara N, Saito H, Nakae S. IL-33 and IL-33 receptors in host defense and diseases. *Allergol Int* 2010;**59**:143–160.
- 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.
- Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc Natl Acad Sci U S A* 2007;**104**:282–287.
- Roussel L, Erard M, Cayrol C, Girard JP. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. *EMBO Rep* 2008;**9**:1006–1012.
- Werman A, Werman-Venkert R, White R, Lee JK, Werman B, Krelin Y et al. The precursor form of IL-1 α is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci U S A* 2004;**101**:2434–2439.
- Andersson U, Tracey KJ. HMGB1 is a therapeutic target for sterile inflammation and infection. *Annu Rev Immunol* 2011;**29**:139–162.
- Dinarello CA. Interleukin-1 β , interleukin-18, and the interleukin-1 β converting enzyme. *Ann N Y Acad Sci* 1998;**856**:1–11.
- Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009;**27**:519–550.
- Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G. Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem* 2009;**284**:19420–19426.
- 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.
- Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A* 2009;**106**:9021–9026.
- Ohno T, Oboki K, Kajiwara N, Morii E, Aozasa K, Flavell RA et al. Caspase-1, caspase-8, and calpain are dispensable for IL-33 release by macrophages. *J Immunol* 2009;**183**:7890–7897.
- Ali S, Huber M, Kollwe 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.
- Chackerian AA, Oldham ER, Murphy EE, Schmitz J, Pflanz S, Kastelein RA. IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *J Immunol* 2007;**179**:2551–2555.
- Palmer G, Lipsky BP, Smithgall MD, Meininger D, Siu S, Talabot-Ayer D et al. The IL-1 receptor accessory protein (AcP) is required for IL-33 signaling and soluble AcP enhances the ability of soluble ST2 to inhibit IL-33. *Cytokine* 2008;**42**:358–364.
- Smith DE. IL-33: a tissue derived cytokine pathway involved in allergic inflammation and asthma. *Clin Exp Allergy* 2010;**40**:200–208.

17. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;**2**:675–680.
18. Lohning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, Gutierrez-Ramos JC et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc Natl Acad Sci U S A* 1998;**95**:6930–6935.
19. Lecart S, Lecoine N, Subramaniam A, Alkan S, Ni D, Chen R et al. Activated, but not resting human Th2 cells, in contrast to Th1 and T regulatory cells, produce soluble ST2 and express low levels of ST2L at the cell surface. *Eur J Immunol* 2002;**32**:2979–2987.
20. Nakae S, Iwakura Y, Suto H, Galli SJ. Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. *J Leukoc Biol* 2007;**81**:1258–1268.
21. Guo L, Wei G, Zhu J, Liao W, Leonard WJ, Zhao K et al. IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc Natl Acad Sci U S A* 2009;**106**:13463–13468.
22. 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.
23. Kropf P, Schopf LR, Chung CL, Xu D, Liew FY, Sypek JP et al. Expression of Th2 cytokines and the stable Th2 marker ST2L in the absence of IL-4 during Leishmania major infection. *Eur J Immunol* 1999;**29**:3621–3628.
24. Kropf P, Herath S, Tewari R, Syed N, Klemenz R, Muller I. Identification of two distinct subpopulations of Leishmania major-specific T helper 2 cells. *Infect Immun* 2002;**70**:5512–5520.
25. Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N et al. Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int Immunol* 2008;**20**:791–800.
26. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol* 2007;**37**:2779–2786.
27. Lohning M, Grogan JL, Coyle AJ, Yazdanbakhsh M, Meisel C, Gutierrez-Ramos JC et al. T1/ST2 expression is enhanced on CD4⁺ T cells from schistosome egg-induced granulomas: analysis of Th cell cytokine coexpression ex vivo. *J Immunol* 1999;**162**:3882–3889.
28. Hoshino K, Kashiwamura S, Kuribayashi K, Kodama T, Tsujimura T, Nakanishi K et al. The absence of interleukin 1 receptor-related T1/ST2 does not affect T helper cell type 2 development and its effector function. *J Exp Med* 1999;**190**:1541–1548.
29. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000;**191**:1069–1076.
30. Oboki K, Ohno T, Kajiura 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.
31. 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.
32. Blom L, Poulsen BC, Jensen BM, Hansen A, Poulsen LK. IL-33 induces IL-9 production in human CD4⁺ T cells and basophils. *PLoS ONE* 2011;**6**:e21695.
33. Uytendhove C, Brombacher F, Van Snick J. TGF-beta interactions with IL-1 family members trigger IL-4-independent IL-9 production by mouse CD4⁺ T cells. *Eur J Immunol* 2010;**40**:2230–2235.
34. McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. *J Exp Med* 2002;**195**:221–231.
35. Chan WL, Pejnovic N, Lee CA, Al-Ali NA. Human IL-18 receptor and ST2L are stable and selective markers for the respective type 1 and type 2 circulating lymphocytes. *J Immunol* 2001;**167**:1238–1244.
36. Yang Q, Li G, Zhu Y, Liu L, Chen E, Turnquist H et al. IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8⁺ T cells. *Eur J Immunol* 2011;**41**:3351–3360.
37. 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.
38. Bourgeois E, Van LP, Samson M, Diem S, Barra A, Roga S et al. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. *Eur J Immunol* 2009;**39**:1046–1055.
39. Bourgeois EA, Levescot A, Diem S, Chauvineau A, Berges H, Milpied P et al. A natural protective function of invariant NKT cells in a mouse model of innate-cell-driven lung inflammation. *Eur J Immunol* 2011;**41**:299–305.
40. Dorshkind K, Montecino-Rodriguez E. Fetal B-cell lymphopoiesis and the emergence of B-1-cell potential. *Nat Rev Immunol* 2007;**7**:213–219.
41. Martin F, Oliver AM, Kearney JF. Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity* 2001;**14**:617–629.
42. 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.
43. Takatsu K, Kouro T, Nagai Y. Interleukin 5 in the link between the innate and acquired immune response. *Adv Immunol* 2009;**101**:191–236.
44. Ho LH, Ohno T, Oboki K, Kajiura N, Suto H, Iikura M et al. IL-33 induces IL-13 production by mouse mast cells independently of IgE-Fc epsilon RI signals. *J Leukoc Biol* 2007;**82**:1481–1490.
45. Iikura M, Suto H, Kajiura N, Oboki K, Ohno T, Okayama Y et al. IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest* 2007;**87**:971–978.
46. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: the ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol* 2007;**179**:2051–2054.
47. Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J Immunol* 1998;**161**:4866–4874.
48. Moulin D, Donze O, Talabot-Ayer D, Mezin F, Palmer G, Gabay C. Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine* 2007;**40**:216–225.
49. Kaieda S, Shin K, Nigrovic PA, Seki K, Lee RT, Stevens RL et al. Synovial fibroblasts promote the expression and granule accumulation of tryptase via interleukin-33 and its receptor ST-2 (IL1RL1). *J Biol Chem* 2010;**285**:21478–21486.
50. Pushparaj PN, Tay HK, H'Ng SC, Pitman N, Xu D, McKenzie A et al. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A* 2009;**106**:9773–9778.
51. Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-

- independent agents to promote mast cell and basophil activation. *Inflamm Res* 2010;**59**:207–218.
52. Hueber AJ, Alves-Filho JC, Asquith DL, Michels C, Millar NL, Reilly JH et al. IL-33 induces skin inflammation with mast cell and neutrophil activation. *Eur J Immunol* 2011;**41**:2229–2237.
 53. Pecaric-Petkovic T, Didichenko SA, Kampfer 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.
 54. Suzukawa M, Iikura M, Koketsu R, Nagase H, Tamura C, Komiya A et al. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol* 2008;**181**:5981–5989.
 55. Schneider E, Petit-Bertron AF, Bricard R, Levasseur M, Ramadan A, Girard JP et al. IL-33 activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production. *J Immunol* 2009;**183**:3591–3597.
 56. Kroeger KM, Sullivan BM, Locksley RM. IL-18 and IL-33 elicit Th2 cytokines from basophils via a MyD88- and p38alpha-dependent pathway. *J Leukoc Biol* 2009;**86**:769–778.
 57. Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H et al. Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest* 2008;**88**:1245–1253.
 58. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol* 2008;**121**:1484–1490.
 59. Chow JY, Wong CK, Cheung PF, Lam CW. Intracellular signaling mechanisms regulating the activation of human eosinophils by the novel Th2 cytokine IL-33: implications for allergic inflammation. *Cell Mol Immunol* 2010;**7**:26–34.
 60. Roth N, Stadler S, Lemann M, Hosli S, Simon HU, Simon D. Distinct eosinophil cytokine expression patterns in skin diseases – the possible existence of functionally different eosinophil subpopulations. *Allergy* 2011;**66**:1477–1486.
 61. Mayuzumi N, Matsushima H, Takashima A. IL-33 promotes DC development in BM culture by triggering GM-CSF production. *Eur J Immunol* 2009;**39**:3331–3342.
 62. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. *J Allergy Clin Immunol* 2009;**123**:1047–1054.
 63. Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol* 2011;**41**:1675–1686.
 64. Steinman RM. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol* 2012;**30**:1–22.
 65. Oshikawa K, Yanagisawa K, Tominaga S, Sugiyama Y. ST2 protein induced by inflammatory stimuli can modulate acute lung inflammation. *Biochem Biophys Res Commun* 2002;**299**:18–24.
 66. Sweet MJ, Leung BP, Kang D, Sogaard M, Schulz K, Trajkovic V et al. A novel pathway regulating lipopolysaccharide-induced shock by ST2/T1 via inhibition of Toll-like receptor 4 expression. *J Immunol* 2001;**166**:6633–6639.
 67. Espinassous Q, Garcia-de-Paco E, Garcia-Verdugo I, Synguelakis M, von Aulock S, Sallenave JM et al. IL-33 enhances lipopolysaccharide-induced inflammatory cytokine production from mouse macrophages by regulating lipopolysaccharide receptor complex. *J Immunol* 2009;**183**:1446–1455.
 68. Kumar S, Tzimas MN, Griswold DE, Young PR. Expression of ST2, an interleukin-1 receptor homologue, is induced by proinflammatory stimuli. *Biochem Biophys Res Commun* 1997;**235**:474–478.
 69. Saccani S, Polentarutti N, Penton-Rol G, Sims JE, Mantovani A. Divergent effects of LPS on expression of IL-1 receptor family members in mononuclear phagocytes in vitro and in vivo. *Cytokine* 1998;**10**:773–780.
 70. Joshi AD, Oak SR, Hartigan AJ, Finn WG, Kunkel SL, Duffy KE et al. Interleukin-33 contributes to both M1 and M2 chemokine marker expression in human macrophages. *BMC Immunol* 2010;**11**:52.
 71. 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.
 72. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. *Circ Res* 2011;**107**:650–658.
 73. Ohno T, Oboki K, Morita H, Kajiura N, Arae K, Tanaka S et al. Paracrine IL-33 stimulation enhances lipopolysaccharide-mediated macrophage activation. *PLoS ONE* 2011;**6**:e18404.
 74. Brint EK, Xu D, Liu H, Dunne A, McKenzie AN, O'Neill LA et al. ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol* 2004;**5**:373–379.
 75. Schulze J, Bickert T, Beil FT, Zaiss MM, Albers J, Wintges K et al. Interleukin-33 is expressed in differentiated osteoblasts and blocks osteoclast formation from bone marrow precursor cells. *J Bone Miner Res* 2011;**26**:704–717.
 76. Saidi S, Bouri F, Lencel P, Duplomb L, Baud'huin M, Delplace S et al. IL-33 is expressed in human osteoblasts, but has no direct effect on bone remodeling. *Cytokine* 2011;**53**:347–354.
 77. Saleh H, Eeles D, Hodge JM, Nicholson GC, Gu R, Pompolo S et al. Interleukin-33, a target of parathyroid hormone and oncostatin m, increases osteoblastic matrix mineral deposition and inhibits osteoclast formation in vitro. *Endocrinology* 2011;**152**:1911–1922.
 78. Mun SH, Ko NY, Kim HS, Kim JW, Kim do K, Kim AR et al. Interleukin-33 stimulates formation of functional osteoclasts from human CD14⁺ monocytes. *Cell Mol Life Sci* 2010;**67**:3883–3892.
 79. Zaiss MM, Kurowska-Stolarska M, Bohm C, Gary R, Schlotysek C, Stolarski B et al. IL-33 shifts the balance from osteoclast to alternatively activated macrophage differentiation and protects from TNF- α -mediated bone loss. *J Immunol* 2011;**186**:6097–6105.
 80. Andre R, Lerouet D, Kimber I, Pinteaux E, Rothwell NJ. Regulation of expression of the novel IL-1 receptor family members in the mouse brain. *J Neurochem* 2005;**95**:324–330.
 81. Yasuoka S, Kawanokuchi J, Parajuli B, Jin S, Doi Y, Noda M et al. Production and functions of IL-33 in the central nervous system. *Brain Res* 2011;**1385**:8–17.
 82. Hudson CA, Christophi GP, Gruber RC, Wilmore JR, Lawrence DA, Massa PT. Induction of IL-33 expression and activity in central nervous system glia. *J Leukoc Biol* 2008;**84**:631–643.
 83. Allakhverdi Z, Comeau MR, Smith DE, Toy D, Endam LM, Desrosiers M et al. CD34⁺ hemopoietic progenitor cells are potent effectors of allergic inflammation. *J Allergy Clin Immunol* 2009;**123**:472–478.
 84. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H et al. Innate production of TH2 cytokines by adipose tissue-associated c-Kit⁺ Sca-1⁺ lymphoid cells. *Nature* 2010;**463**:540–544.
 85. Koyasu S, Moro K. Type 2 innate immune responses and the natural helper cell. *Immunology* 2011;**132**:475–481.
 86. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010;**464**:1367–1370.
 87. Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF Jr, Tocker JE et al.

- IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. *Nature* 2010;**464**:1362–1366.
88. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisle CJ, Erle DJ et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A* 2010;**107**:11489–11494.
 89. Saenz SA, Noti M, Artis D. Innate immune cell populations function as initiators and effectors in Th2 cytokine responses. *Trends Immunol* 2010;**31**:407–413.
 90. Barlow JL, McKenzie AN. Nuocytes: expanding the innate cell repertoire in type-2 immunity. *J Leukoc Biol* 2011;**90**:867–874.
 91. Koyasu S, Moro K. Innate Th2-type immune responses and the natural helper cell, a newly identified lymphocyte population. *Curr Opin Allergy Clin Immunol* 2011;**11**:109–114.
 92. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat Immunol* 2011;**13**:58–66.
 93. Wilhelm C, Hirota K, Stieglitz B, Van Snick J, Tolaini M, Lahl K et al. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat Immunol* 2011;**12**:1071–1077.
 94. Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, Cruickshank JP et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J Allergy Clin Immunol* 2012;**129**:191–198. e1–4.
 95. Chang YJ, Kim HY, Albacker LA, Baumgarth N, McKenzie AN, Smith DE et al. Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nat Immunol* 2011;**12**:631–638.
 96. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol* 2011;**12**:1045–1054.
 97. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR2 and CD161. *Nat Immunol* 2011;**12**:1055–1062.
 98. Choi YS, Choi HJ, Min JK, Pyun BJ, Maeng YS, Park H et al. Interleukin-33 induces angiogenesis and vascular permeability through ST2/TRAF6-mediated endothelial nitric oxide production. *Blood* 2009;**114**:3117–3126.
 99. Yagami A, Orihara K, Morita H, Futamura K, Hashimoto N, Matsumoto K et al. IL-33 mediates inflammatory responses in human lung tissue cells. *J Immunol* 2010;**185**:5743–5750.
 100. Aoki S, Hayakawa M, Ozaki H, Takezako N, Obata H, Ibaraki N et al. ST2 gene expression is proliferation-dependent and its ligand, IL-33, induces inflammatory reaction in endothelial cells. *Mol Cell Biochem* 2010;**335**:75–81.
 101. Demyanets S, Konya V, Kastl SP, Kaun C, Rauscher S, Niessner A et al. Interleukin-33 induces expression of adhesion molecules and inflammatory activation in human endothelial cells and in human atherosclerotic plaques. *Arterioscler Thromb Vasc Biol* 2011;**31**:2080–2089.
 102. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M et al. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. *Proc Natl Acad Sci U S A* 2010;**107**:8017–8022.
 103. Fujita J, Kawaguchi M, Kokubu F, Ohara G, Ota K, Huang SK et al. Interleukin-33 induces interleukin-17F in bronchial epithelial cells. *Allergy* 2012;**67**:744–750.
 104. Tominaga S. Murine mRNA for the beta-subunit of integrin is increased in BALB/c-3T3 cells entering the G1 phase from the G0 state. *FEBS Lett* 1988;**238**:315–319.
 105. Kurokawa M, Matsukura S, Kawaguchi M, Ieki K, Suzuki S, Odaka M et al. Expression and effects of IL-33 and ST2 in allergic bronchial asthma: IL-33 induces eosinophil production in lung fibroblasts. *Int Arch Allergy Immunol* 2011;**155**(Suppl 1):12–20.
 106. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadóttir A, Sulem P, Jonsdóttir GM et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009;**41**:342–347.
 107. Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S et al. Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med* 2001;**164**:277–281.
 108. Ali M, Zhang G, Thomas WR, McLean CJ, Bizzantino JA, Laing IA et al. Investigations into the role of ST2 in acute asthma in children. *Tissue Antigens* 2009;**73**:206–212.
 109. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol* 2009;**183**:5094–5103.
 110. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**:752–754.
 111. Oshikawa K, Yanagisawa K, Tominaga S, Sugiyama Y. Expression and function of the ST2 gene in a murine model of allergic airway inflammation. *Clin Exp Allergy* 2002;**32**:1520–1526.
 112. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* 2007;**282**:26369–26380.
 113. Kim HY, Chang YJ, Subramanian S, Lee HH, Albacker LA, Matangkasombut P et al. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J Allergy Clin Immunol* 2012;**129**:216–227.
 114. Wills-Karp M, Rani R, Dienger K, Lewkowich I, Fox JG, Perkins C et al. Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med* 2012;**209**:607–622.
 115. Mangan NE, Dasvarma A, McKenzie AN, Fallon PG. T1/ST2 expression on Th2 cells negatively regulates allergic pulmonary inflammation. *Eur J Immunol* 2007;**37**:1302–1312.
 116. Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. *Allergol Int* 2008;**57**:121–134.
 117. Besnard AG, Guillou N, Tschopp J, Erard F, Couillin I, Iwakura Y et al. NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. *Allergy* 2011;**66**:1047–1057.
 118. Louten J, Rankin AL, Li Y, Murphy EE, Beaumont M, Moon C et al. Endogenous IL-33 enhances Th2 cytokine production and T-cell responses during allergic airway inflammation. *Int Immunol* 2011;**23**:307–315.
 119. Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ et al. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 2004;**169**:378–385.
 120. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;**15**:410–416.
 121. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009;**457**:585–588.
 122. Milne J, Brand S. Occupational asthma after inhalation of dust of the proteolytic enzyme, papain. *Br J Ind Med* 1975;**32**:302–307.
 123. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;**36**:451–463.

124. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* 2007;**447**:92–96.
125. Yasuda K, Muto T, Kawagoe T, Matsumoto M, Sasaki Y, Matsushita K et al. Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proc Natl Acad Sci U S A* 2012;**109**:3451–3456.
126. 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.
127. Castano R, Bosse Y, Endam LM, Desrosiers M. Evidence of association of interleukin-1 receptor-like 1 gene polymorphisms with chronic rhinosinusitis. *Am J Rhinol Allergy* 2009;**23**:377–384.
128. Hong YS, Moon SJ, Joo YB, Jeon CH, Cho ML, Ju JH et al. Measurement of interleukin-33 (IL-33) and IL-33 receptors (sST2 and ST2L) in patients with rheumatoid arthritis. *J Korean Med Sci* 2011;**26**:1132–1139.
129. Kim YH, Yang TY, Park CS, Ahn SH, Son BK, Kim JH et al. Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis. *Allergy* 2012;**67**:183–190.
130. Matsuda A, Okayama Y, Terai N, Yokoi N, Ebihara N, Tanioka H et al. The role of interleukin-33 in chronic allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 2009;**50**:4646–4652.
131. Matsuba-Kitamura S, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Taki Y, Muto T et al. Contribution of IL-33 to induction and augmentation of experimental allergic conjunctivitis. *Int Immunol* 2010;**22**:479–489.
132. 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.
133. Paliwal V, Tsuji RF, Szczepanik M, Kawkikova I, Campos RA, Kneilling M et al. Subunits of IgM reconstitute defective contact sensitivity in B-1 cell-deficient xid mice: kappa light chains recruit T cells independent of complement. *J Immunol* 2002;**169**:4113–4123.
134. Itakura A, Kikuchi Y, Kouro T, Ikutani M, Takaki S, Askenase PW et al. Interleukin 5 plays an essential role in elicitation of contact sensitivity through dual effects on eosinophils and B-1 cells. *Int Arch Allergy Immunol* 2006;**140**(Suppl 1): 8–16.
135. Komai-Koma M, Brombacher F, Pushparaj PN, Arendse B, McSharry C, Alexander J et al. Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naive mice. *Allergy* 2012. doi: 10.1111/j.1398-9995.2012.02859.x. [Epub ahead of print]
136. Hsu CL, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS One* 2010;**5**: e11944.